

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 01-012068- -00017-0000

Fecha: 2009-06-01 16:44:23 Dep. 2020 NUECREACION
Tra. 2 PATENTES Eve: 1 REGDEPOSITO
Act. 444 RESPUESTAREQUE Folios: 135

Re: Expediente No. 01.012.068

Solicitud de patente titulada: "INDOLINONAS SUSTITUIDAS CON PIRROLES,
INHIBIDORAS DE PROTEIN QUINASAS"

Solicitante: SUGEN INC. Y PHARMACIA & UPJOHN COMPANY.

RESPUESTA AL OFICIO No. 0499, NOTIFICADO POR FIJACIÓN EN LISTA No. 06,
DE FECHA 22 DE ENERO DE 2009.
(TERCER EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal de prórroga previsto en el artículo 45 de la Decisión 486:

1. En primer lugar y con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio presentado junto con mi memorial del 22 de enero de 2007.

Así entonces, el Capítulo Reivindicatorio Modificado contiene las siguientes modificaciones:

Reivindicación original	Modificaciones
1	Se limita el significado de los grupos arilo, heteroarilo o heterociclilo, así: R^2 = fenil, -SO ₂ -NH-fenil/piridina; R^3 =fenilo o piridina; R^7 =fenilo; R^{12} =pirrolidin(2-ona), piperazina, imidazol(2-ona), Pyridin(2-ona), tetrahidropirimidin-2-ona. Adicionalmente, se elimina OH dentro de la definición de R^{12} .

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial. Por el contrario, la materia contenida en el Capítulo Reivindicatorio Modificado es una franca limitación del alcance originalmente pretendido en la presente solicitud.

2. a- El Examinador insiste en que las Reivindicaciones 1-8 son muy amplias, no claras e inconcisas. En particular, el Examinador menciona ciertos términos genéricos tales como arilo y heteroarilo, lo cuales no se encuentran limitados. Adicionalmente, el Examinador anota que aún cuando la Descripción contiene 175 ejemplos, las Reivindicaciones comprenden compuestos que se extienden más allá de ésta divulgación, y por lo tanto una persona medianamente versada en la materia no encontraría enseñanzas suficientes que le permitiesen reproducir cada una de las modalidades cobijadas por las Reivindicaciones.

Específicamente, el Examinador identifica como compuestos no soportados enteramente por la descripción aquellos en los que R^3 es aril, o heteroaril; R^7 es aril; R^8 y R^9 son arilo, R^{12} es heteroarilo; R^{13} y R^{14} son arilo o heteroarilo; R^{13} y R^{14} tomados juntos forman un heterociclo; y en los que R^{15} es arilo.

Asimismo, el Examinador argumenta que dada la amplitud de las reivindicaciones pueden existir modalidades que no se encuentren vinculadas por un único concepto inventivo.

b- En respuesta a los argumentos del Examinador, me permito señalar respetuosamente que en el Capítulo Reivindicatorio Modificado, los sustituyentes R^2 , R^3 , R^7 y R^{12} se han limitado drásticamente, hasta el punto que ahora se restringen a aquellos grupos químicos que encuentran ilustrados en los ejemplos de la Descripción:

R^2 = fenil, $-\text{SO}_2\text{-NH-fenil/piridina}$;

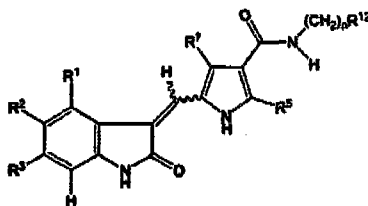
R^3 =fenilo o piridina;

R^7 =fenilo;

R^{12} =pirrolidin(2-ona), piperazina, imidazol(2-ona), Piridin(2-ona), tetrahidropirimidin-2-ona.

Es más, de manera preferente la presente solicitud se dirige a Compuestos de Formula:

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1132



en los cuales el grupo amido en la posición 3 (R^6), a los que se limita completamente esta invención, proporciona una actividad mejorada frente a compuestos similares con diferentes grupos sustituyentes R^6 del anillo de pirrol.

3. a- El Examinador establece que las patentes WO98/07695 (D1) y WO98/50356 (D2) afectan el nivel inventivo de la presente invención.

El Examinador argumenta que la única diferencia entre los compuestos de la presente invención y los compuestos de D1 y D2, es que en los compuestos de la presente invención la amida del grupo lateral se encuentra unida al anillo de pirrol a través del carbono carbonílico, mientras que el estado del arte enseña un unión directa entre un nitrógeno y el anillo de pirrol. De cualquier manera, D2 revela la posibilidad de incluir grupos arilo o N-dialquilo como sustituyentes del grupo carbamilo en la posición 3 del anillo de pirrol. Así, el Examinador concluye que los compuestos del arte previo son tanto estructural como funcionalmente similares a la presente invención.

Asimismo, el Examinador anota que D1 y D2 divulgaron previamente la actividad de este tipo de compuestos como moduladores de las señales de proteína-quinasa. El Examinador también anota que los resultados que demuestran la superioridad comparativa de los compuestos de la invención no fueron objeto de la divulgación inicial de la solicitud. Adicionalmente, los compuestos allí ilustrados, que tienen un grupo amida como sustituyente R^6 , fueron anticipados por D2, como se entiende a partir de la ilustración de derivados 2-indolinonas.

b- En relación con el arte previo citado por el Examinador, me permito señalar lo siguiente:

i) D1 revela muchas estructuras genéricas, cada una de las cuales presenta sustituyentes opcionales que pueden ser combinados en innumerables combinaciones. A manera de ilustración, adjunto al presente memorial (como ANEXO 1) un reporte de SciFinder, un servicio de Chemical Abstracts Service (CAS), que extrae compuestos de la publicaciones y proporciona su estructura química, en donde se evidencia que SciFinder encuentra 1325 ejemplos específicos en D1, en donde la mayoría de indolinonas allí reveladas no se encuentran sustituidos

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H

por pirrol, si no por fenilo u otro grupo. Así las cosas, para una persona medianamente versada en la materia es difícil, a partir de la simple lectura de D1, determinar cuáles son los compuestos específicamente allí revelados.

Es evidente que solo una pequeña fracción de los compuestos revelados en D1 están sustituidos por pirrol (ver anexo 1), y de estos, muchos presentan estructuras de anillos fusionados y/o unidos a la posición equivocada del anillo de pirrol. De la pequeña cantidad de ejemplos que indolinonas sustituidas por pirrol de D1, los únicos sustituyentes en el anillo de pirrol son H, alquilo, halógeno, éster o alquilester. De hecho, esto es consistente con la divulgación general de D1. En las estructuras genéricas de D1 en las que el grupo unido en la posición 3 de la indolinona es usualmente un anillo A (ver pagina 36), la amida se encuentra enunciada entre un sinnúmero de posibles sustituyentes (aunque no una alquilaminoamida como en la presente invención); sin embargo, cuando A se limita a pirrol, los únicos sustituyente posibles, aún en la estructura genérica, son hidrógeno, alquilo, halógeno, éster y alquilester.

Así, una persona medianamente versada en la materia leyendo D1, sería conducida inevitablemente a pensar que el sustituyente preferido para el anillo indolinona es el grupo fenilo. Es más, si por alguna razón no sugerida en D1 se seleccionara un grupo pirrol, las enseñanzas de esta referencia indican que el sitio de unión del pirrol no importaría. Adicionalmente, D1 indica que el anillo podrá estar fusionado o no y los sustituyentes apropiados para el pirrol serían H, alquilo, halógeno, éster o alquilester. No existe razón alguna para incluir un grupo amido en la posición 3 del anillo de pirrol.

Ahora bien, como se muestra en las tablas de resultados presentadas en respuesta al primer examen de fondo¹, los compuestos de la invención son inhibidores potentes tanto de PDGFR y VEGFR. Por el contrario, los compuestos de D1 correspondientes a indolinonas sustituidas por pirrol, muestran ser activos sobre quinasas EGFR y Met, pero no muestran ningún tipo de actividad sobre PDGFR.

Con respecto a la Tabla 13 de D1, los compuestos que presentan un sustituyente pirrol se encuentran ilustrados específicamente en 10717/04, 10717/A04, 10717/A11, 10717/B4, etc. Sin embargo, como se mencionó anteriormente, ninguno de dichos compuestos presenta una amida

¹ El Examinador parece sugerir en su concepto técnico que las tablas de resultados anexadas al expediente mediante mi memorial en respuesta al primer examen de fondo no serían tenidas en cuenta, por cuanto éstas no fueron incluidas en el Capítulo Descriptivo inicial. Sin embargo, me permito recordar respetuosamente al Examinador, que tal como lo enseña el Manual Andino de Patentes en su sección 1.7, ante una objeción por falta de nivel inventivo, el solicitante puede aportar pruebas en forma de argumentos o documentos, para demostrar la presencia de un efecto técnico o ventajas de la invención (ej. ensayos comparativos). Por lo tanto, los resultados evidenciando la potente inhibición tanto de PDGFR y VEGFR deben ser tenidos en cuenta en la evaluación de la patentabilidad de la presente invención.

como sustituyente en el anillo de pirrol. Ahora, al evaluar la actividad de estos compuestos en la Tabla 14, se encuentra que los mismos no presentan actividad o presentan una actividad insipiente sobre FLK quinasa, y solo una actividad moderada sobre quinasa EGFR o Met.

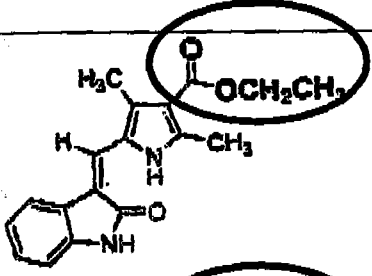
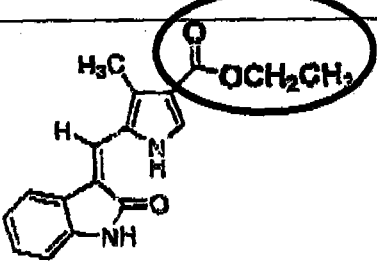
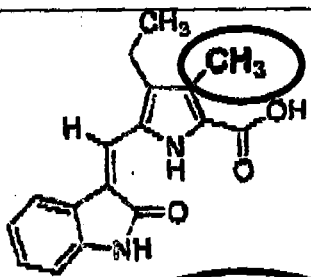
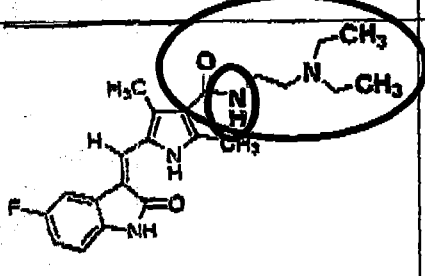
Por lo tanto, una persona medianamente versada en la materia, con la tarea de desarrollar un compuesto con actividad inhibitoria sobre VEGFR y PDGFR, no habría encontrado motivación alguna para seleccionar y modificar alguno de los compuestos de D1, que enseñan ser más activos sobre otro tipo de quinasas, y cuyo efecto sobre PDGFR es completamente desconocido. Particularmente, en vista de los datos presentados previamente, las indolinonas sustituidas con pirrol, con grupos éster como sustituyentes del anillo de pirrol (según D1), presentaron inhibición de PDGFR muy pobre.

De otra parte, aún cuando D2 revela compuestos que son estructuralmente similares a los de la presente invención, que corresponden a 3-((pirrol-2-il)metilideno)indolin-2-onas, D2 no proporciona enseñanzas o sugerencias respecto al uso de un grupo alquil-carboxamida sustituido en la posición 3 del anillo de pirrol.

Así, la configuración estructural específica de los compuestos de la presente invención, no ha sido sugerida en el arte previo y por lo tanto no puede ser considerada obvia para una persona medianamente versada en la materia. Es decir, los compuestos de la presente invención no se derivan evidentemente del arte previo.

ii) Nuevamente, deseo señalar que el concepto inventivo clave involucrado en la presente invención corresponde precisamente al grupo amida (R⁶) que sustituye el anillo pirrol de los compuestos de la invención. Como lo demuestra la siguiente tabla, la presencia del grupo amida proporciona una mejor actividad, en comparación con compuestos que tienen sustituyentes diferentes en esa posición:

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Compuesto	PDGFRb IC ₅₀ (μM) ²	VEGFR2 IC ₅₀ (μM) ³
	> 100	0.578±0.035 (n=2)
	> 100	0.465±0.068 (n=2)
	> 100	> 10 (n=2)
 <u>Presente invención</u> <u>(R⁶=amida)</u>	0.01	0.008±0.002 (n=5)

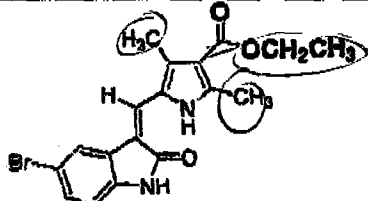
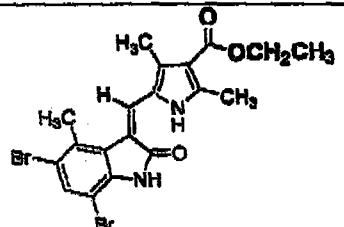
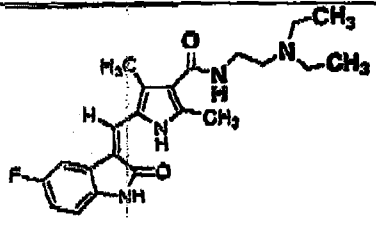
En gracia de discusión, aunque los compuestos de la presente invención compartan un núcleo común con otros compuestos correspondiente a indolinonas sustituidas con pirrol, la sustitución de la posición 3 del anillo de pirrol en la presente invención (R⁶) resulta completamente novedosa e inventiva, pues compuestos similares del estado del arte, que a lo sumo presentan un grupo éster (-C(O)OR) como en los compuestos ilustrados en la anterior tabla, enseñan claramente que el reemplazo de dicho éster o alquil en la posición 3 por grupo amida, proporciona un incremento significativo en la actividad, produciendo compuestos altamente útiles en la inhibición de ambas kinasas (4 órdenes de magnitud para PDGFR y 2 órdenes de magnitud para VEGFR2), lo que significa una importante ventaja en la terapia anticancerígena.

² Actividad celular en células 3T3.

³ Actividad celular en células PAE-VEGFR2.

1136
1403
2

La siguiente tabla, presentada también en respuesta al primer examen de fondo, enseña dos compuestos adicionales que incluyen sustituyentes dimetil y etoxicarbonil, que además, están sustituidos con un halo en el grupo fenil del sistema indolinona, siendo así aún más cercanos a los compuestos de la presente invención. Pero incluso con esta mayor cercanía, los resultados obtenidos demuestran claramente que los compuestos de la presente invención resultan mucho más potentes en los ensayos PDGFR y VEGFR2 en comparación con el primer compuesto que proporciona simplemente una leve inhibición de ambas kinasas. El segundo compuesto no fue medido contra PDGFR, sin embargo, no mostró efecto inhibitorio alguno contra VEGFR2, como lo evidencia el % de inhibición obtenido.

Compuesto	PDGFRb (μ M)	VEGFR2 (μ M) ⁴
	15	Flk-1R IC ₅₀ : 4.2
	--	Flk Kinasa % de inhibición: - 1.4%
 <u>Presente invención</u>	0.01	0.008±0.002 (n=5)

En conclusión, los ensayos comparativos realizados enseñan que:

- los compuestos de la presente invención son inhibidores potentes de VEGFR2 y PDGFR, mientras que compuestos similares del estado de la técnica proporcionan, a lo sumo, solamente una inhibición débil o la inhibición de sólo una, pero no ambas kinasas; y
- la potencia multi-kinasa aumentada de los compuestos de la invención de debe principalmente a la presencia del grupo amida en la posición 3 del anillo pirrol (R⁶).

⁴ Flk es ortólogo de ratón del VEGFR2 humano.

Me permito recordar al Examinador que los resultados ilustrados en el artículo "Discovery of 5-[5-fluoro-2-oxo-1,2-dihydroindol-(3z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic Acid (2-diethylaminoethyl)amide, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial and Platelet-Derived Growth Factor Receptor Tyrosine Kinase"⁵, también son concluyentes demostrando que la inclusión del grupo amida en la posición 4 del anillo pirrol (R⁶), proporciona resultados ventajosos e inesperados (ver tabla 1).

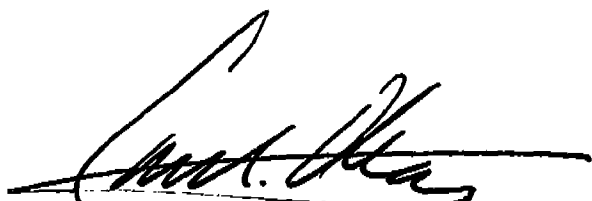
4. Por lo anterior, considero superadas las objeciones del Examinador en cuanto a la unidad de invención de la materia reivindicada en la presente solicitud.

5. Para terminar, deseo subrayar que en relación con la invención de la presente solicitud, que, además de Australia No. 2001239770, Canadá No. 2399358, Eurasia No. 005996, Japón No. 3663382, Nueva Zelanda No. 520640, Perú No. 0400-2006, Sur África No. 2002/6469, Singapur No. 90936, Ucrania No. 73976, Estados Unidos No. 6573293, Ecuador No. 7125905 y la Oficina Europea de Patentes EP 1.255.752, han sido otorgados privilegios de patente en Malaysia, China, México, India, Argentina, Chile, Noruega, Indonesia, Israel, Islandia y Taiwán.

Aunque patentes otorgadas en otras jurisdicciones no son vinculantes para la oficina nacional, sí corresponden a un fuerte indicio sobre el cumplimiento de los requisitos de patentabilidad de la invención reivindicada, puesto que los requisitos de novedad, nivel inventivo son requisitos universales y objetivos que se evalúan alrededor del mundo bajo estándares similares.

6. Por todo lo anterior, considero que las objeciones presentadas por el Examinador han sido resueltas y por consiguiente solicito respetuosamente que se proceda con la concesión de la patente.

Atentamente,



CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295

⁵ Li Sun et al., Discovery of 5-[5-fluoro-2-oxo-1,2-dihydroindol-(3z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic Acid (2-diethylaminoethyl)amide, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial and Platelet-Derived Growth Factor Receptor Tyrosine Kinase. J. Med. Chem. 2003. Vol 46. No. 7. (ANEXO 2)

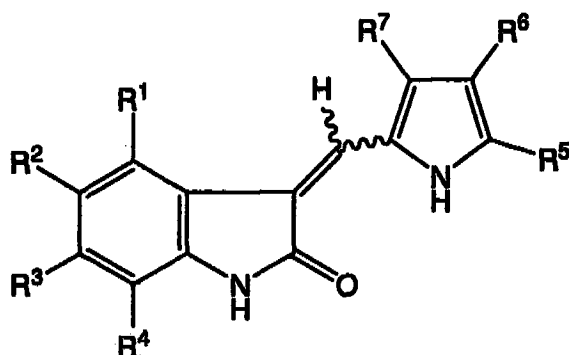
Anexos:

- Capítulo Reivindicatorio Modificado
- ANEXO 1: Resultados del reporte de SciFinder relacionado con en análisis de compuestos incluidos en D1, mostrando 1325 compuestos.
- ANEXO 2: Copia de Li Sun et al., Discovery of 5-[5-fluoro-2-oxo-1,2-dihydroindol-(3z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic Acid (2-diethylaminoethyl)amide, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial and Platelet-Derived Growth Factor Receptor Tyrosine Kinase. J. Med. Chem. 2003. Vol 46. No. 7.

CAPÍTULO REIVINDICATORIO MODIFICADO
(TERCER EXAMEN DE FONDO)

1. Un compuesto de fórmula (I)

5



(I)

o una sal farmacéuticamente aceptable del mismo, donde:

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R¹ se selecciona de hidrógeno, alquilo(C₁-C₄), -(CH₂)_rR¹⁶ y -C(O)NR⁸R⁹;

R² se selecciona de hidrógeno, halo, fenilo, -S(O)₂NHfenilo, y -S(O)₂NHpiridinil;

R³ se selecciona de hidrógeno, alquilo(C₁-C₄), alcoxi(C₁-C₄), fenilo, piridilo y -C(O)R¹⁵;

R⁴ es hidrógeno;

R⁵ se selecciona de hidrógeno y alquilo(C₁-C₄);

15

R⁶ es -C(O)R¹⁰;

R⁷ se selecciona de hidrógeno, alquilo(C₁-C₄) y fenilo;

R⁸ y R⁹ se seleccionan independientemente de hidrógeno, alquilo(C₁-C₆) y fenilo;

R¹⁰ es -N(R¹¹)(CH₂)_nR¹², donde n es 1, 2 o 3;

R¹¹ es hidrógeno;

20

R¹² se selecciona de, alcoxi(C₁-C₄), -C(O)R¹⁵, pirrolidina, pirrolidin-2-ona, piperazina, imidazol-2-ona, piridin-2-ona, tetrahidropirimidin-2-ona, 2-oxo-azepan y -NR¹³R¹⁴;

R¹³ y R¹⁴ se seleccionan independientemente del grupo formado por hidrógeno, alquilo(C₁-C₄); o

25

R¹³ y R¹⁴ se pueden combinar para formar un grupo -(CH₂)₄-, -(CH₂)₅-, -(CH₂)₂-O-(CH₂)₂- y -(CH₂)₂N(CH₃)(CH₂)₂-

R¹⁵ se selecciona del grupo formado por hidrógeno, hidroxi, alcoxi(C₁-C₄) y fenoxi;

30

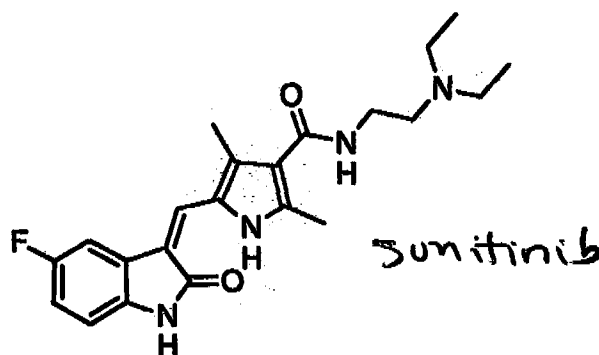
R¹⁶ se selecciona del grupo formado por hidroxi, -C(O)R¹⁵; y r es 2 o 3;

2. El compuesto de la Reivindicación 1, donde n es 2 o 3 y R^{12} es $-NR^{13}R^{14}$ donde R^{13} y R^{14} son independientemente alquil(C_1-C_4). *w*

3. El compuesto de la Reivindicación 1, donde n es 2 o 3 y R^{12} es $-NR^{13}R^{14}$ donde R^{13} y R^{14} se combinan para formar un grupo seleccionado de $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2-O-(CH_2)_2-$ o $-(CH_2)_2N(CH_3)(CH_2)_2-$. *w*

4. El compuesto de la Reivindicación 1, donde R^1 es $-C(O)NR^8R^9$, donde R^8 es hidrógeno y R^9 es fenilo. *w*

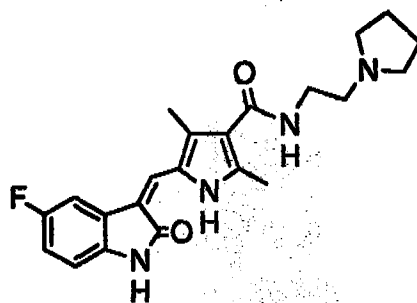
5. El compuesto de la invención que tiene la fórmula



o una sal farmacéuticamente aceptable del mismo.

6. El compuesto de la Reivindicación 1 o 5 que es la sal L-malato del ácido 5-(5-fluoro-2-oxo-1,2-dihidroindol-3-ilidenmetil)-2,4-dimetil-1H-pirrol-3-carboxílico (2-diethylaminoetil)amida. *w*

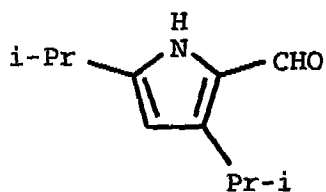
7. El compuesto de la invención que tiene la fórmula



o una sal farmacéuticamente aceptable del mismo.

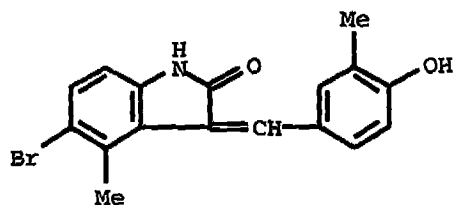
8. Una composición farmacéutica que comprende un compuesto de las Reivindicaciones 1, 5 - 7 o una sal farmacéuticamente aceptable del mismo, y un vehículo o excipiente farmacéuticamente aceptable. *w*

Answer 1:



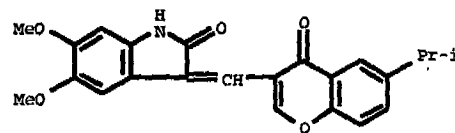
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Answer 2:



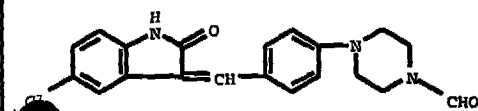
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Answer 3:



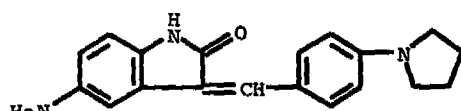
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Answer 4:



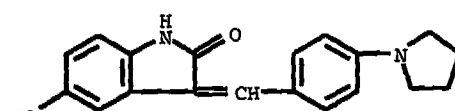
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Answer 5:



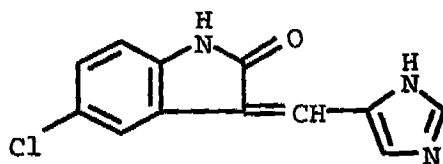
REGISTRY (Copyright (C) 2009 ACS)

Answer 6:



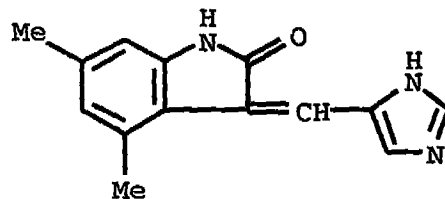
REGISTRY (Copyright (C) 2009 ACS)

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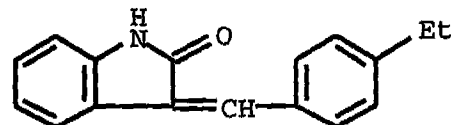
REGISTRY (Copyright (C) 2009 ACS)

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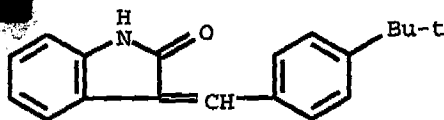
REGISTRY (Copyright (C) 2009 ACS)

Answer 9:



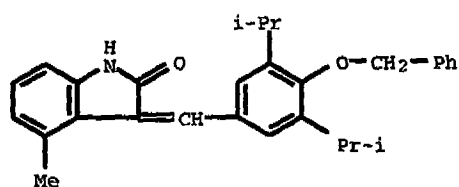
REGISTRY (Copyright (C) 2009 ACS)

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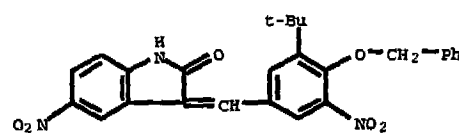
REGISTRY (Copyright (C) 2009 ACS)

Answer 11:



REGISTRY (Copyright (C) 2009 ACS)

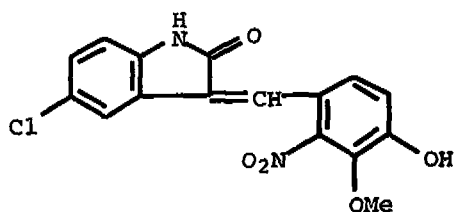
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REGISTRY (Copyright (C) 2009 ACS)

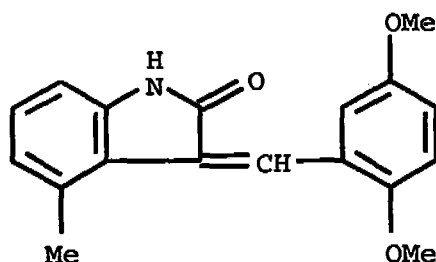
1109
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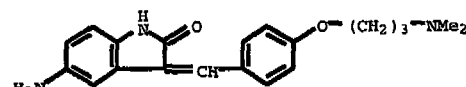
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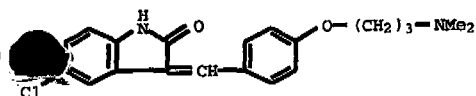
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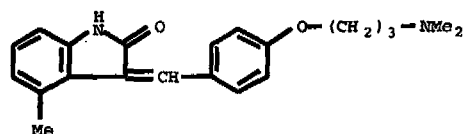
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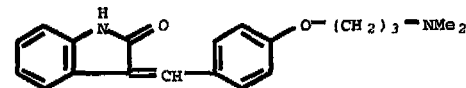
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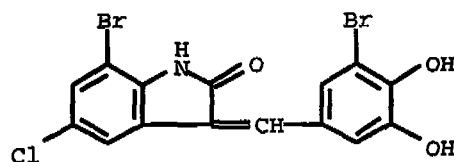
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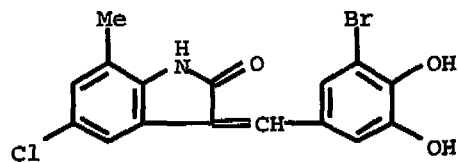
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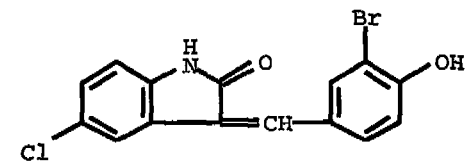
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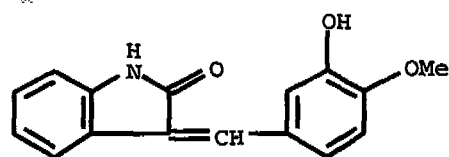
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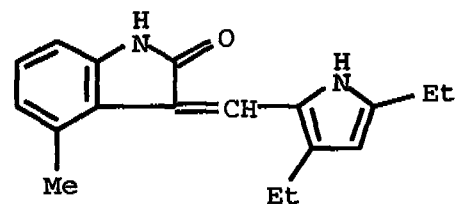
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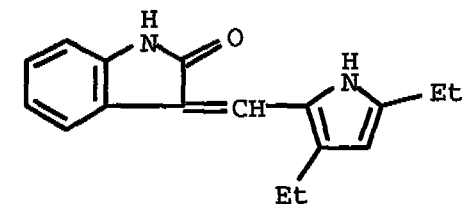
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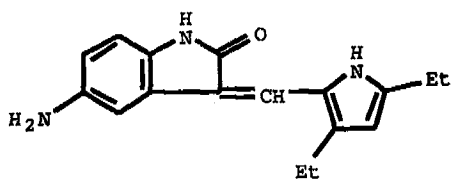
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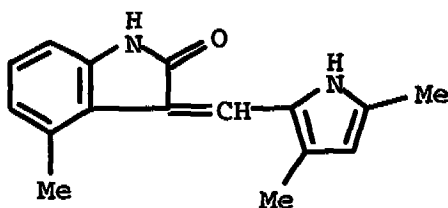
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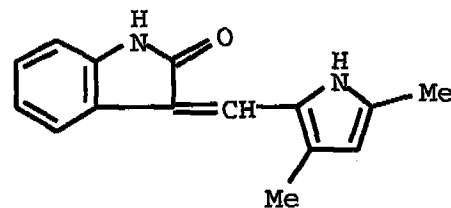
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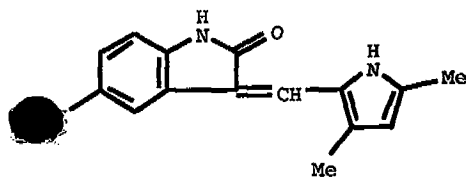
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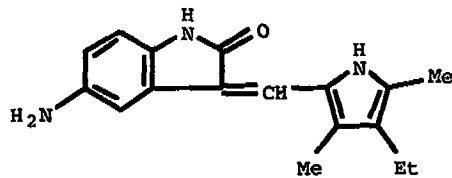
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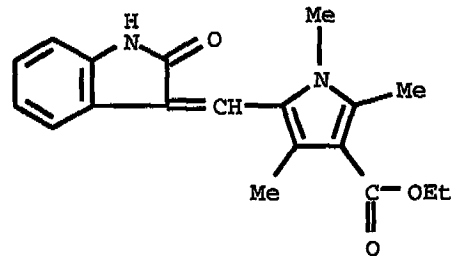
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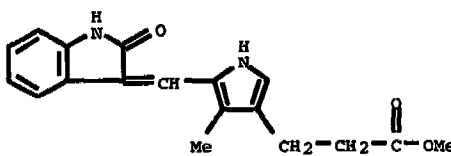
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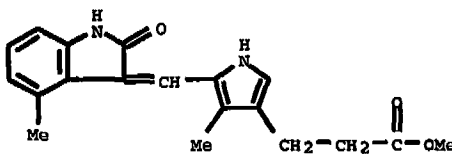
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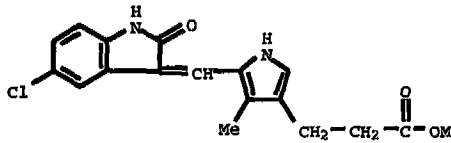
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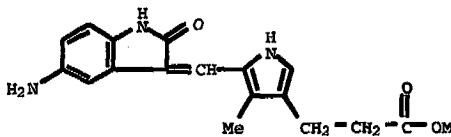
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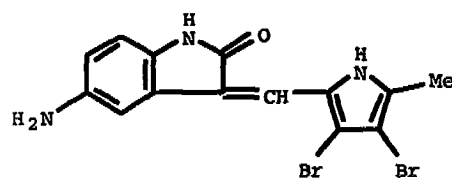
REGISTRY (Copyright (C) 2009 ACS)

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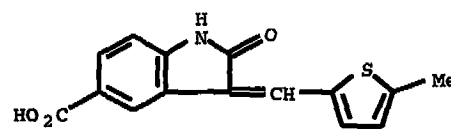
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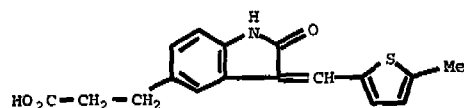
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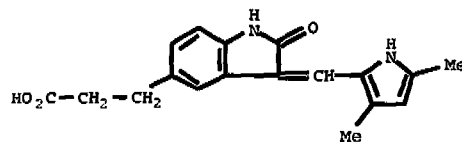
REGISTRY (Copyright (C) 2009 ACS)

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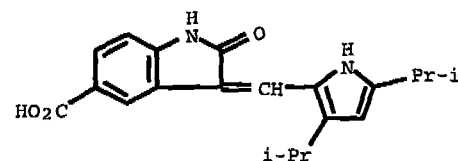
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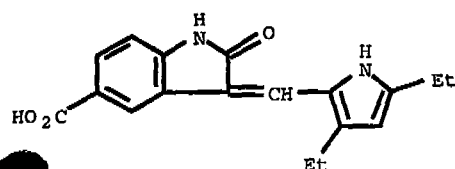
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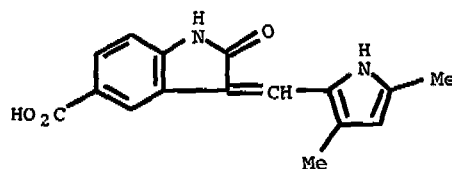
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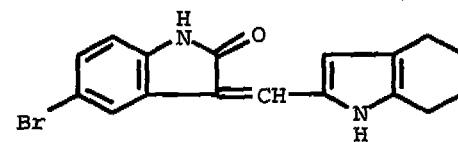
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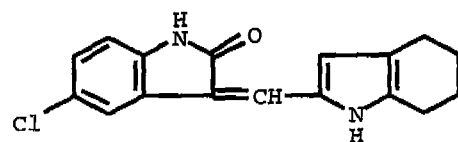
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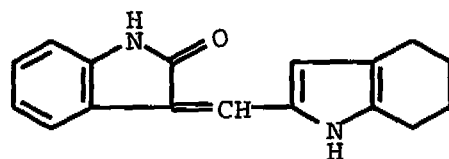
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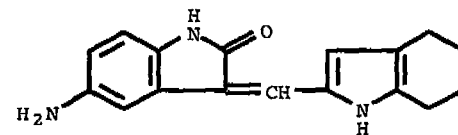
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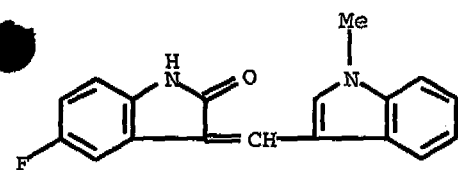
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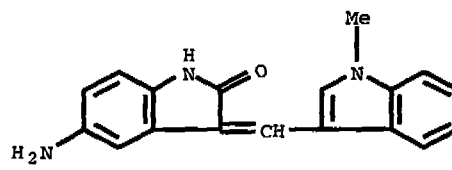
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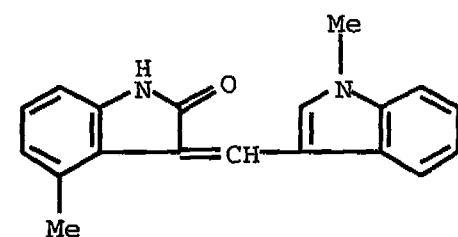
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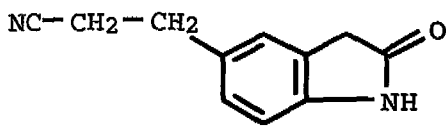
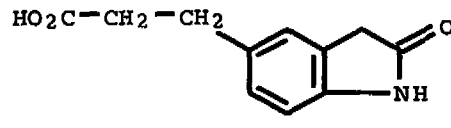
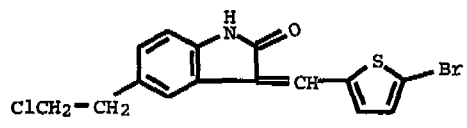
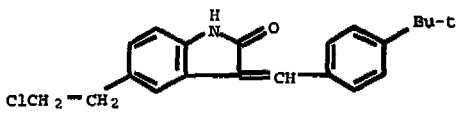
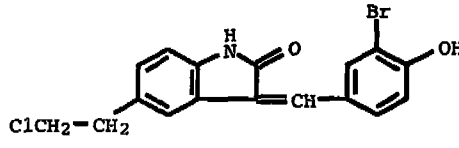
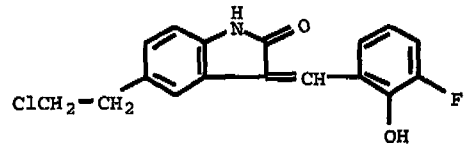
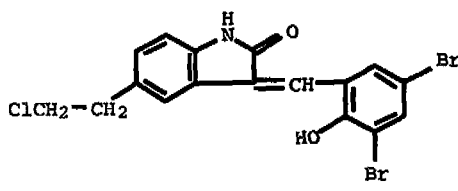
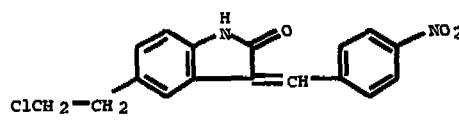
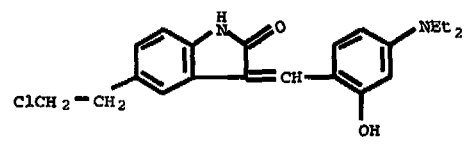
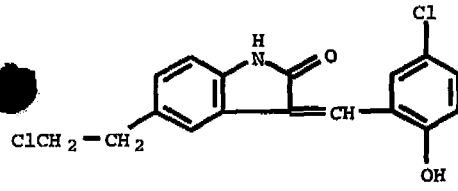
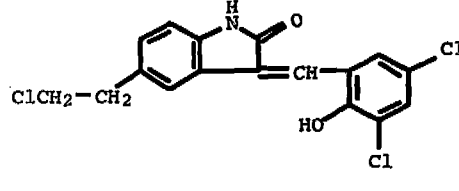
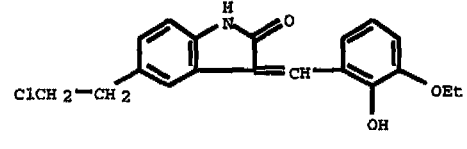


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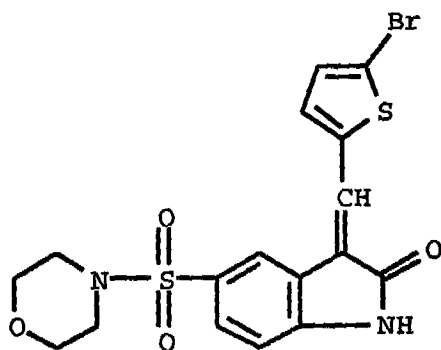


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Answer 49:  REGISTRY (Copyright (C) 2009 ACS)	Answer 50:  REGISTRY (Copyright (C) 2009 ACS)	Answer 51:  REGISTRY (Copyright (C) 2009 ACS)
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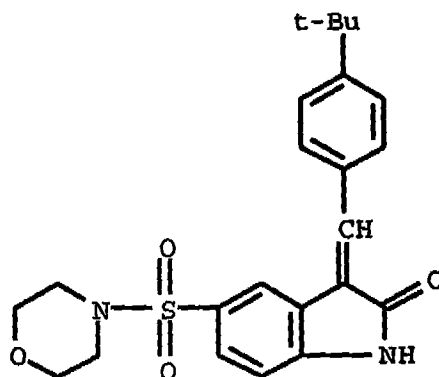
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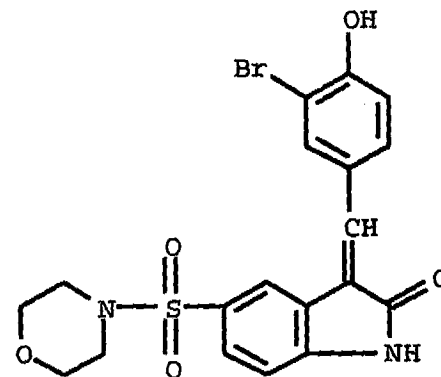
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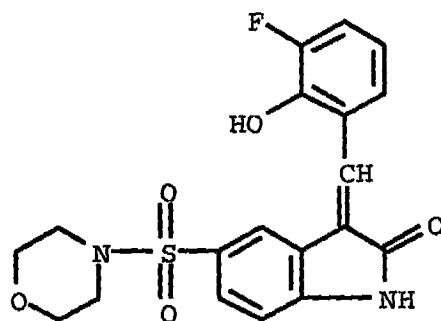
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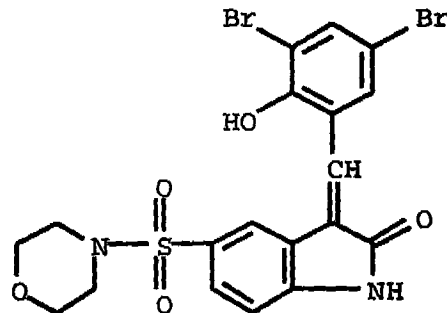
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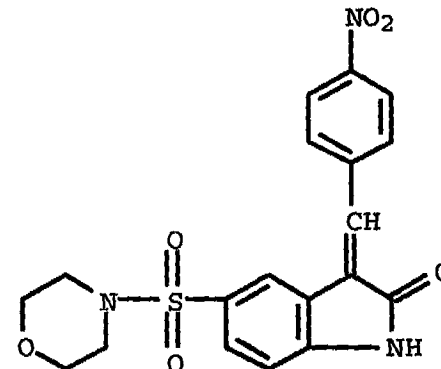
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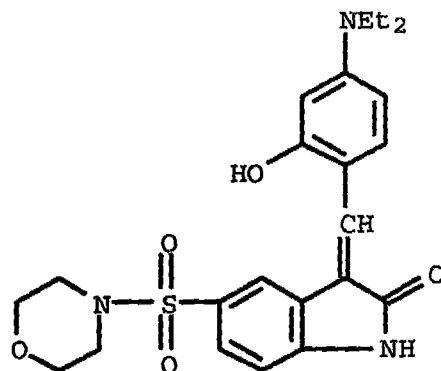
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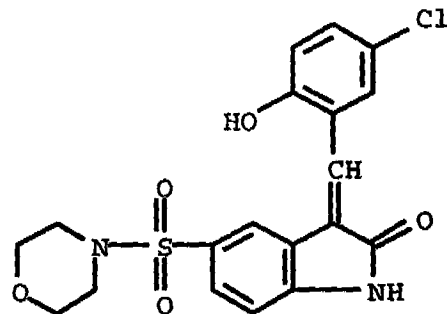
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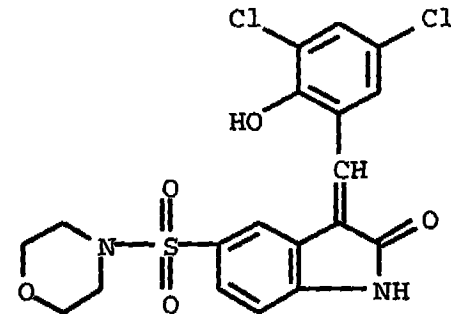
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Answer 68:



REGISTRY (Copyright (C) 2009 ACS)

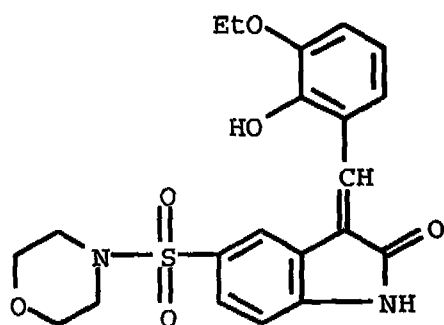
Answer 69:



REGISTRY (Copyright (C) 2009 ACS)

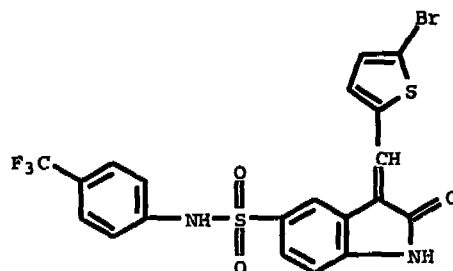
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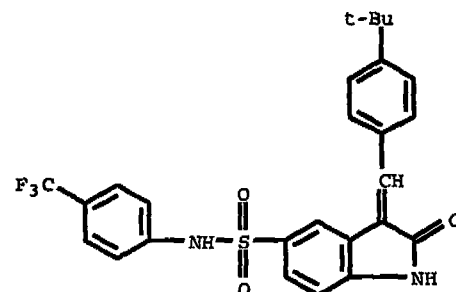
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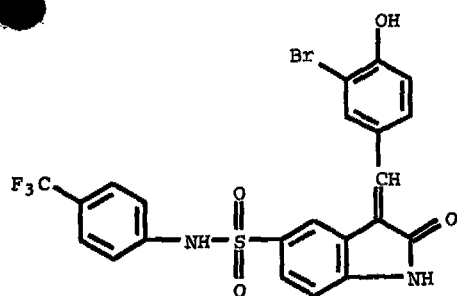
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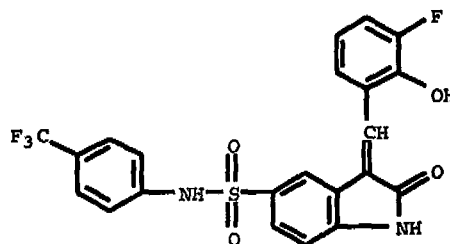
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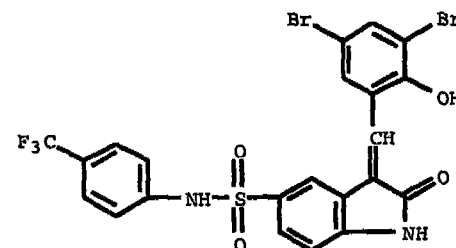
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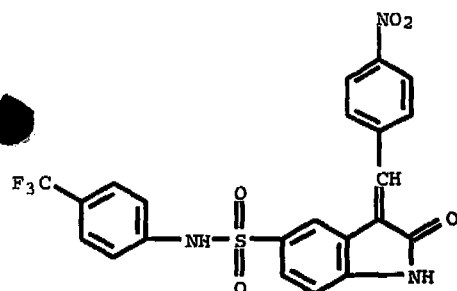
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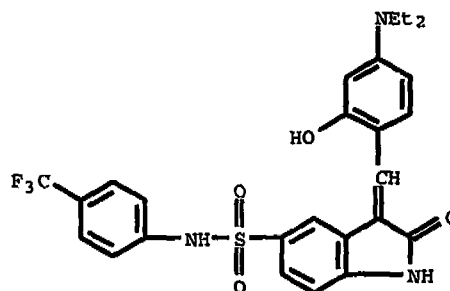
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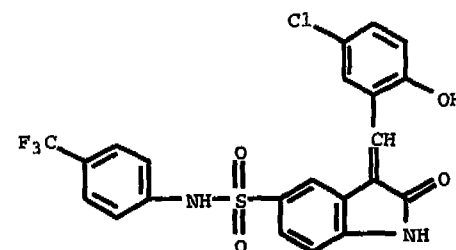
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Answer 77:



REGISTRY (Copyright (C) 2009 ACS)

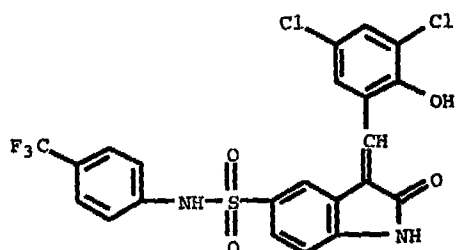
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REGISTRY (Copyright (C) 2009 ACS)

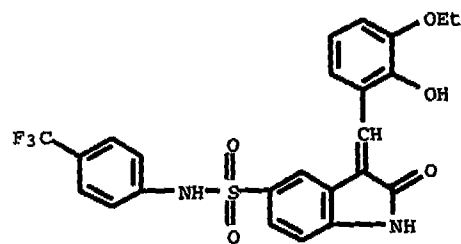
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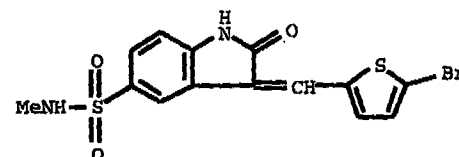
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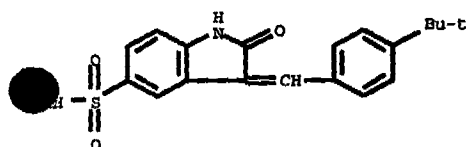
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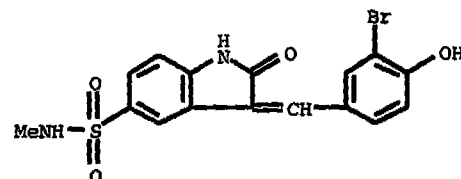
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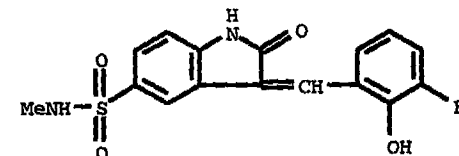
REGISTRY (Copyright (C) 2009 ACS)

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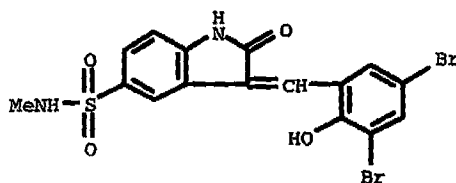
REGISTRY (Copyright (C) 2009 ACS)

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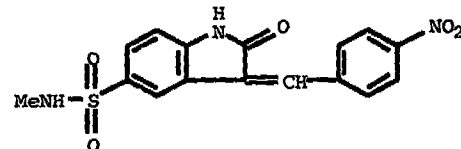
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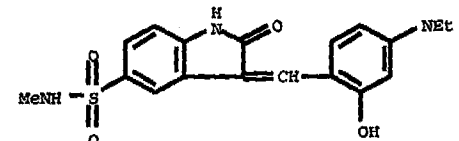
REGISTRY (Copyright (C) 2009 ACS)

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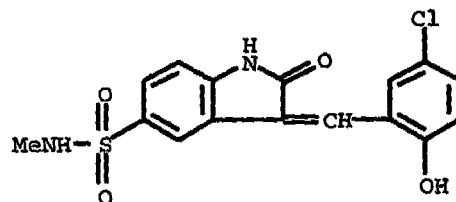
REGISTRY (Copyright (C) 2009 ACS)

Answer 87:



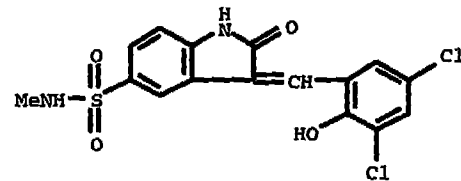
REGISTRY (Copyright (C) 2009 ACS)

Answer 88:



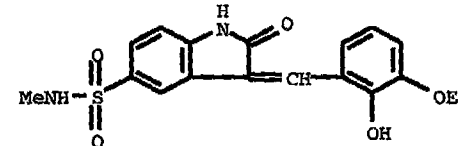
REGISTRY (Copyright (C) 2009 ACS)

Answer 89:



REGISTRY (Copyright (C) 2009 ACS)

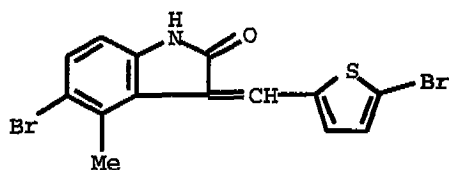
Answer 90:



REGISTRY (Copyright (C) 2009 ACS)

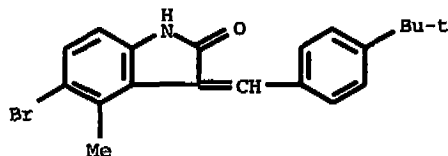
4446
1149

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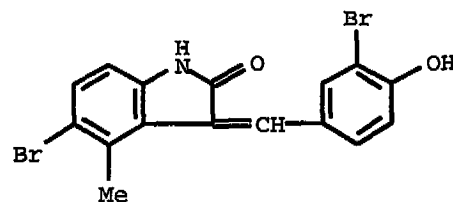
REGISTRY (Copyright (C) 2009 ACS)

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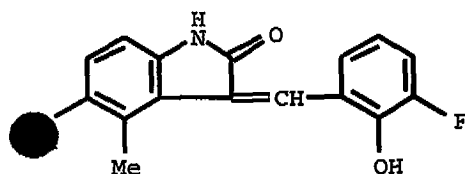
REGISTRY (Copyright (C) 2009 ACS)

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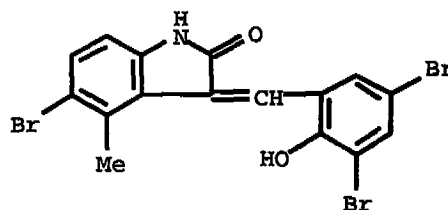
REGISTRY (Copyright (C) 2009 ACS)

Answer 94:



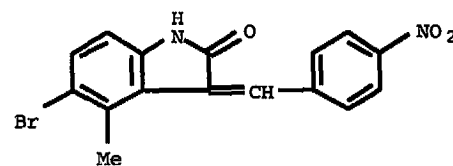
REGISTRY (Copyright (C) 2009 ACS)

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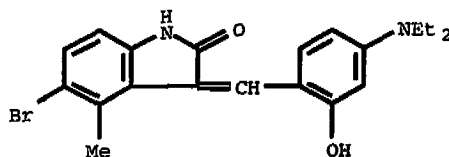
REGISTRY (Copyright (C) 2009 ACS)

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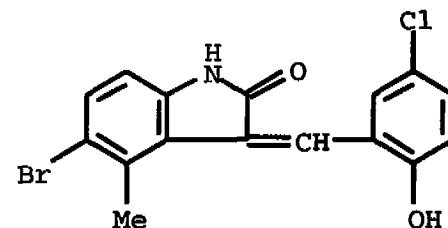
REGISTRY (Copyright (C) 2009 ACS)

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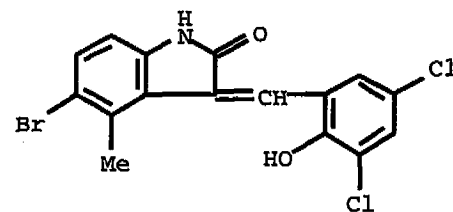
REGISTRY (Copyright (C) 2009 ACS)

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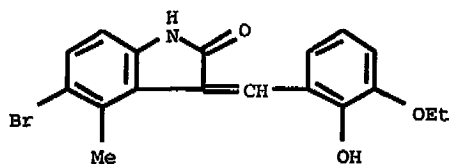
REGISTRY (Copyright (C) 2009 ACS)

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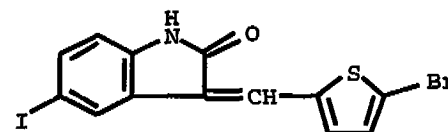
REGISTRY (Copyright (C) 2009 ACS)

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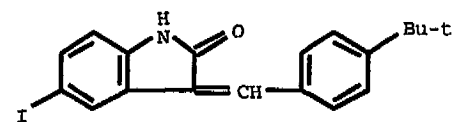
REGISTRY (Copyright (C) 2009 ACS)

Answer 101:



REGISTRY (Copyright (C) 2009 ACS)

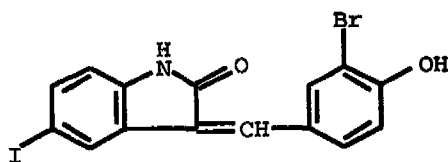
Answer 102:



REGISTRY (Copyright (C) 2009 ACS)

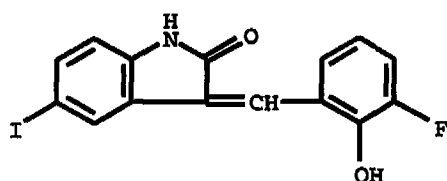
1147
2/ 1150

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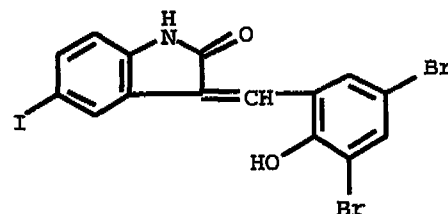
REGISTRY (Copyright (C) 2009 ACS)

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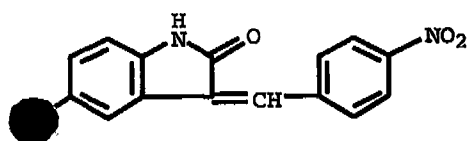
REGISTRY (Copyright (C) 2009 ACS)

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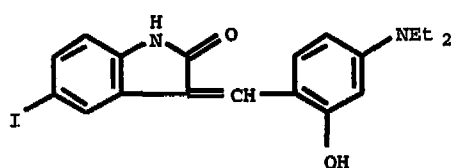
REGISTRY (Copyright (C) 2009 ACS)

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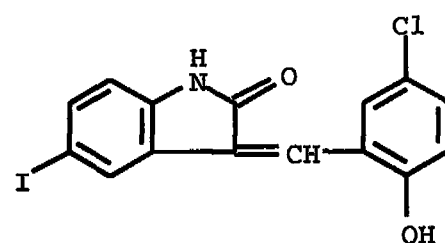
REGISTRY (Copyright (C) 2009 ACS)

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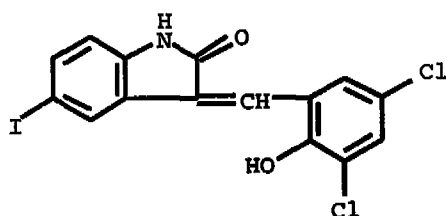
REGISTRY (Copyright (C) 2009 ACS)

Answer 108:



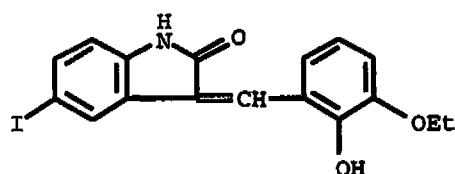
REGISTRY (Copyright (C) 2009 ACS)

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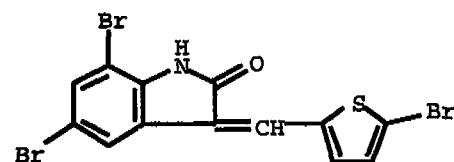
REGISTRY (Copyright (C) 2009 ACS)

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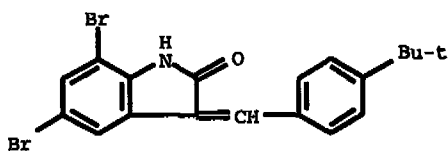
REGISTRY (Copyright (C) 2009 ACS)

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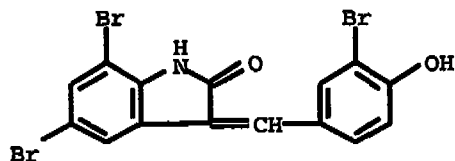
REGISTRY (Copyright (C) 2009 ACS)

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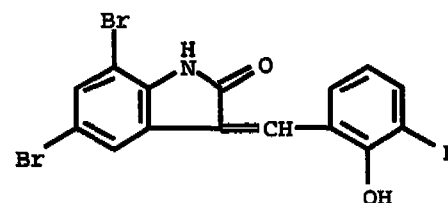
REGISTRY (Copyright (C) 2009 ACS)

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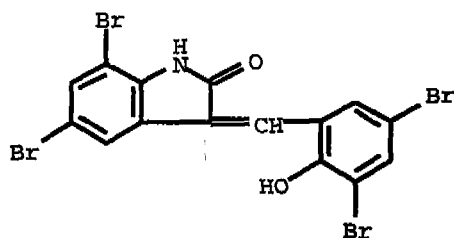
REGISTRY (Copyright (C) 2009 ACS)

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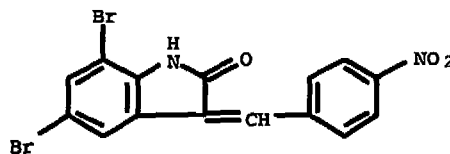
REGISTRY (Copyright (C) 2009 ACS)

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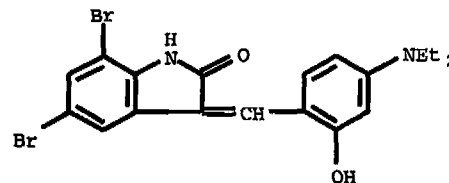
REGISTRY (Copyright (C) 2009 ACS)

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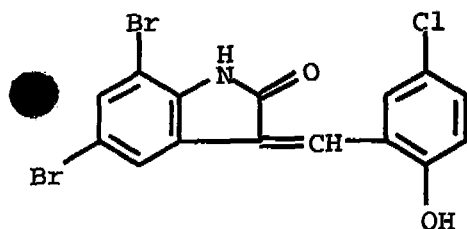
REGISTRY (Copyright (C) 2009 ACS)

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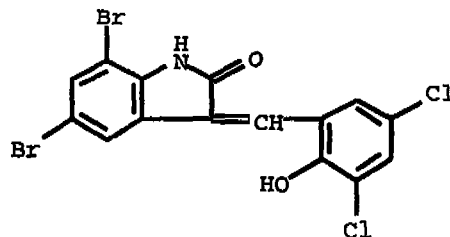
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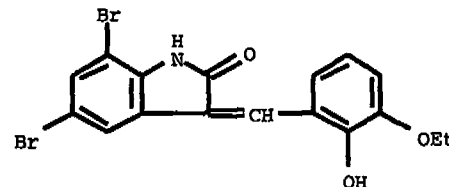
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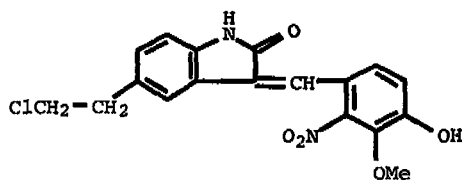
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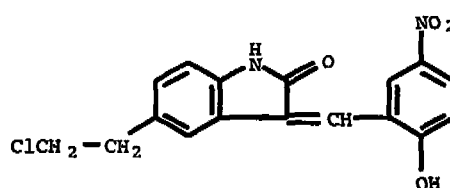
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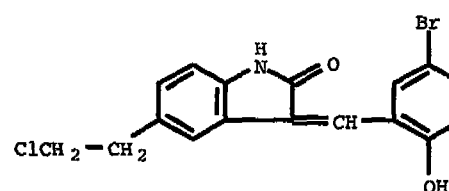
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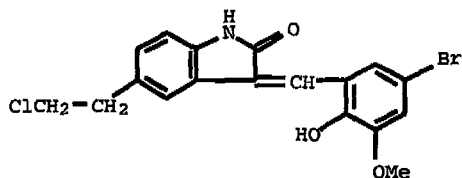
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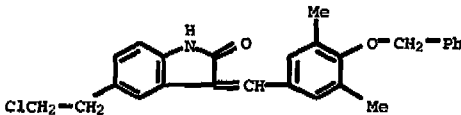
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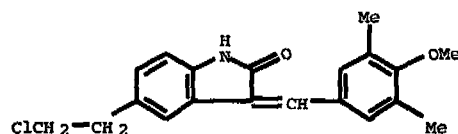
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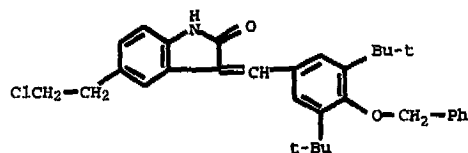
REGISTRY (Copyright (C) 2009 ACS)

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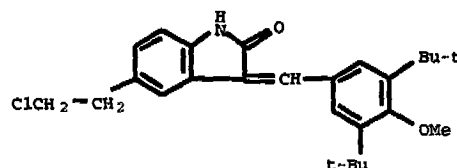
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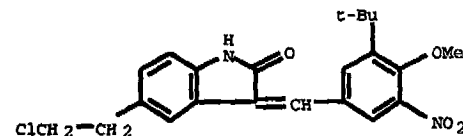
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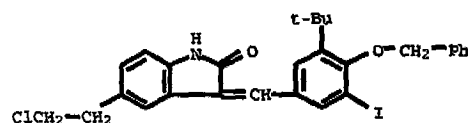
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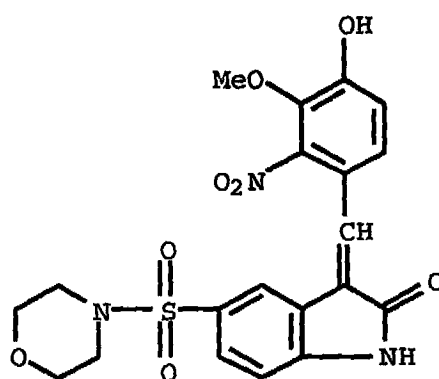
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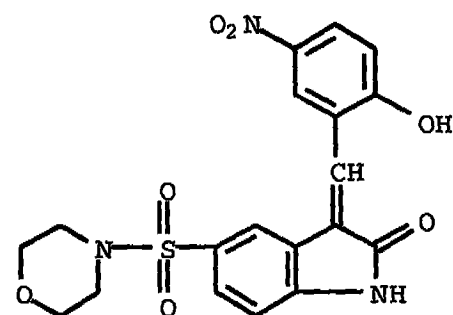
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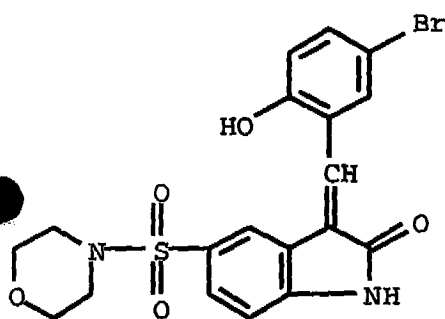
REGISTRY (Copyright (C) 2009 ACS)

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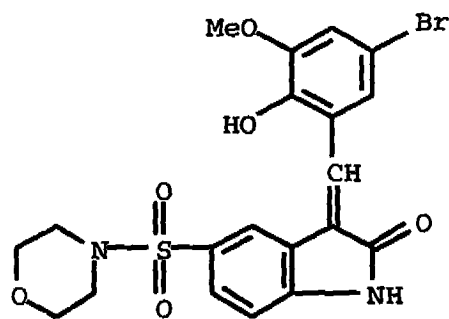
REGISTRY (Copyright (C) 2009 ACS)

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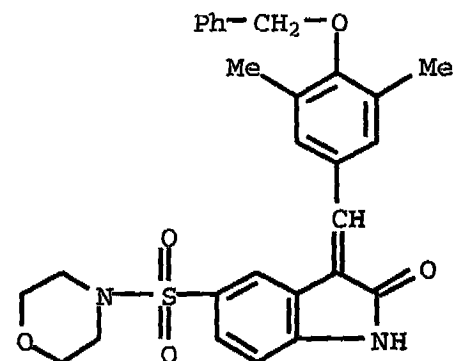
REGISTRY (Copyright (C) 2009 ACS)

Answer 134:



REGISTRY (Copyright (C) 2009 ACS)

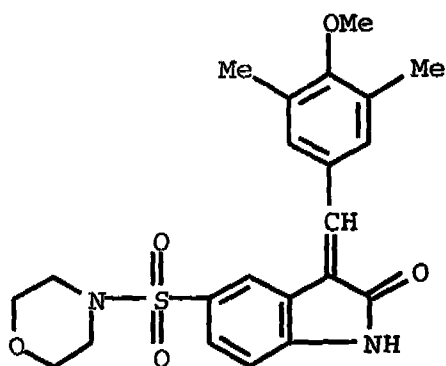
Answer 135:



REGISTRY (Copyright (C) 2009 ACS)

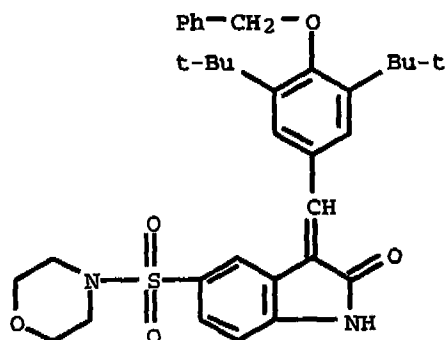
1120
24
1153

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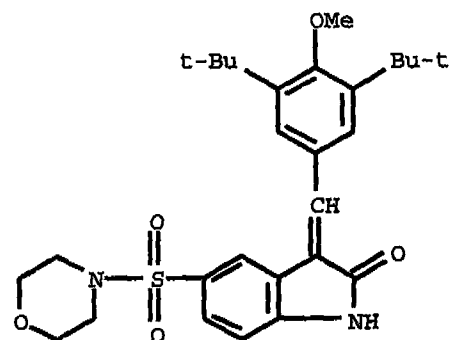
REGISTRY (Copyright (C) 2009 ACS)

Answer 137:



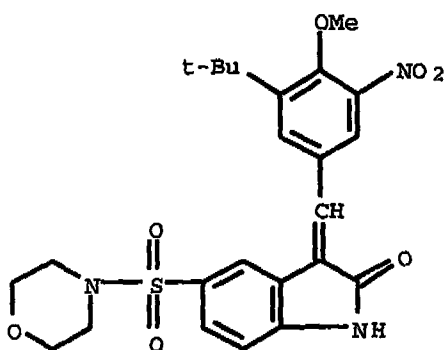
REGISTRY (Copyright (C) 2009 ACS)

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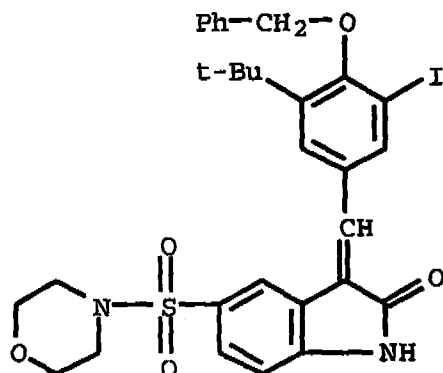
REGISTRY (Copyright (C) 2009 ACS)

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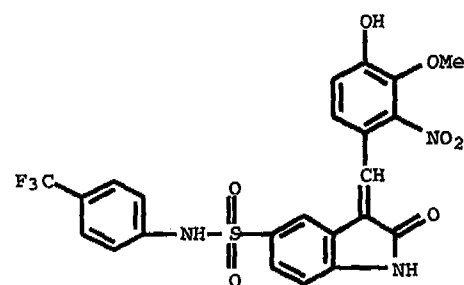
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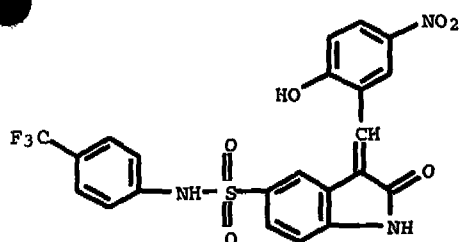
REGISTRY (Copyright (C) 2009 ACS)

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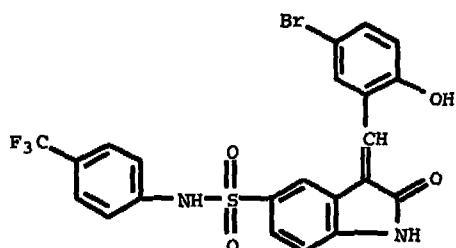
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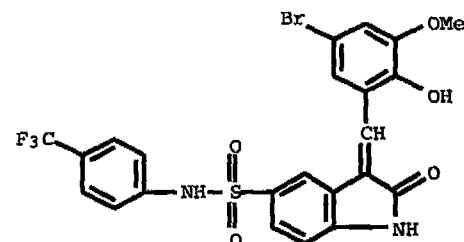
REGISTRY (Copyright (C) 2009 ACS)

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REGISTRY (Copyright (C) 2009 ACS)

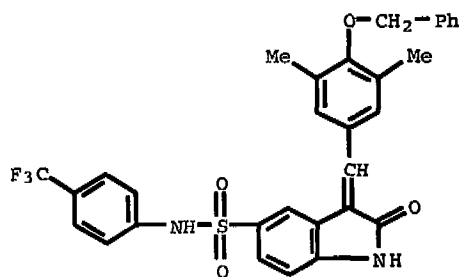
Answer 144:



REGISTRY (Copyright (C) 2009 ACS)

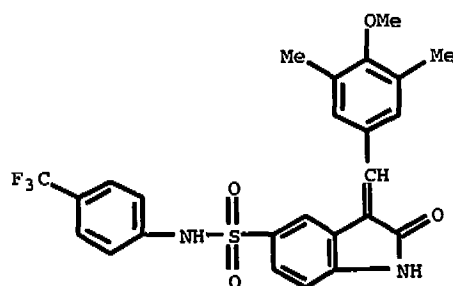
4424
AS 1154

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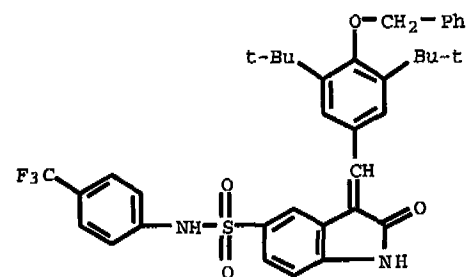
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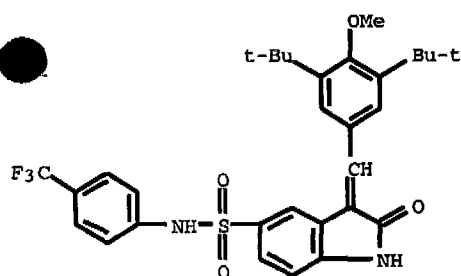
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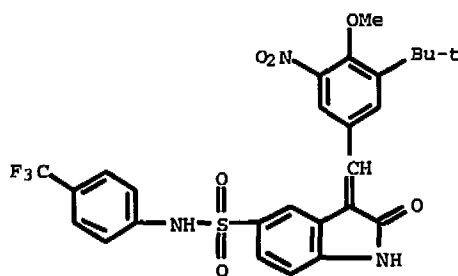
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Answer 148:



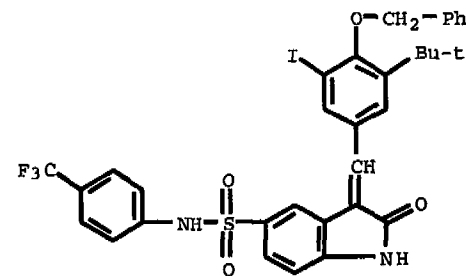
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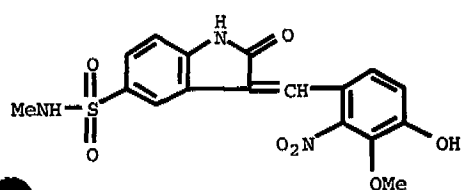
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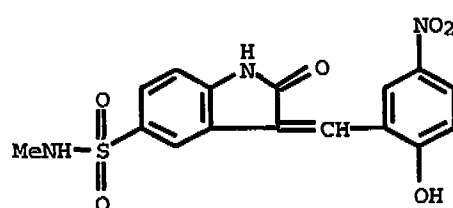
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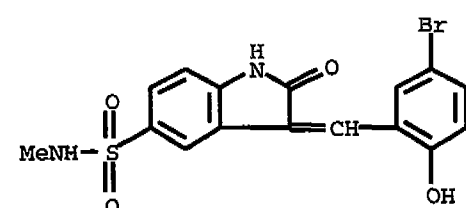
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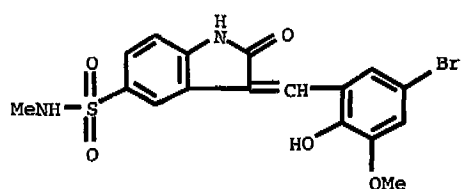
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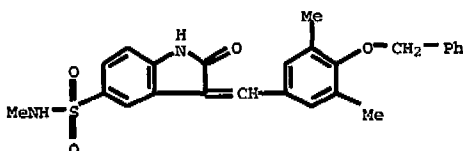
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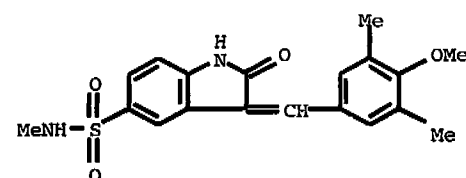
REGISTRY (Copyright (C) 2009 ACS)

Answer 155:



REGISTRY (Copyright (C) 2009 ACS)

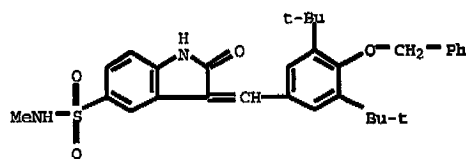
Answer 156:



REGISTRY (Copyright (C) 2009 ACS)

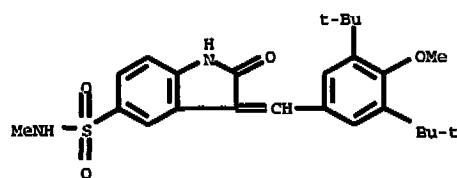
1122
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Answer 157:



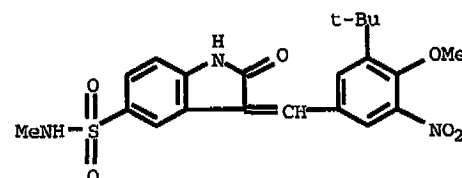
REGISTRY (Copyright (C) 2009 ACS)

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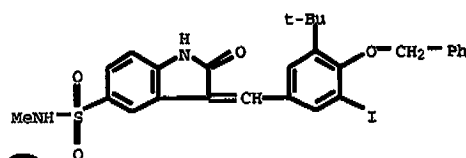
REGISTRY (Copyright (C) 2009 ACS)

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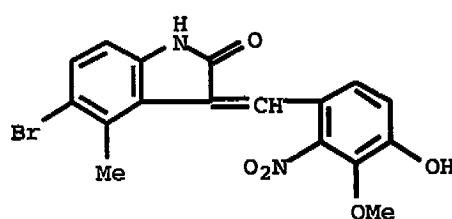
REGISTRY (Copyright (C) 2009 ACS)

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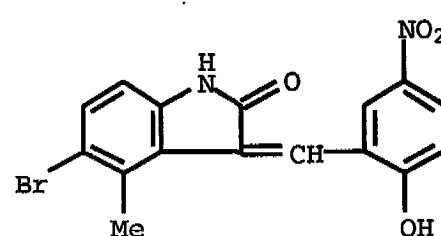
REGISTRY (Copyright (C) 2009 ACS)

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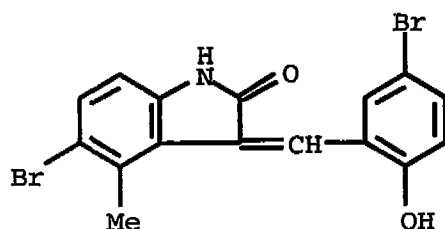
REGISTRY (Copyright (C) 2009 ACS)

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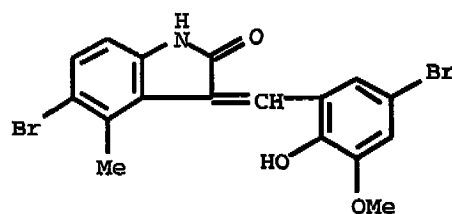
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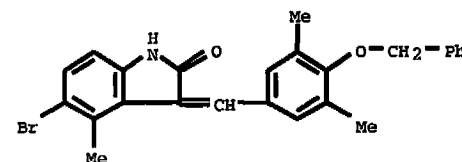
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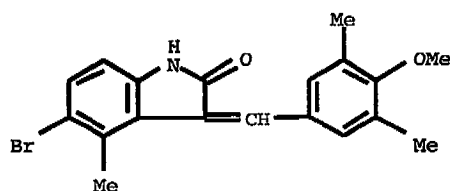
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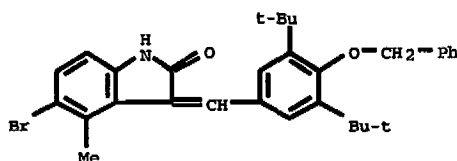
REGISTRY (Copyright (C) 2009 ACS)

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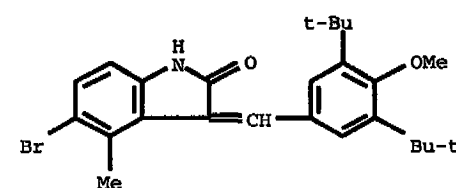
REGISTRY (Copyright (C) 2009 ACS)

Answer 167:



REGISTRY (Copyright (C) 2009 ACS)

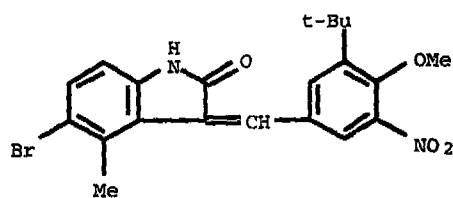
Answer 168:



REGISTRY (Copyright (C) 2009 ACS)

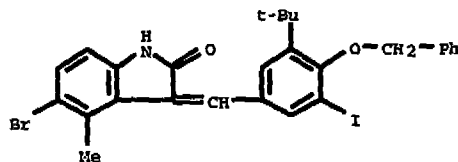
4423
1156

Answer 169:



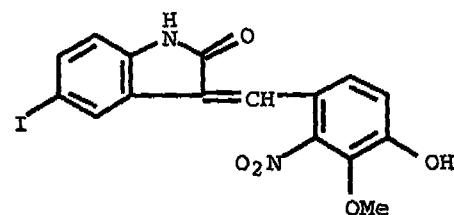
REGISTRY (Copyright (C) 2009 ACS)

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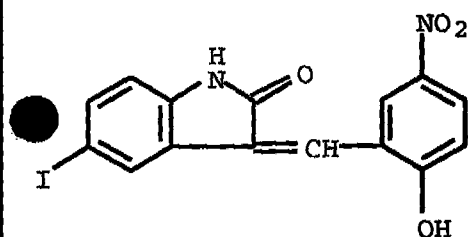
REGISTRY (Copyright (C) 2009 ACS)

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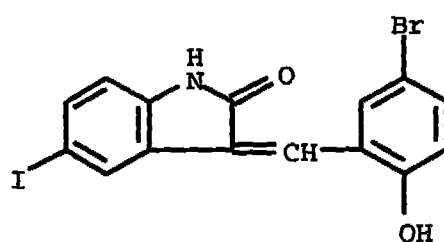
REGISTRY (Copyright (C) 2009 ACS)

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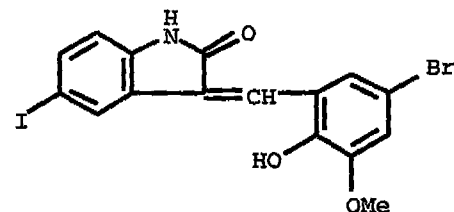
REGISTRY (Copyright (C) 2009 ACS)

Answer 173:



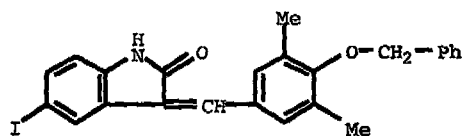
REGISTRY (Copyright (C) 2009 ACS)

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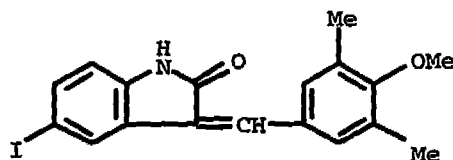
REGISTRY (Copyright (C) 2009 ACS)

Answer 175:



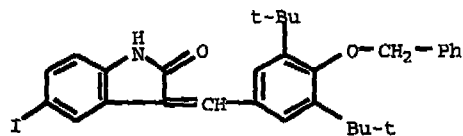
REGISTRY (Copyright (C) 2009 ACS)

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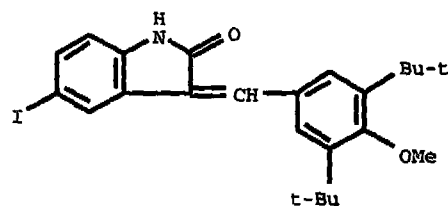
REGISTRY (Copyright (C) 2009 ACS)

Answer 177:



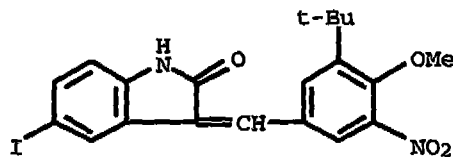
REGISTRY (Copyright (C) 2009 ACS)

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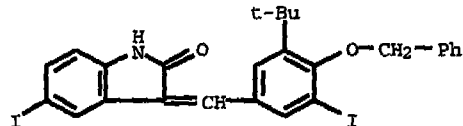
REGISTRY (Copyright (C) 2009 ACS)

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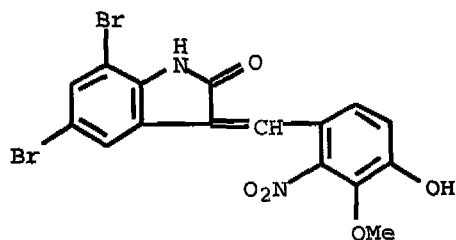
REGISTRY (Copyright (C) 2009 ACS)

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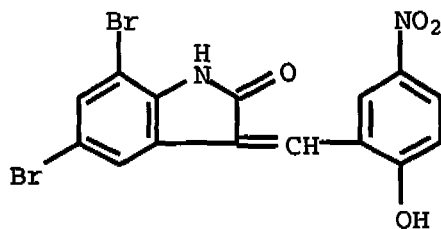
REGISTRY (Copyright (C) 2009 ACS)

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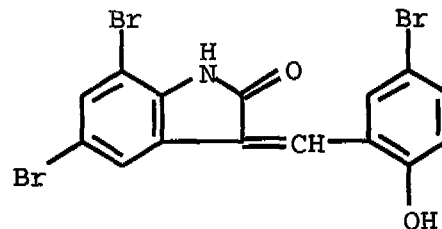
REGISTRY (Copyright (C) 2009 ACS)

Answer 182:



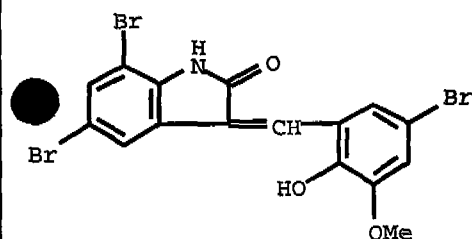
REGISTRY (Copyright (C) 2009 ACS)

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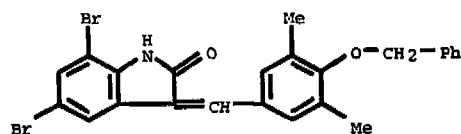
REGISTRY (Copyright (C) 2009 ACS)

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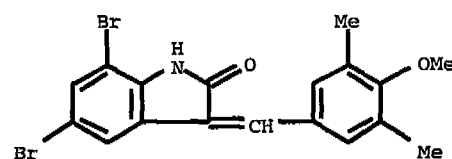
REGISTRY (Copyright (C) 2009 ACS)

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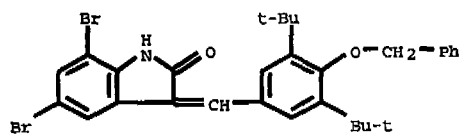
REGISTRY (Copyright (C) 2009 ACS)

Answer 186:



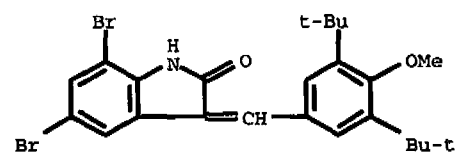
REGISTRY (Copyright (C) 2009 ACS)

Answer 187:



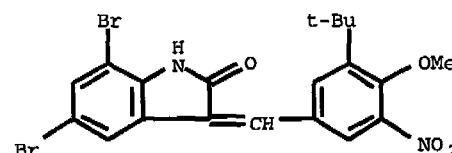
REGISTRY (Copyright (C) 2009 ACS)

Answer 188:



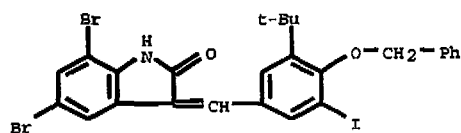
REGISTRY (Copyright (C) 2009 ACS)

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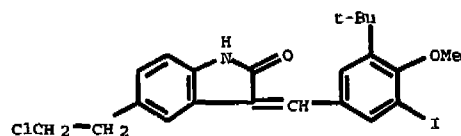
REGISTRY (Copyright (C) 2009 ACS)

Answer 190:



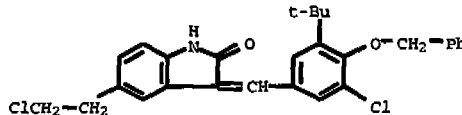
REGISTRY (Copyright (C) 2009 ACS)

Answer 191:



REGISTRY (Copyright (C) 2009 ACS)

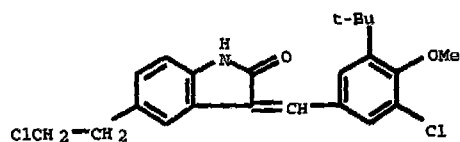
Answer 192:



REGISTRY (Copyright (C) 2009 ACS)

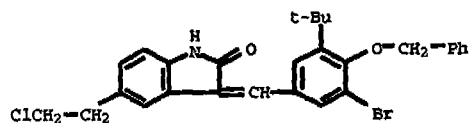
4425
1158

Answer 193:



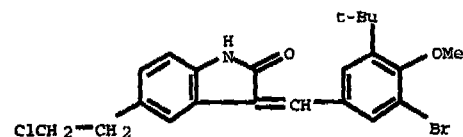
REGISTRY (Copyright (C) 2009 ACS)

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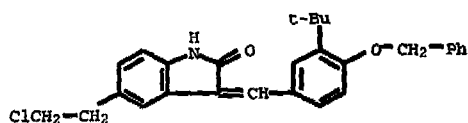
REGISTRY (Copyright (C) 2009 ACS)

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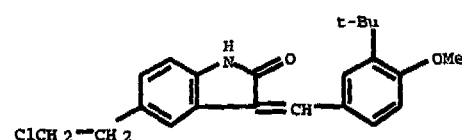
REGISTRY (Copyright (C) 2009 ACS)

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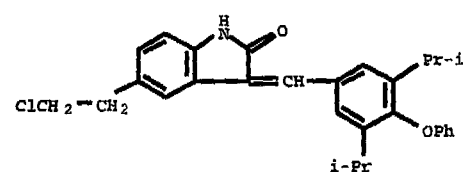
REGISTRY (Copyright (C) 2009 ACS)

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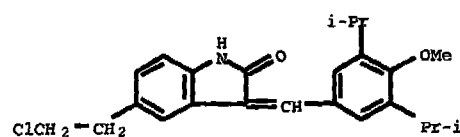
REGISTRY (Copyright (C) 2009 ACS)

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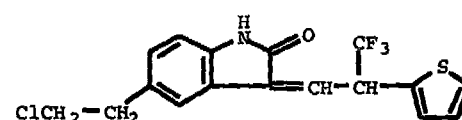
REGISTRY (Copyright (C) 2009 ACS)

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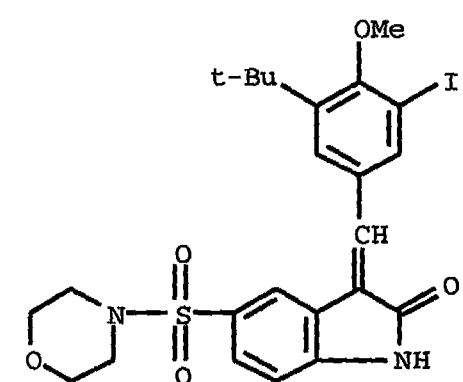
REGISTRY (Copyright (C) 2009 ACS)

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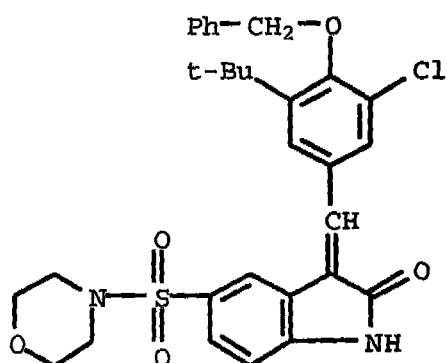
REGISTRY (Copyright (C) 2009 ACS)

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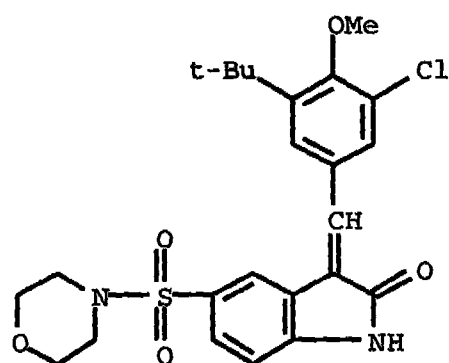


REGISTRY (Copyright (C) 2009 ACS)

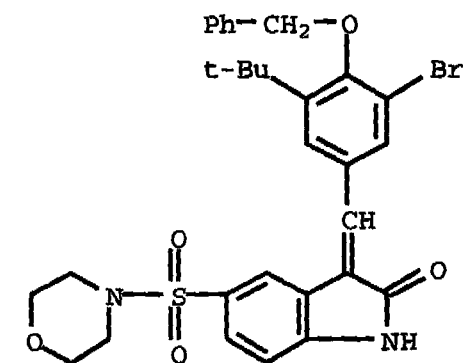
Answer 202:



Answer 203:



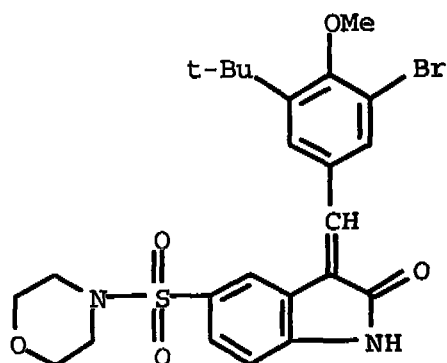
Answer 204:



4126
1159

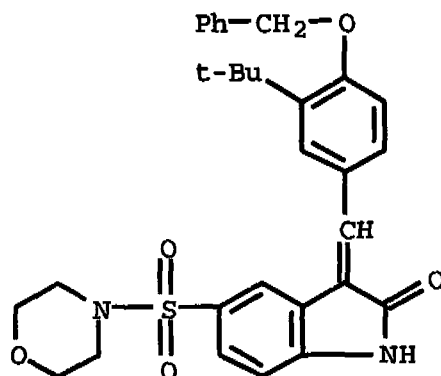
REGISTRY (Copyright (C) 2009 ACS)

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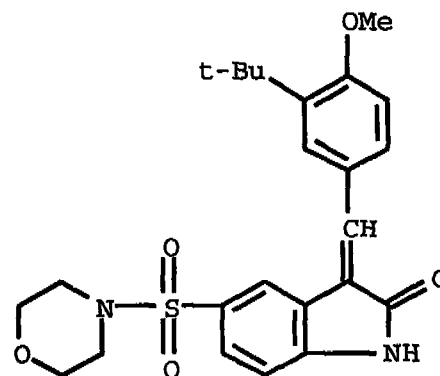
REGISTRY (Copyright (C) 2009 ACS)

Answer 206:



REGISTRY (Copyright (C) 2009 ACS)

Answer 207:

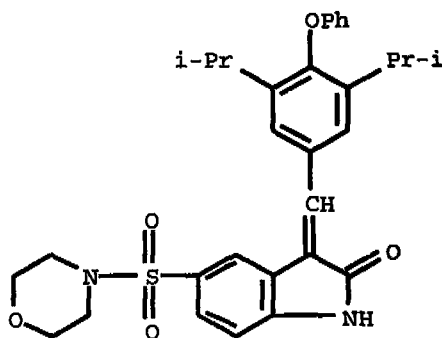


REGISTRY (Copyright (C) 2009 ACS)

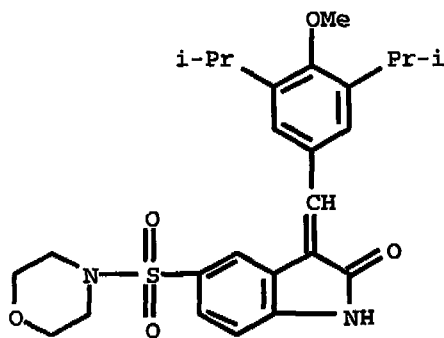
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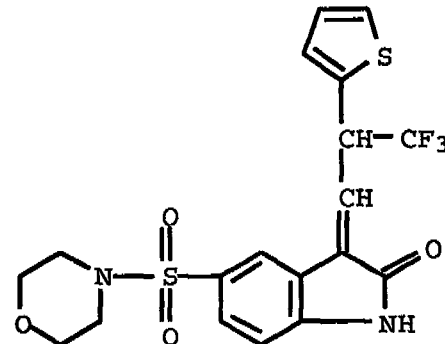
Answer 208:



Answer 209:



Answer 210:

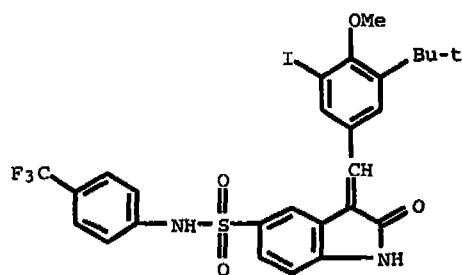


REGISTRY (Copyright (C) 2009 ACS)

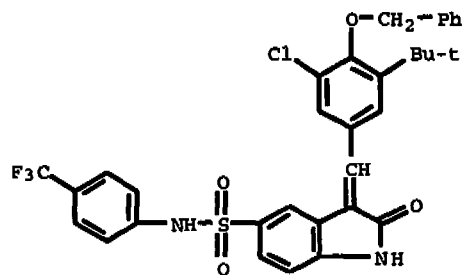
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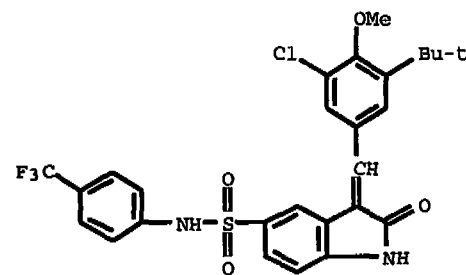
Answer 211:



Answer 212:



Answer 213:



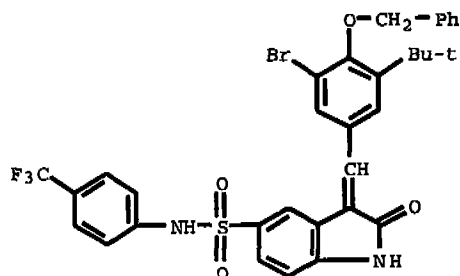
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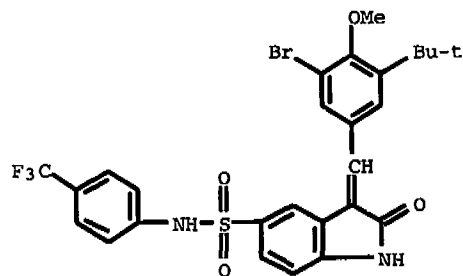
4127
1160

Answer 214:



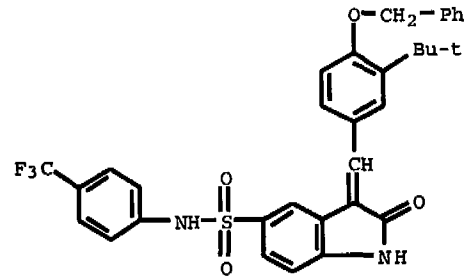
REGISTRY (Copyright (C) 2009 ACS)

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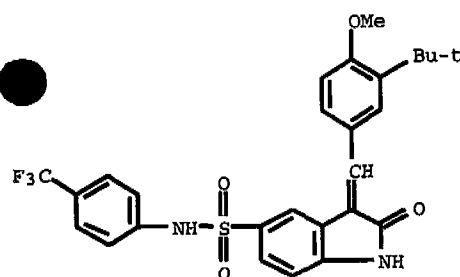
REGISTRY (Copyright (C) 2009 ACS)

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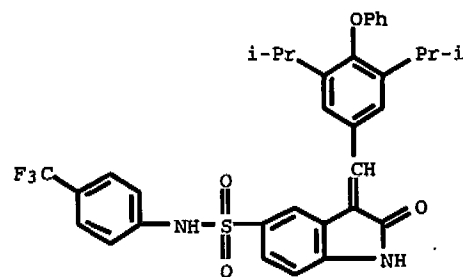
REGISTRY (Copyright (C) 2009 ACS)

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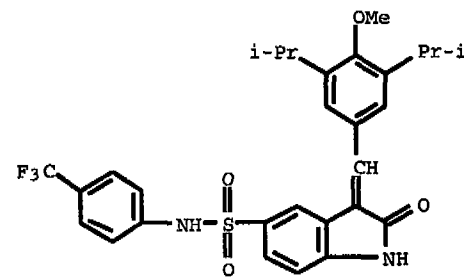
REGISTRY (Copyright (C) 2009 ACS)

Answer 218:



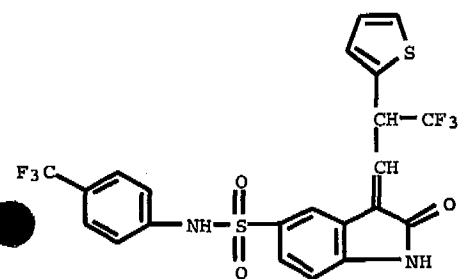
REGISTRY (Copyright (C) 2009 ACS)

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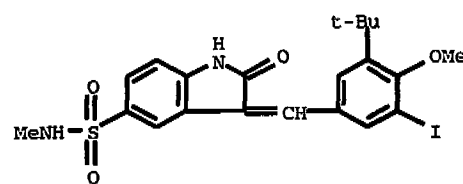
REGISTRY (Copyright (C) 2009 ACS)

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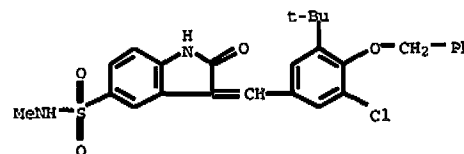
REGISTRY (Copyright (C) 2009 ACS)

Answer 221:



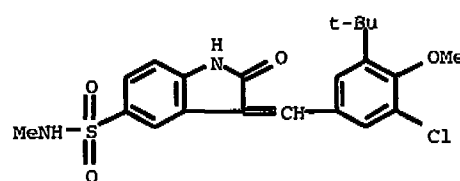
REGISTRY (Copyright (C) 2009 ACS)

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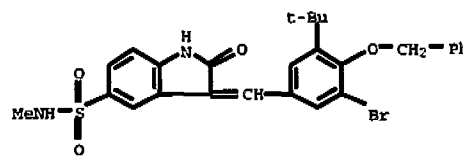
REGISTRY (Copyright (C) 2009 ACS)

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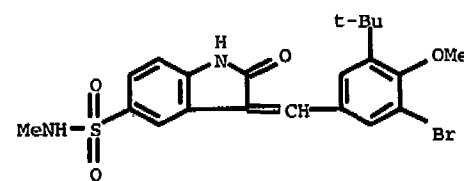
REGISTRY (Copyright (C) 2009 ACS)

Answer 224:



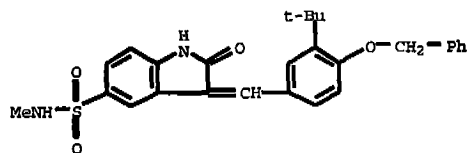
REGISTRY (Copyright (C) 2009 ACS)

Answer 225:



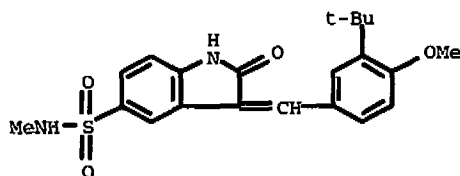
REGISTRY (Copyright (C) 2009 ACS)

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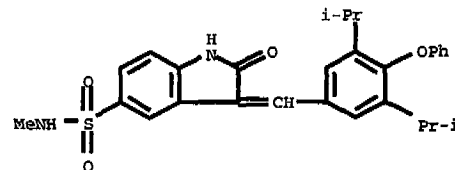
REGISTRY (Copyright (C) 2009 ACS)

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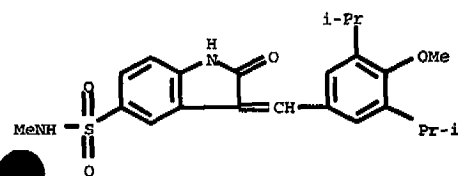
REGISTRY (Copyright (C) 2009 ACS)

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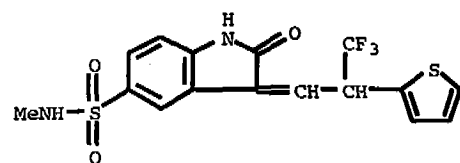
REGISTRY (Copyright (C) 2009 ACS)

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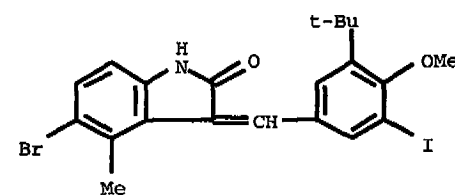
REGISTRY (Copyright (C) 2009 ACS)

Answer 230:



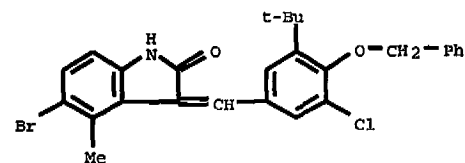
REGISTRY (Copyright (C) 2009 ACS)

Answer 231:



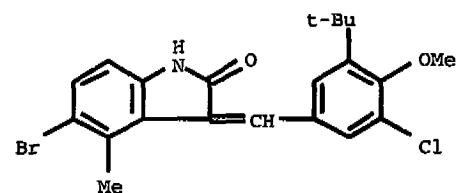
REGISTRY (Copyright (C) 2009 ACS)

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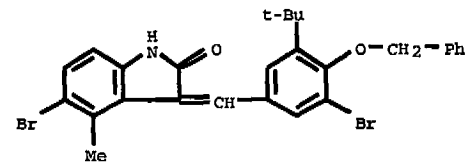
REGISTRY (Copyright (C) 2009 ACS)

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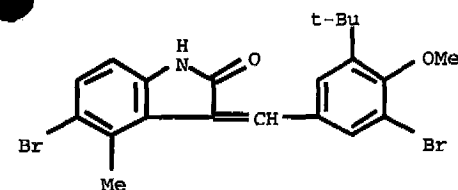
REGISTRY (Copyright (C) 2009 ACS)

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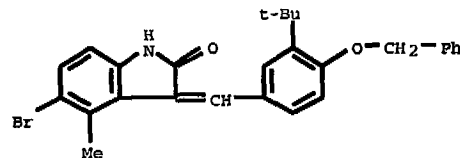
REGISTRY (Copyright (C) 2009 ACS)

Answer 235:



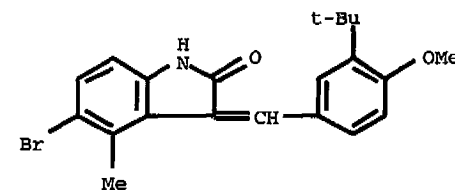
REGISTRY (Copyright (C) 2009 ACS)

Answer 236:



REGISTRY (Copyright (C) 2009 ACS)

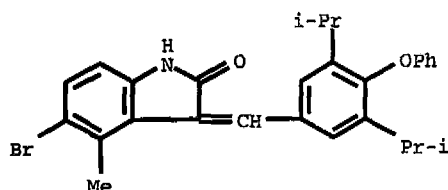
Answer 237:



REGISTRY (Copyright (C) 2009 ACS)

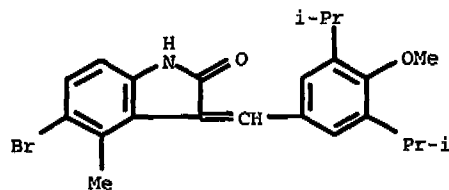
4429
1162

Answer 238:



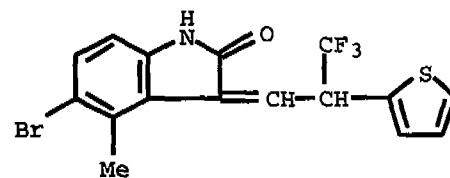
REGISTRY (Copyright (C) 2009 ACS)

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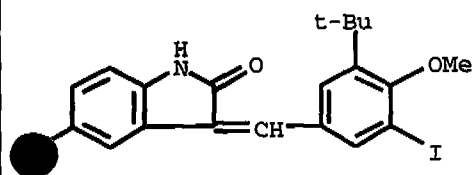
REGISTRY (Copyright (C) 2009 ACS)

Answer 240:



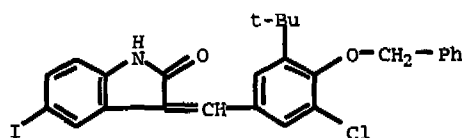
REGISTRY (Copyright (C) 2009 ACS)

Answer 241:



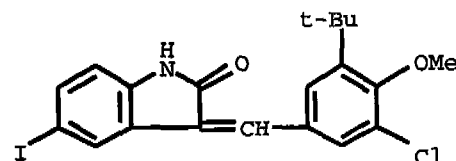
REGISTRY (Copyright (C) 2009 ACS)

Answer 242:



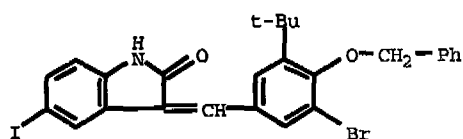
REGISTRY (Copyright (C) 2009 ACS)

Answer 243:



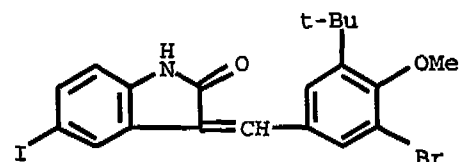
REGISTRY (Copyright (C) 2009 ACS)

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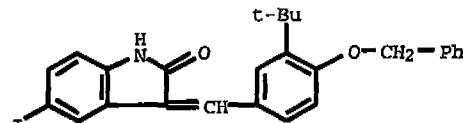
REGISTRY (Copyright (C) 2009 ACS)

Answer 245:



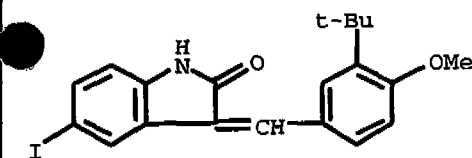
REGISTRY (Copyright (C) 2009 ACS)

Answer 246:



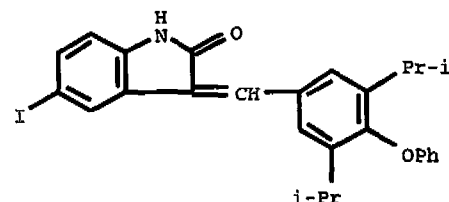
REGISTRY (Copyright (C) 2009 ACS)

Answer 247:



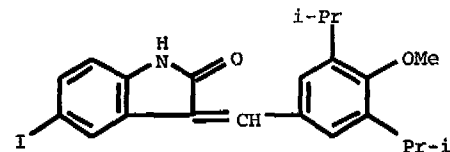
REGISTRY (Copyright (C) 2009 ACS)

Answer 248:



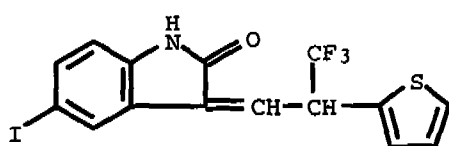
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Answer 249:

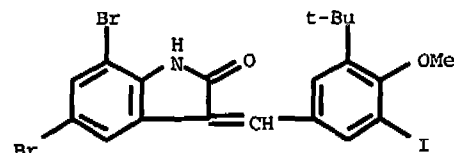


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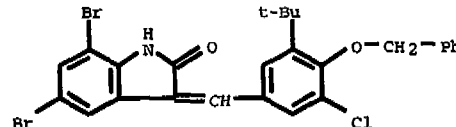
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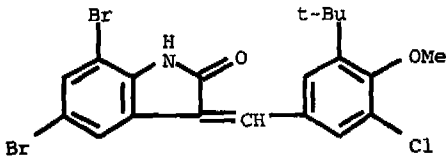
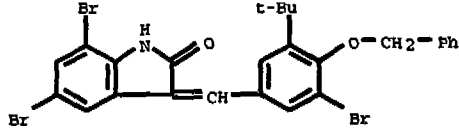
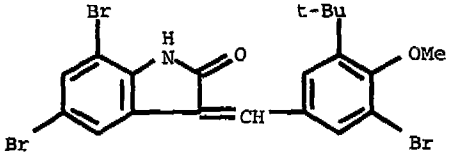
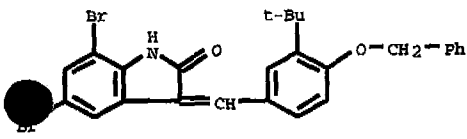
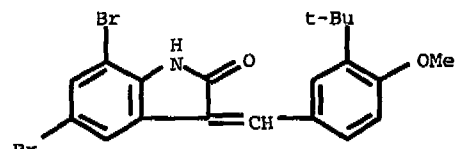
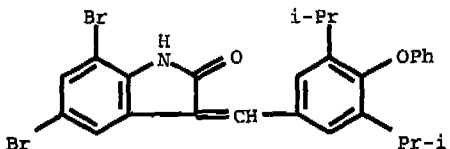
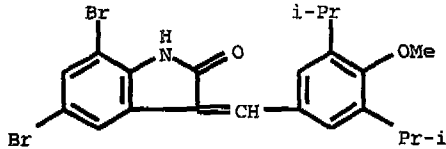
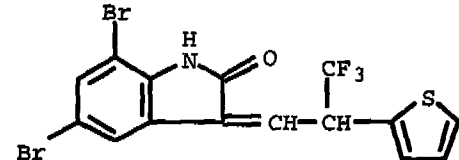
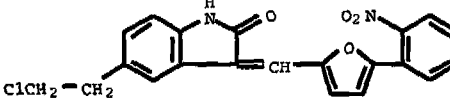
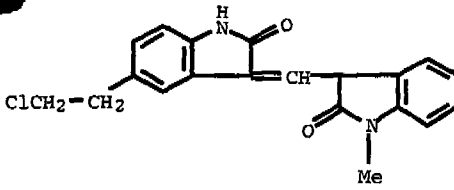
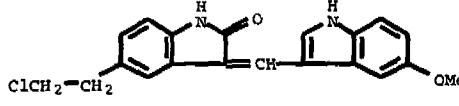
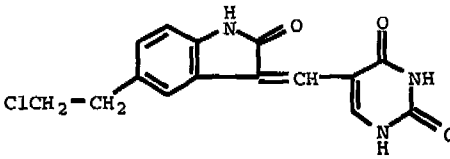


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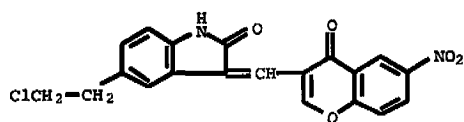


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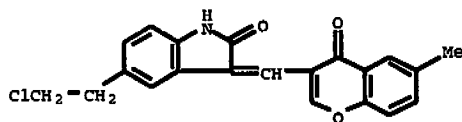
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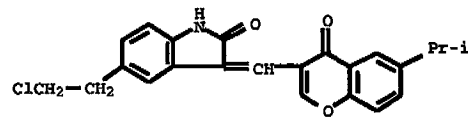
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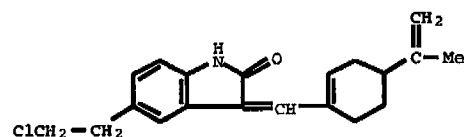
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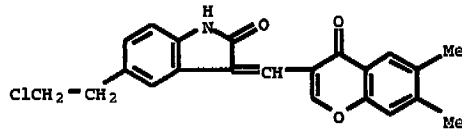
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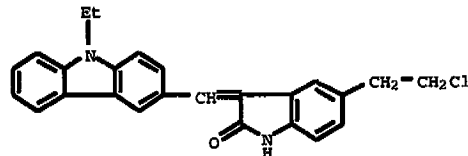
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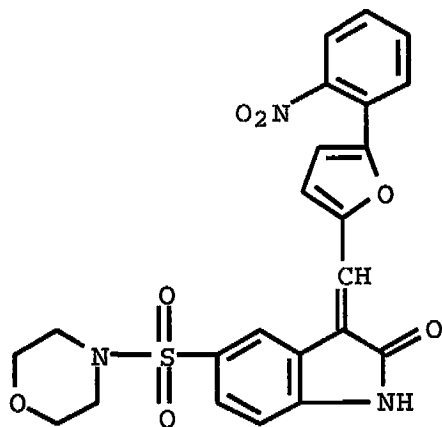
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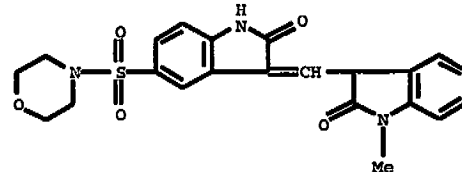
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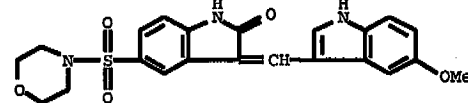
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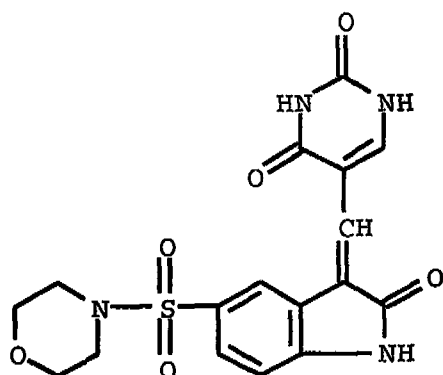


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432

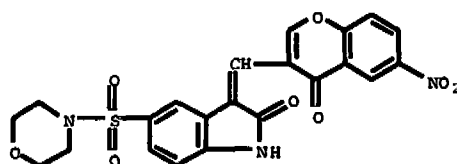
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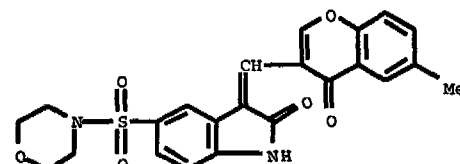
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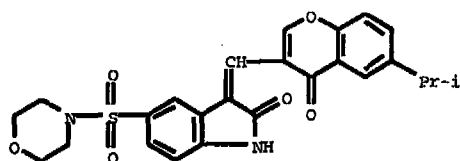
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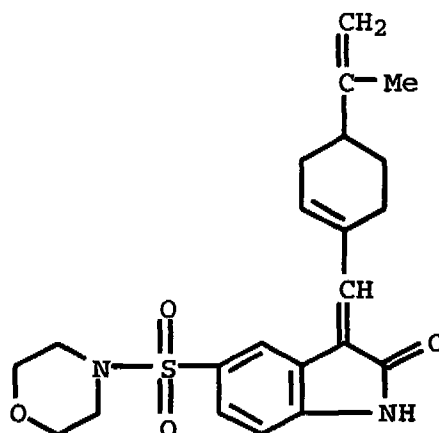
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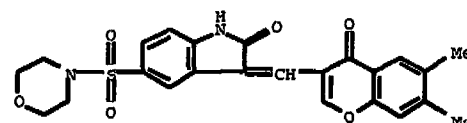
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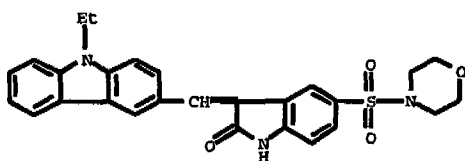
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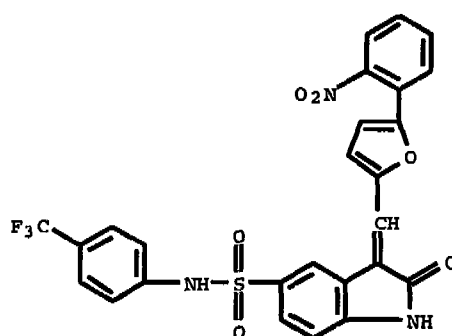
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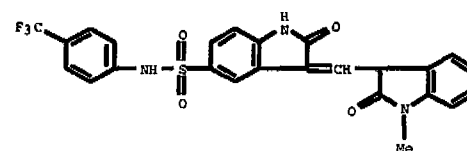
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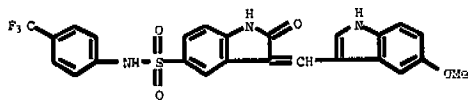


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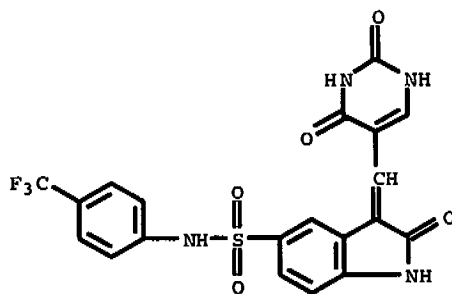
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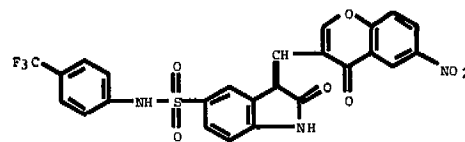
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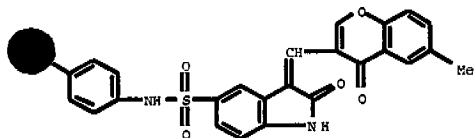
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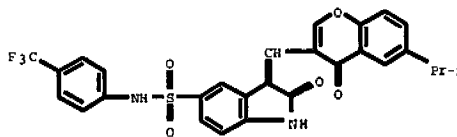
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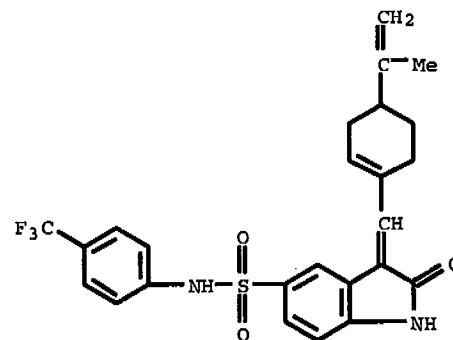
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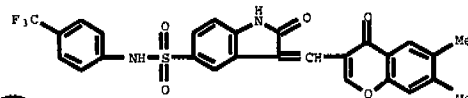
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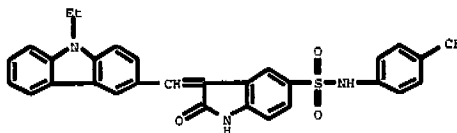
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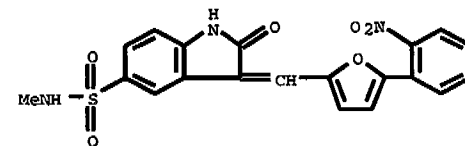
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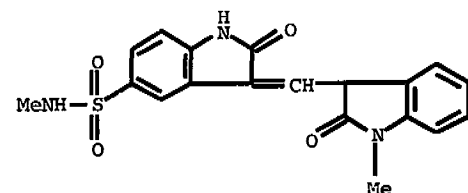
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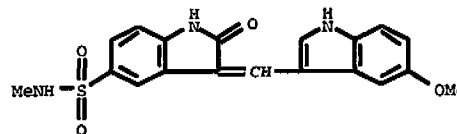
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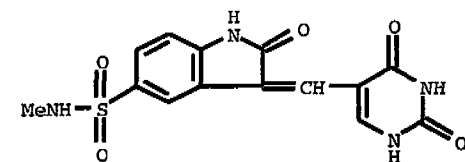
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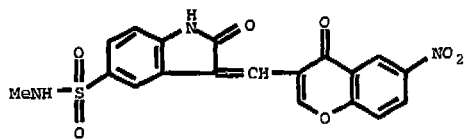
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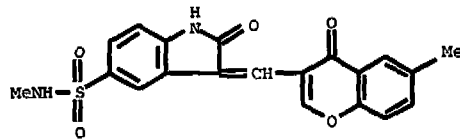
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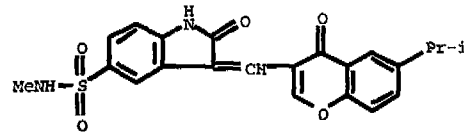
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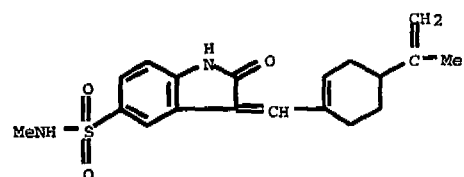
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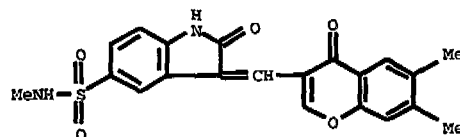
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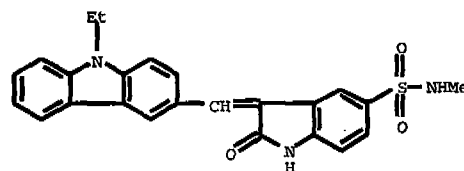
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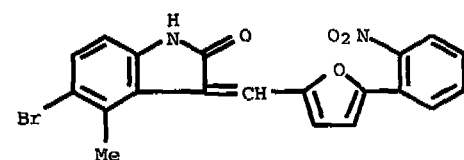
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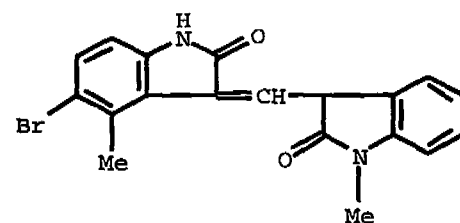
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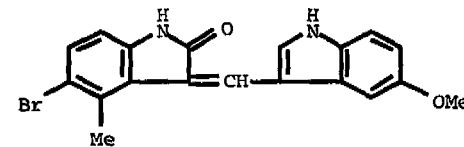
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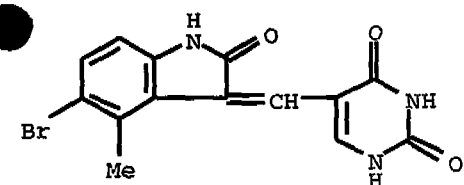
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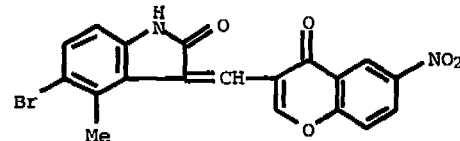
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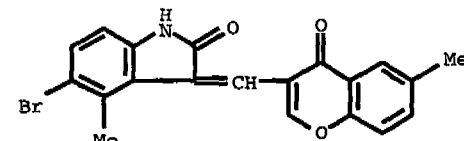
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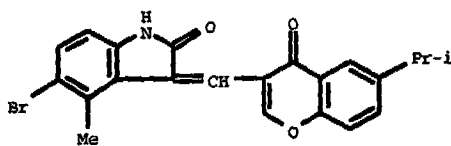
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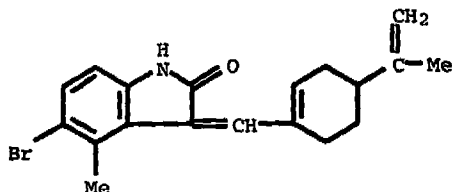
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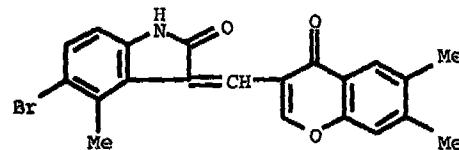
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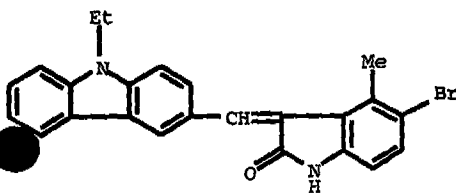
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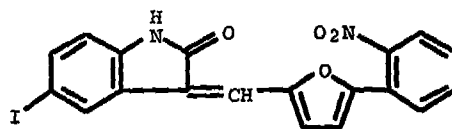
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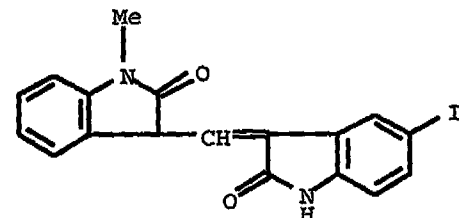
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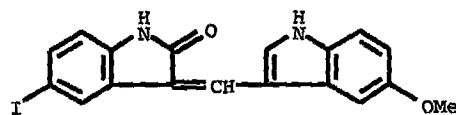
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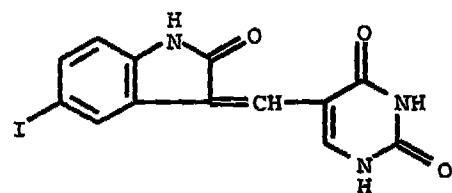
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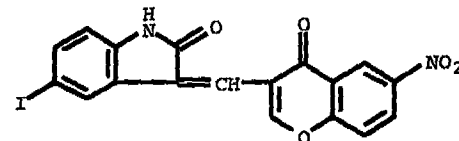
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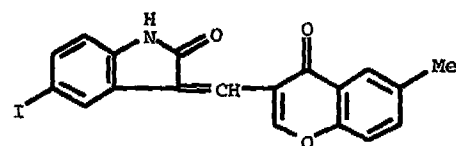
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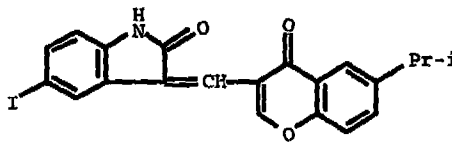
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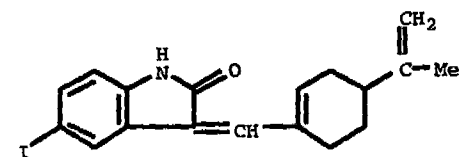
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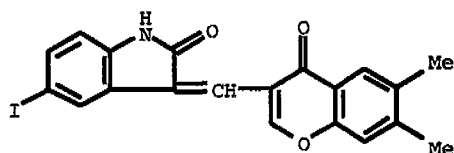
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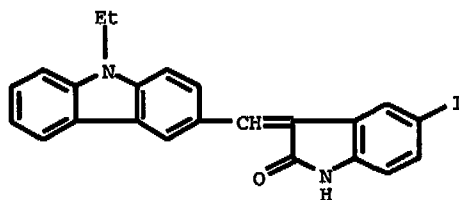
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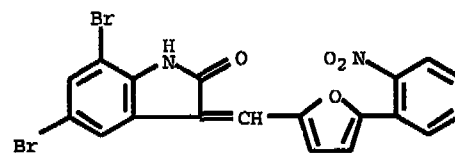
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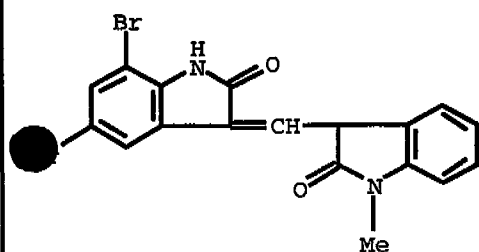
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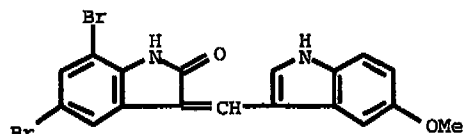
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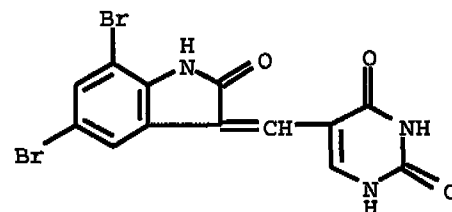
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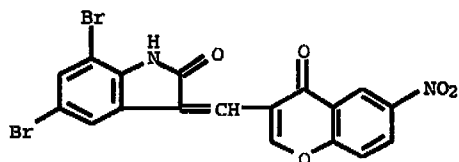
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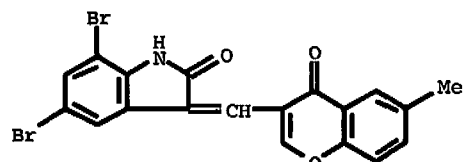
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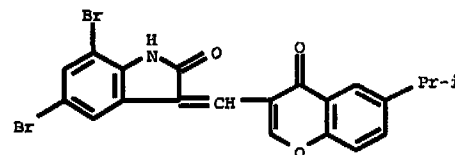
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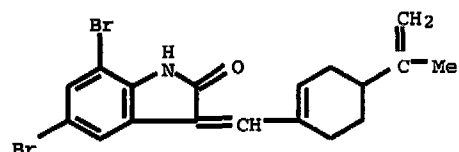
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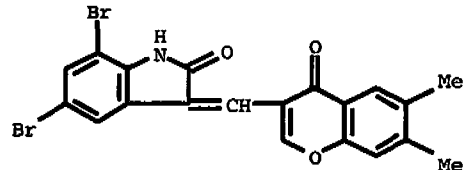
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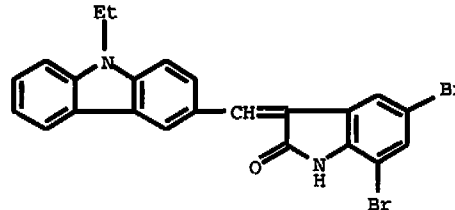
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Answer 329:



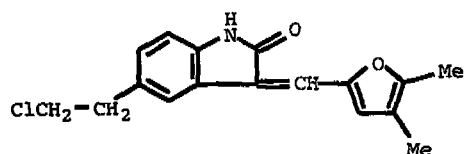
REGISTRY (Copyright (C) 2009 ACS)

Answer 330:



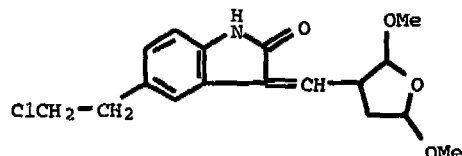
REGISTRY (Copyright (C) 2009 ACS)

Answer 331:



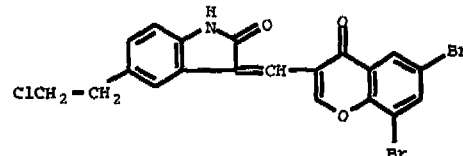
REGISTRY (Copyright (C) 2009 ACS)

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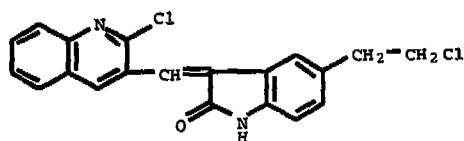
REGISTRY (Copyright (C) 2009 ACS)

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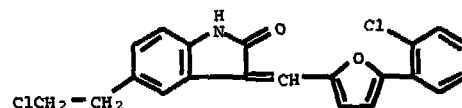
REGISTRY (Copyright (C) 2009 ACS)

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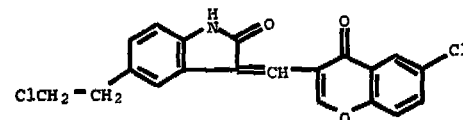
REGISTRY (Copyright (C) 2009 ACS)

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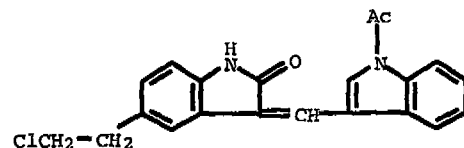
REGISTRY (Copyright (C) 2009 ACS)

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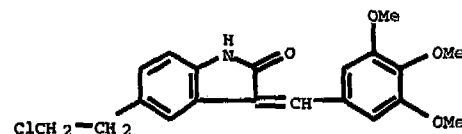
REGISTRY (Copyright (C) 2009 ACS)

Answer 337:



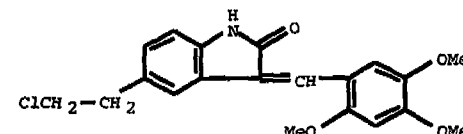
REGISTRY (Copyright (C) 2009 ACS)

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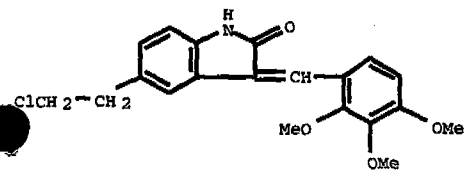
REGISTRY (Copyright (C) 2009 ACS)

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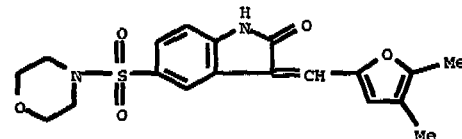
REGISTRY (Copyright (C) 2009 ACS)

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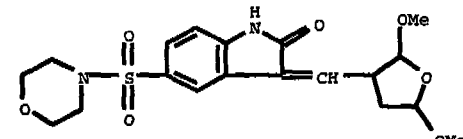
REGISTRY (Copyright (C) 2009 ACS)

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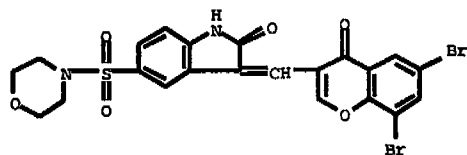
REGISTRY (Copyright (C) 2009 ACS)

Answer 342:



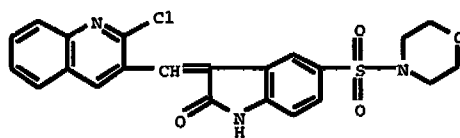
REGISTRY (Copyright (C) 2009 ACS)

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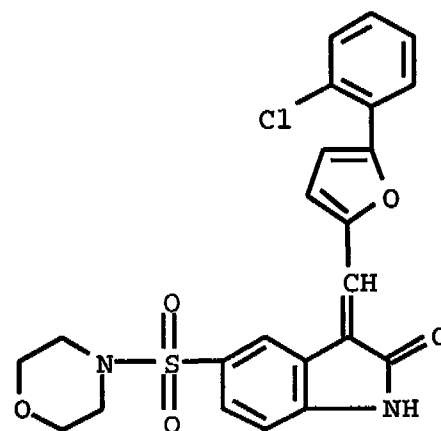
REGISTRY (Copyright (C) 2009 ACS)

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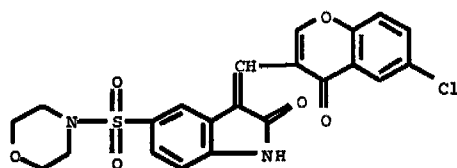
REGISTRY (Copyright (C) 2009 ACS)

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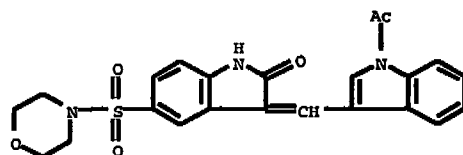
REGISTRY (Copyright (C) 2009 ACS)

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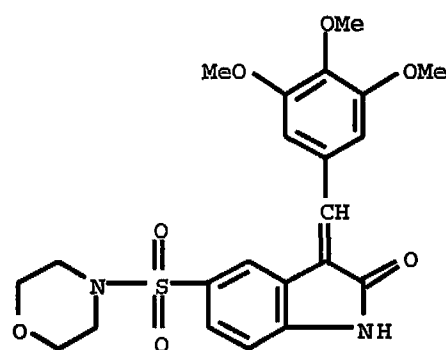
REGISTRY (Copyright (C) 2009 ACS)

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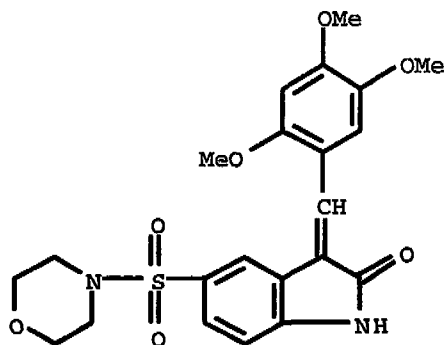
REGISTRY (Copyright (C) 2009 ACS)

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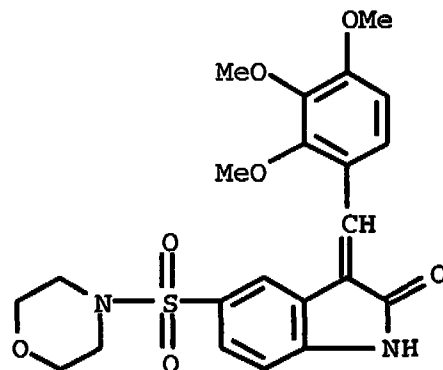
REGISTRY (Copyright (C) 2009 ACS)

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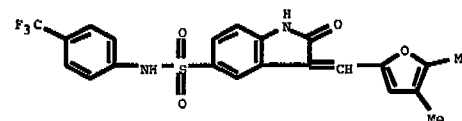
REGISTRY (Copyright (C) 2009 ACS)

Answer 350:



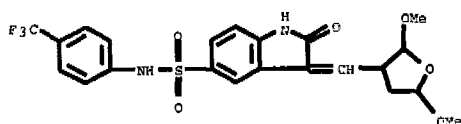
REGISTRY (Copyright (C) 2009 ACS)

Answer 351:



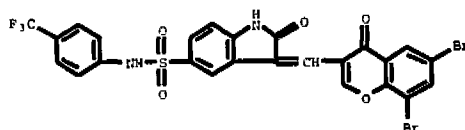
REGISTRY (Copyright (C) 2009 ACS)

Answer 352:



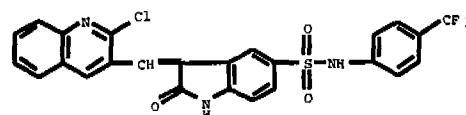
REGISTRY (Copyright (C) 2009 ACS)

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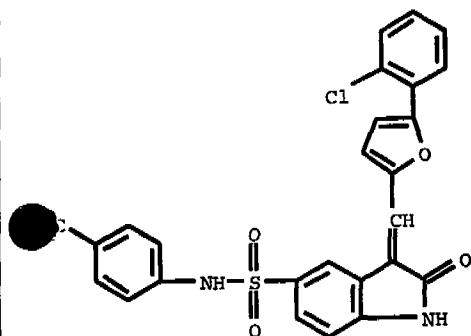
REGISTRY (Copyright (C) 2009 ACS)

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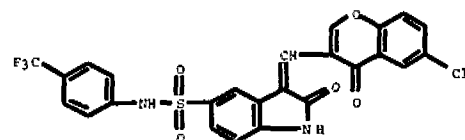
REGISTRY (Copyright (C) 2009 ACS)

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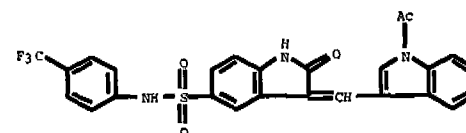
REGISTRY (Copyright (C) 2009 ACS)

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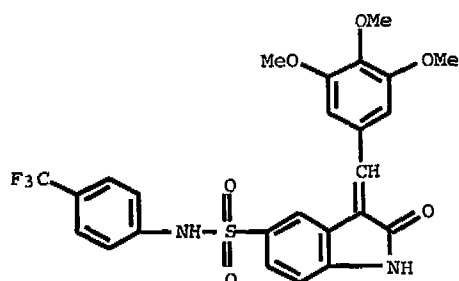
REGISTRY (Copyright (C) 2009 ACS)

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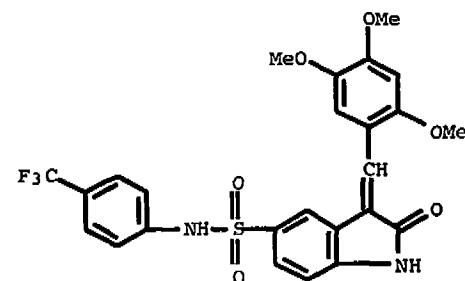
REGISTRY (Copyright (C) 2009 ACS)

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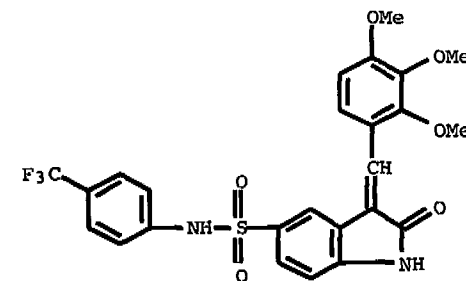
REGISTRY (Copyright (C) 2009 ACS)

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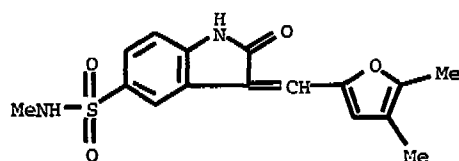
REGISTRY (Copyright (C) 2009 ACS)

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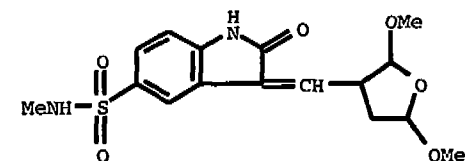
REGISTRY (Copyright (C) 2009 ACS)

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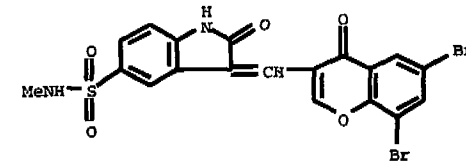
REGISTRY (Copyright (C) 2009 ACS)

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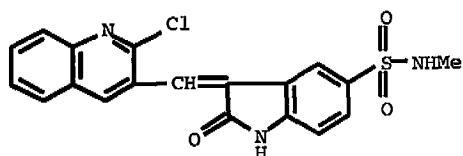
REGISTRY (Copyright (C) 2009 ACS)

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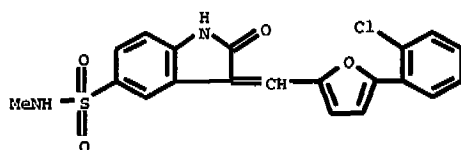
REGISTRY (Copyright (C) 2009 ACS)

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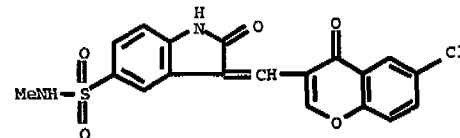
REGISTRY (Copyright (C) 2009 ACS)

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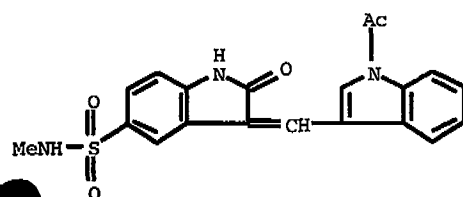
REGISTRY (Copyright (C) 2009 ACS)

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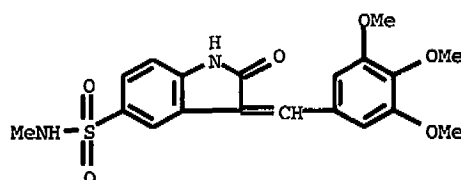
REGISTRY (Copyright (C) 2009 ACS)

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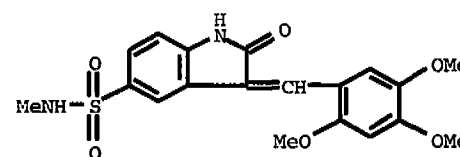
REGISTRY (Copyright (C) 2009 ACS)

Answer 368:



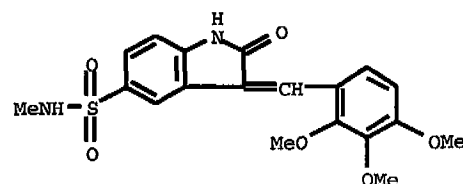
REGISTRY (Copyright (C) 2009 ACS)

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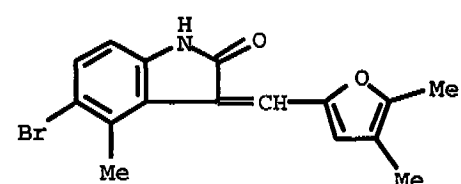
REGISTRY (Copyright (C) 2009 ACS)

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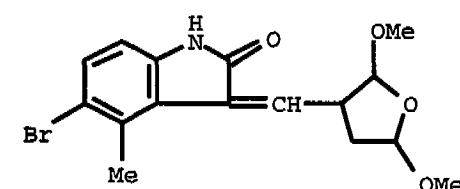


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Answer 371:

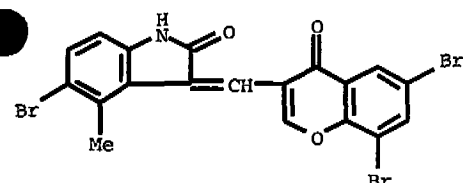


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Answer 372:

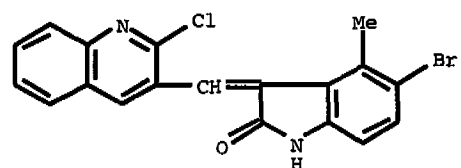
REGISTRY (Copyright (C) 2009 ACS)

Answer 373:



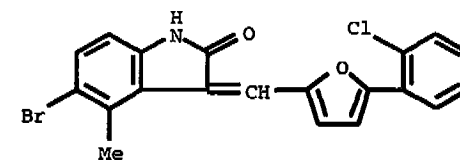
REGISTRY (Copyright (C) 2009 ACS)

Answer 374:



REGISTRY (Copyright (C) 2009 ACS)

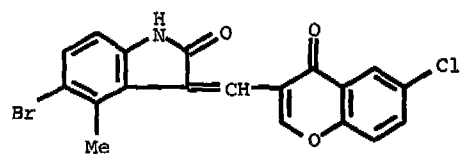
Answer 375:



REGISTRY (Copyright (C) 2009 ACS)

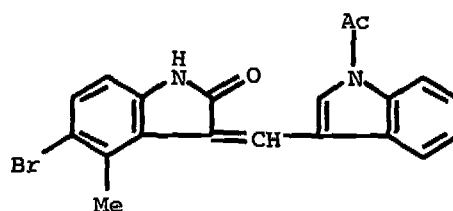
1174
1174

Answer 376:



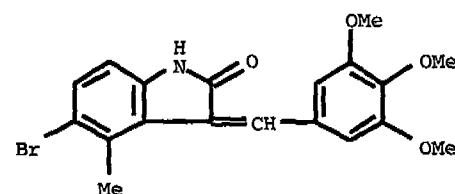
REGISTRY (Copyright (C) 2009 ACS)

Answer 377:



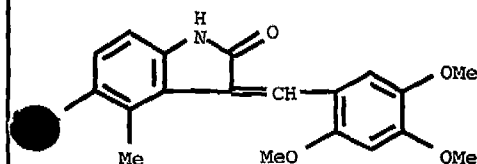
REGISTRY (Copyright (C) 2009 ACS)

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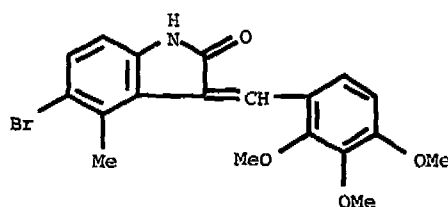
REGISTRY (Copyright (C) 2009 ACS)

Answer 379:



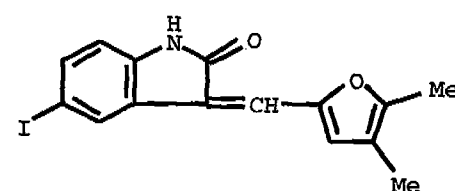
REGISTRY (Copyright (C) 2009 ACS)

Answer 380:



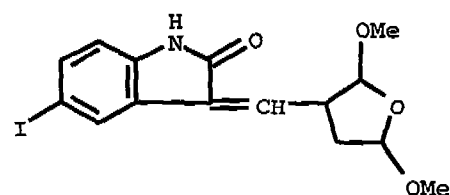
REGISTRY (Copyright (C) 2009 ACS)

Answer 381:



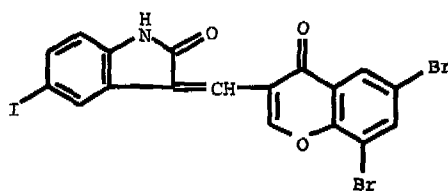
REGISTRY (Copyright (C) 2009 ACS)

Answer 382:



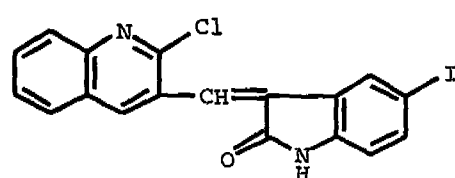
REGISTRY (Copyright (C) 2009 ACS)

Answer 383:



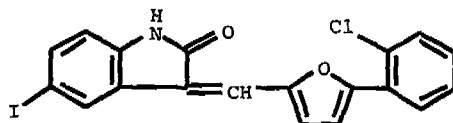
REGISTRY (Copyright (C) 2009 ACS)

Answer 384:



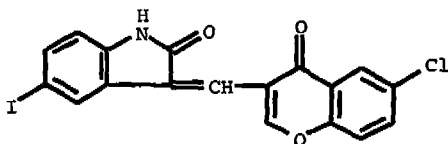
REGISTRY (Copyright (C) 2009 ACS)

Answer 385:



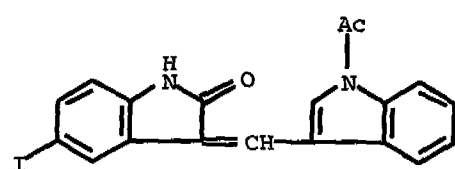
REGISTRY (Copyright (C) 2009 ACS)

Answer 386:



REGISTRY (Copyright (C) 2009 ACS)

Answer 387:

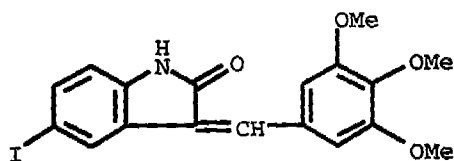


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1142

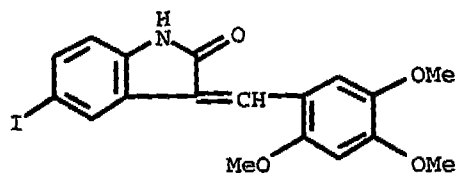
1175

Answer 388:



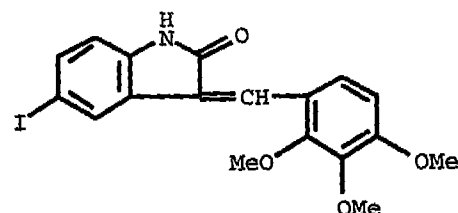
REGISTRY (Copyright (C) 2009 ACS)

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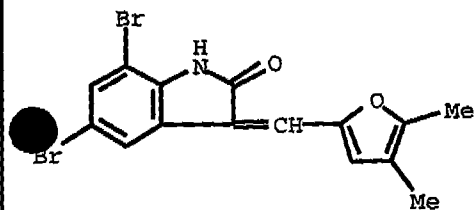
REGISTRY (Copyright (C) 2009 ACS)

Answer 390:



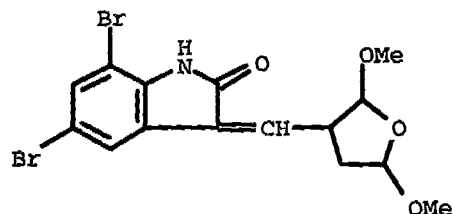
REGISTRY (Copyright (C) 2009 ACS)

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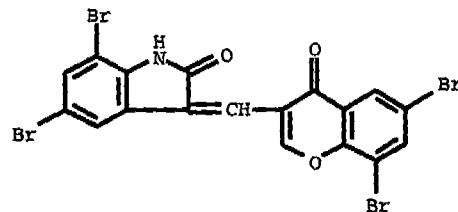
REGISTRY (Copyright (C) 2009 ACS)

Answer 392:



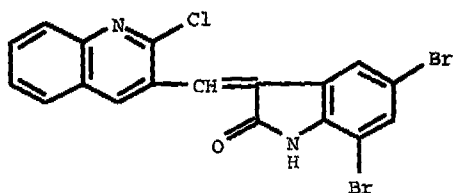
REGISTRY (Copyright (C) 2009 ACS)

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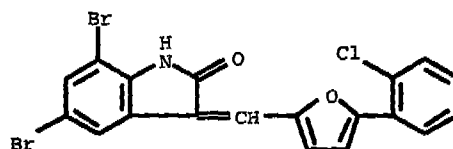
REGISTRY (Copyright (C) 2009 ACS)

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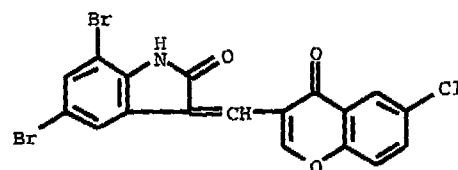
REGISTRY (Copyright (C) 2009 ACS)

Answer 395:



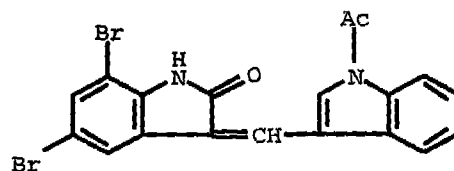
REGISTRY (Copyright (C) 2009 ACS)

Answer 396:



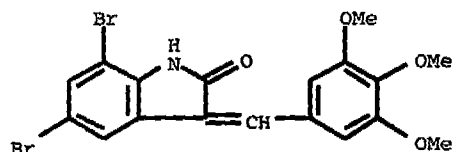
REGISTRY (Copyright (C) 2009 ACS)

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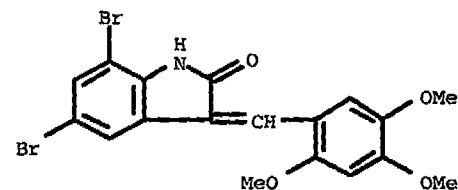
REGISTRY (Copyright (C) 2009 ACS)

Answer 398:



REGISTRY (Copyright (C) 2009 ACS)

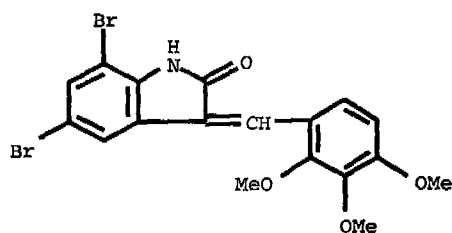
Answer 399:



REGISTRY (Copyright (C) 2009 ACS)

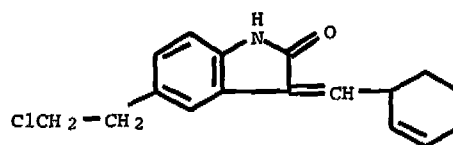
4173
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Answer 400:



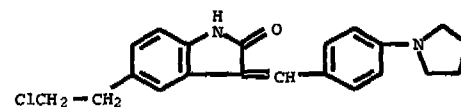
REGISTRY (Copyright (C) 2009 ACS)

Answer 401:



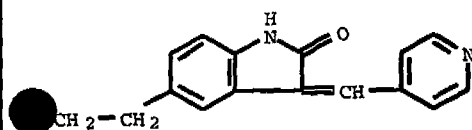
REGISTRY (Copyright (C) 2009 ACS)

Answer 402:



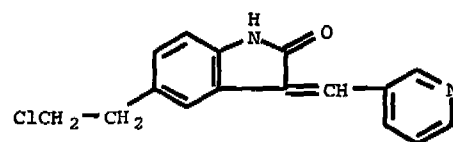
REGISTRY (Copyright (C) 2009 ACS)

Answer 403:



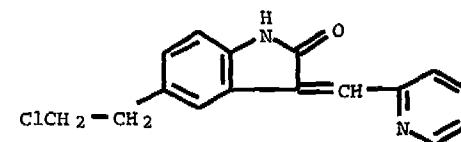
REGISTRY (Copyright (C) 2009 ACS)

Answer 404:



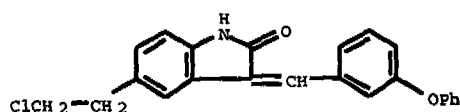
REGISTRY (Copyright (C) 2009 ACS)

Answer 405:



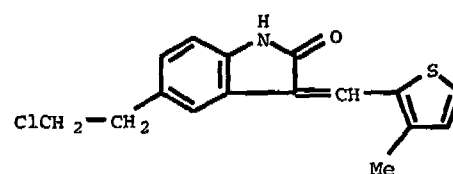
REGISTRY (Copyright (C) 2009 ACS)

Answer 406:



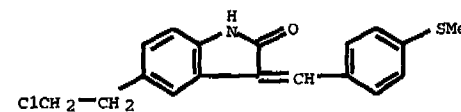
REGISTRY (Copyright (C) 2009 ACS)

Answer 407:



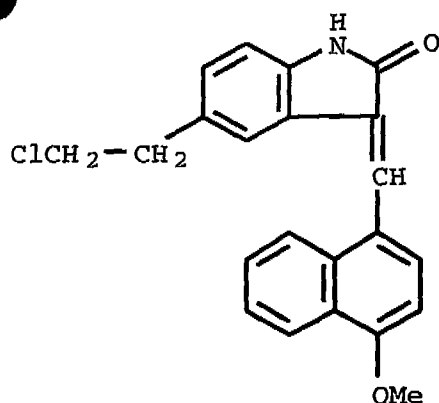
REGISTRY (Copyright (C) 2009 ACS)

Answer 408:



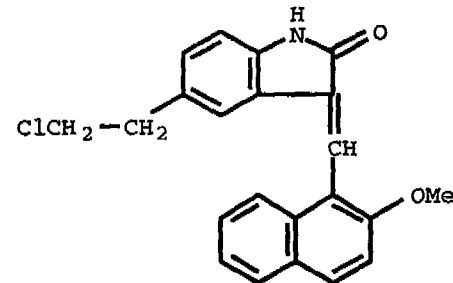
REGISTRY (Copyright (C) 2009 ACS)

Answer 409:



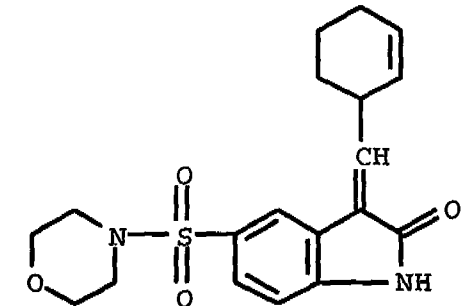
REGISTRY (Copyright (C) 2009 ACS)

Answer 410:



REGISTRY (Copyright (C) 2009 ACS)

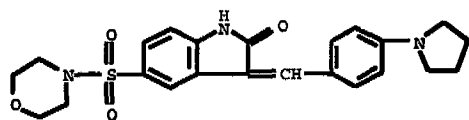
Answer 411:



REGISTRY (Copyright (C) 2009 ACS)

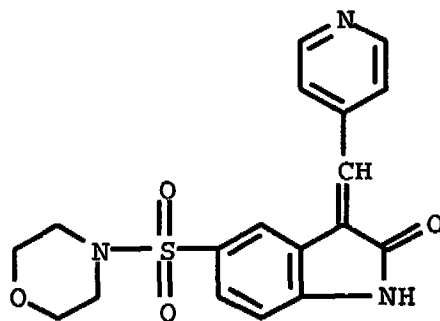
4444
1177

Answer 412:



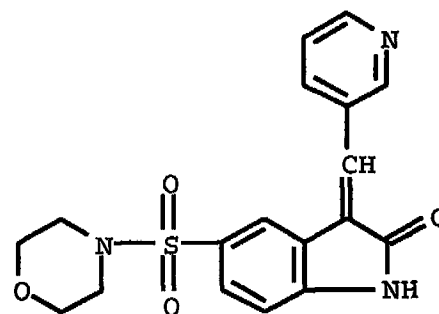
REGISTRY (Copyright (C) 2009 ACS)

Answer 413:



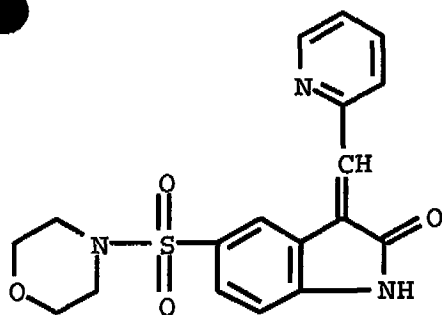
REGISTRY (Copyright (C) 2009 ACS)

Answer 414:



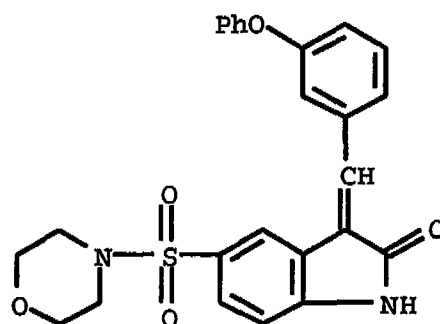
REGISTRY (Copyright (C) 2009 ACS)

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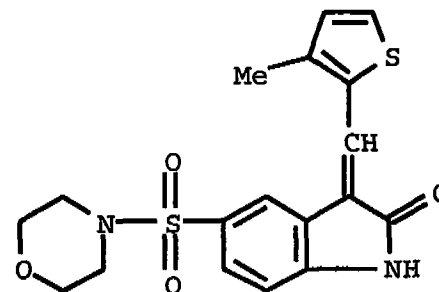
REGISTRY (Copyright (C) 2009 ACS)

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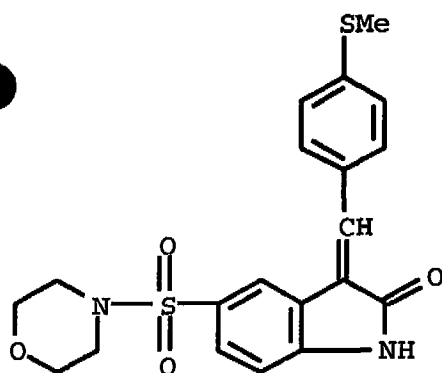
REGISTRY (Copyright (C) 2009 ACS)

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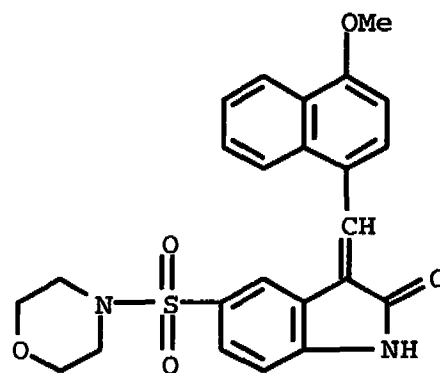
REGISTRY (Copyright (C) 2009 ACS)

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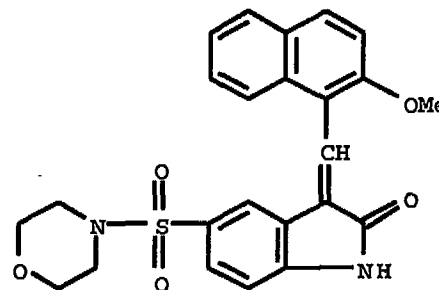
REGISTRY (Copyright (C) 2009 ACS)

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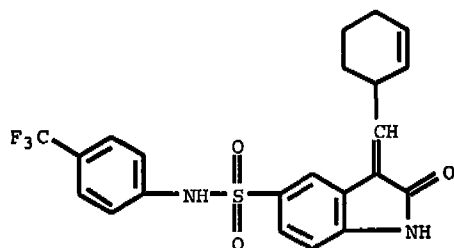
REGISTRY (Copyright (C) 2009 ACS)

Answer 420:



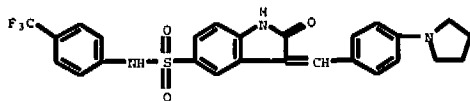
REGISTRY (Copyright (C) 2009 ACS)

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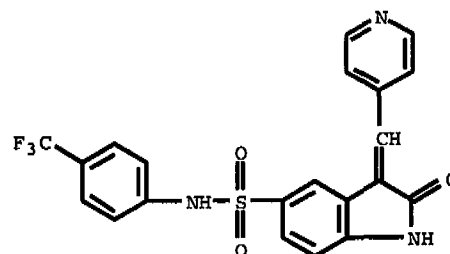
REGISTRY (Copyright (C) 2009 ACS)

Answer 422:



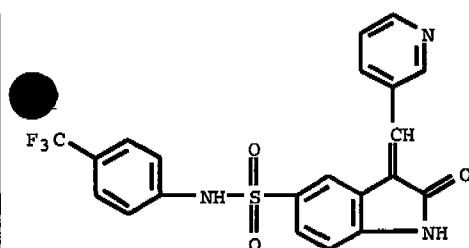
REGISTRY (Copyright (C) 2009 ACS)

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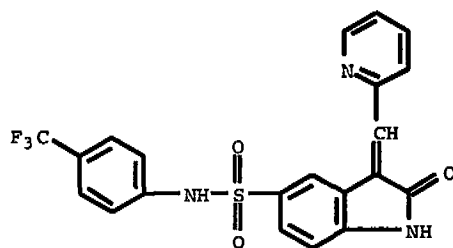
REGISTRY (Copyright (C) 2009 ACS)

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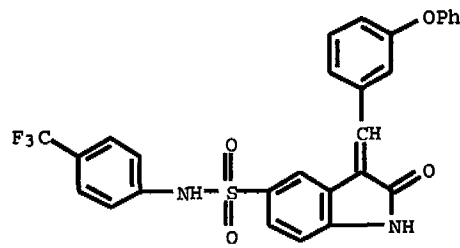
REGISTRY (Copyright (C) 2009 ACS)

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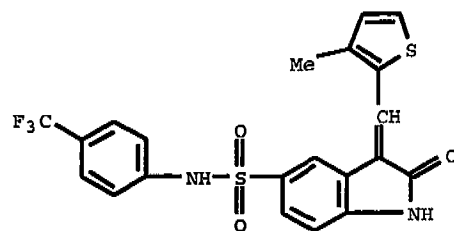
REGISTRY (Copyright (C) 2009 ACS)

Answer 426:



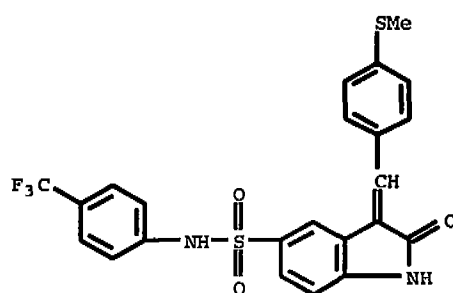
REGISTRY (Copyright (C) 2009 ACS)

Answer 427:



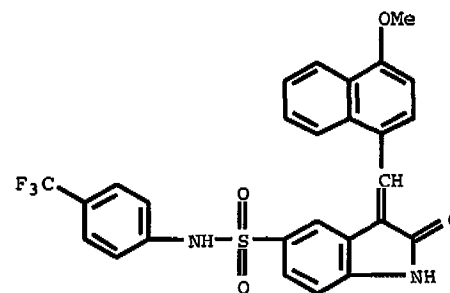
REGISTRY (Copyright (C) 2009 ACS)

Answer 428:



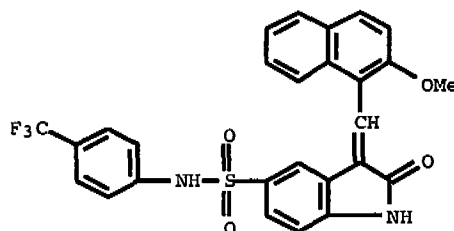
REGISTRY (Copyright (C) 2009 ACS)

Answer 429:

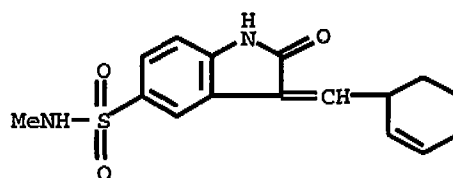


REGISTRY (Copyright (C) 2009 ACS)

Answer 430:

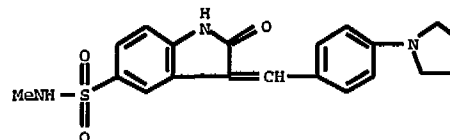


Answer 431:



REGISTRY (Copyright (C) 2009 ACS)

Answer 432:



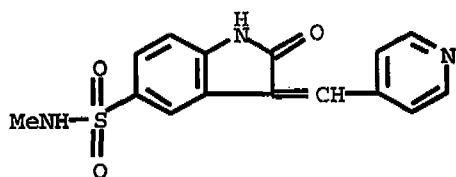
REGISTRY (Copyright (C) 2009 ACS)

1146

1179

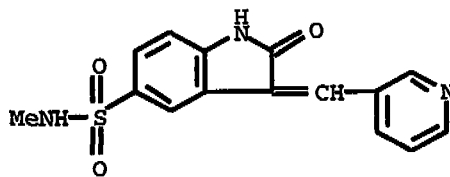
REGISTRY (Copyright (C) 2009 ACS)

Answer 433:



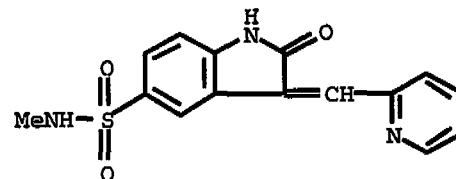
REGISTRY (Copyright (C) 2009 ACS)

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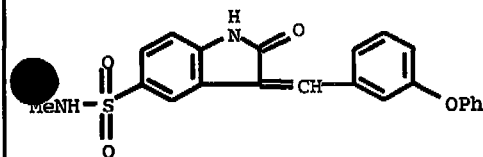
REGISTRY (Copyright (C) 2009 ACS)

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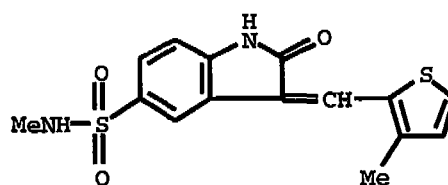
REGISTRY (Copyright (C) 2009 ACS)

Answer 436:



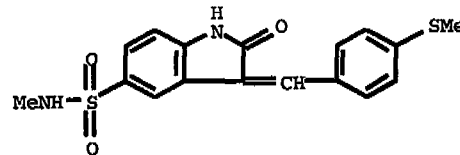
REGISTRY (Copyright (C) 2009 ACS)

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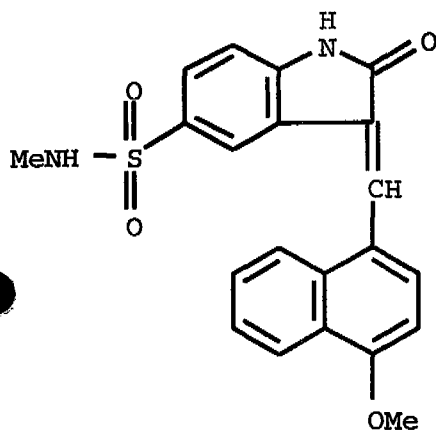
REGISTRY (Copyright (C) 2009 ACS)

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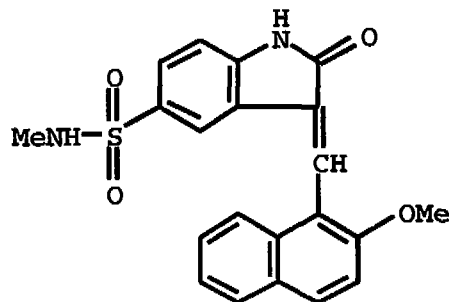
REGISTRY (Copyright (C) 2009 ACS)

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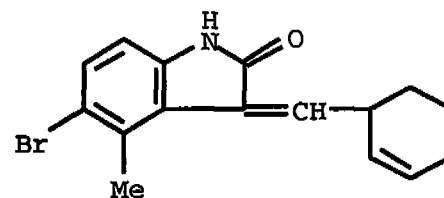
REGISTRY (Copyright (C) 2009 ACS)

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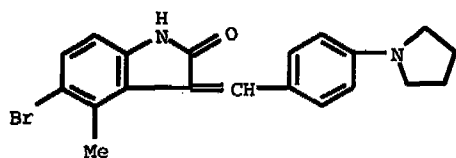
REGISTRY (Copyright (C) 2009 ACS)

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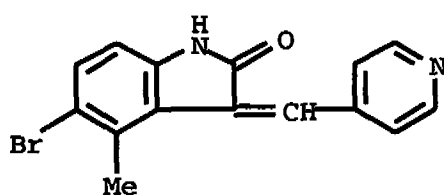
REGISTRY (Copyright (C) 2009 ACS)

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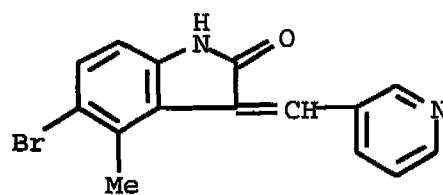
REGISTRY (Copyright (C) 2009 ACS)

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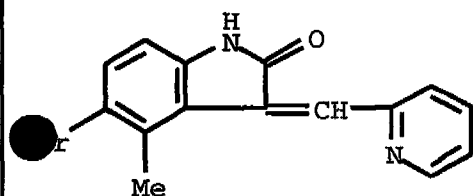
REGISTRY (Copyright (C) 2009 ACS)

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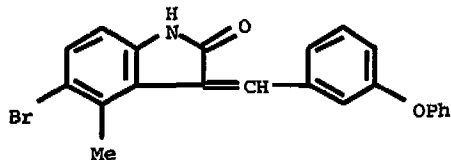
REGISTRY (Copyright (C) 2009 ACS)

Answer 445:



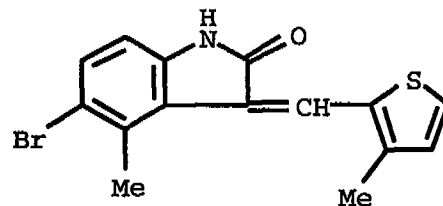
REGISTRY (Copyright (C) 2009 ACS)

Answer 446:



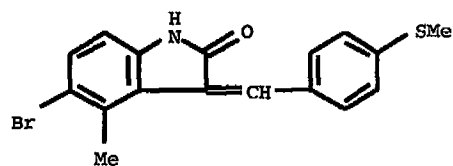
REGISTRY (Copyright (C) 2009 ACS)

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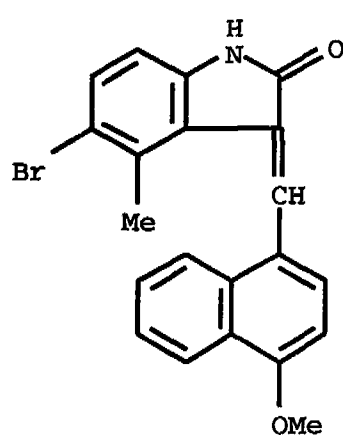
REGISTRY (Copyright (C) 2009 ACS)

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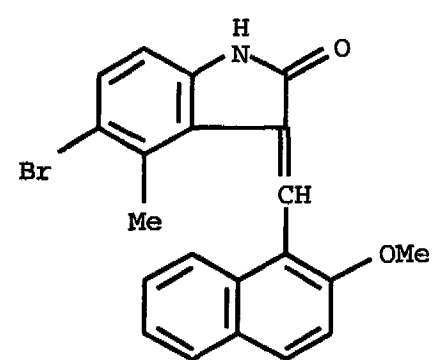
REGISTRY (Copyright (C) 2009 ACS)

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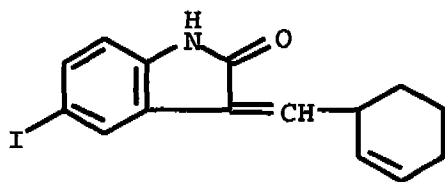
REGISTRY (Copyright (C) 2009 ACS)

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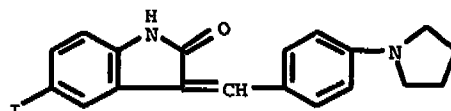


REGISTRY (Copyright (C) 2009 ACS)

Answer 451:

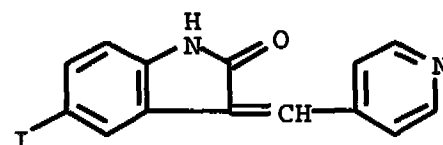


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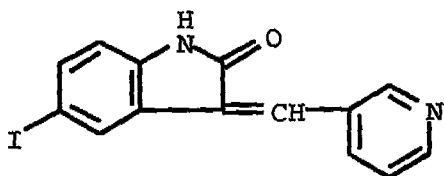
REGISTRY (Copyright (C) 2009 ACS)

Answer 453:



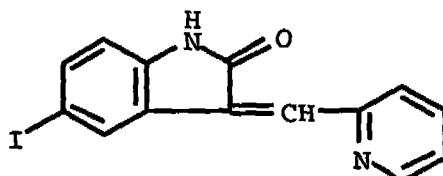
REGISTRY (Copyright (C) 2009 ACS)

Answer 454:



REGISTRY (Copyright (C) 2009 ACS)

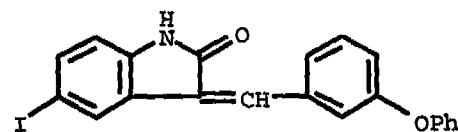
Answer 455:



REGISTRY (Copyright (C) 2009 ACS)

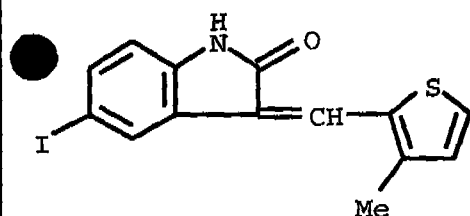
REGISTRY (Copyright (C) 2009 ACS)

Answer 456:



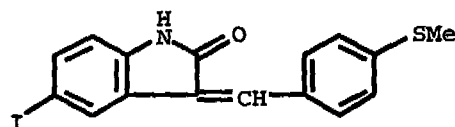
REGISTRY (Copyright (C) 2009 ACS)

Answer 457:



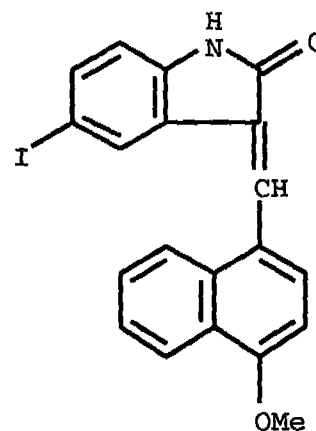
REGISTRY (Copyright (C) 2009 ACS)

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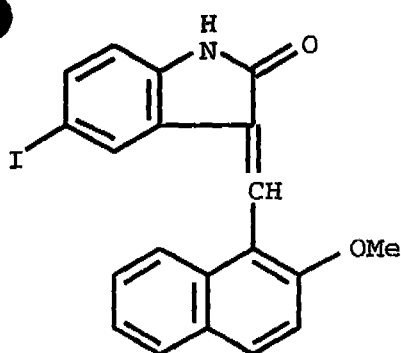
REGISTRY (Copyright (C) 2009 ACS)

Answer 459:



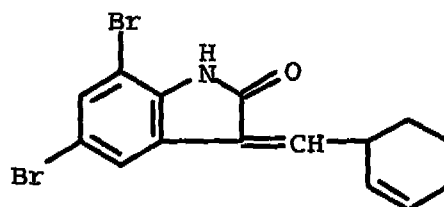
REGISTRY (Copyright (C) 2009 ACS)

Answer 460:



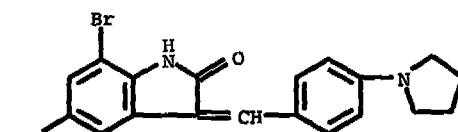
REGISTRY (Copyright (C) 2009 ACS)

Answer 461:



REGISTRY (Copyright (C) 2009 ACS)

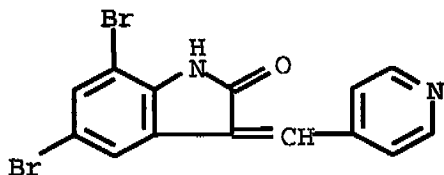
Answer 462:



REGISTRY (Copyright (C) 2009 ACS)

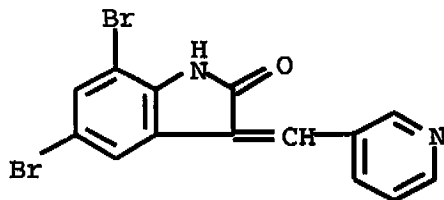
4449
52
1182

Answer 463:



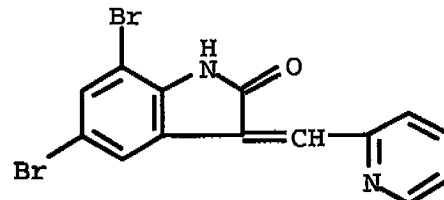
REGISTRY (Copyright (C) 2009 ACS)

Answer 464:



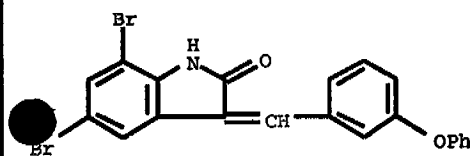
REGISTRY (Copyright (C) 2009 ACS)

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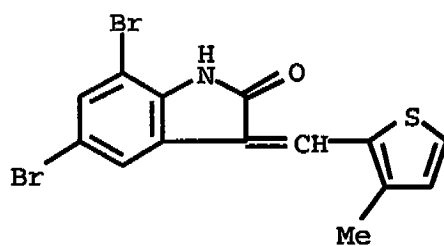
REGISTRY (Copyright (C) 2009 ACS)

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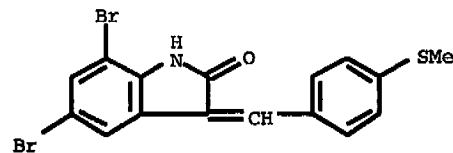
REGISTRY (Copyright (C) 2009 ACS)

Answer 467:



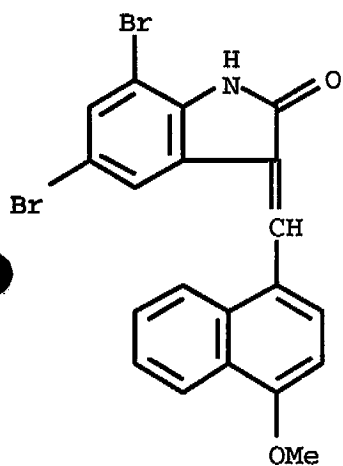
REGISTRY (Copyright (C) 2009 ACS)

Answer 468:



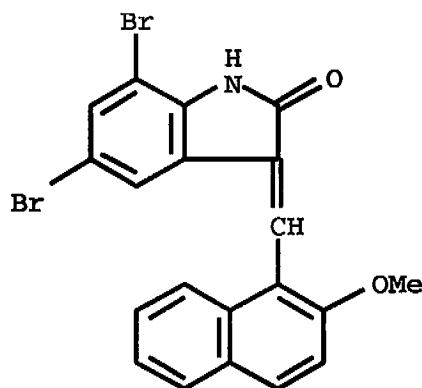
REGISTRY (Copyright (C) 2009 ACS)

Answer 469:



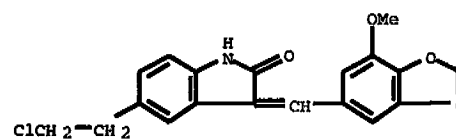
REGISTRY (Copyright (C) 2009 ACS)

Answer 470:



REGISTRY (Copyright (C) 2009 ACS)

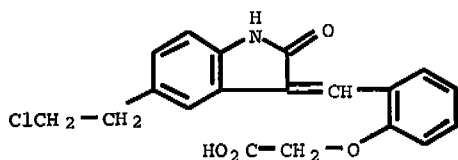
Answer 471:



REGISTRY (Copyright (C) 2009 ACS)

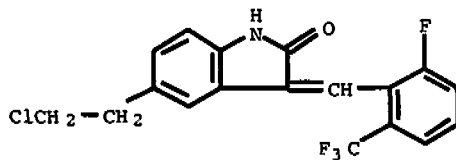
1183

Answer 472:



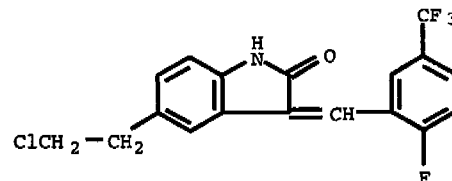
REGISTRY (Copyright (C) 2009 ACS)

Answer 473:



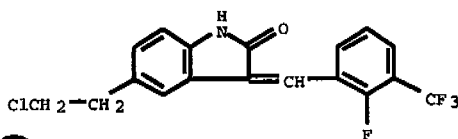
REGISTRY (Copyright (C) 2009 ACS)

Answer 474:



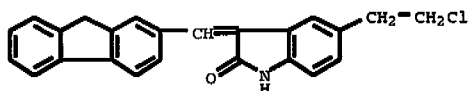
REGISTRY (Copyright (C) 2009 ACS)

Answer 475:



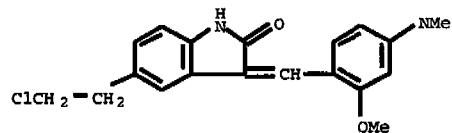
REGISTRY (Copyright (C) 2009 ACS)

Answer 476:



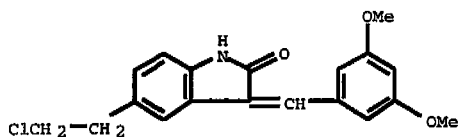
REGISTRY (Copyright (C) 2009 ACS)

Answer 477:



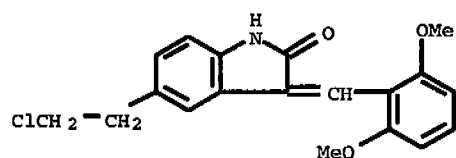
REGISTRY (Copyright (C) 2009 ACS)

Answer 478:



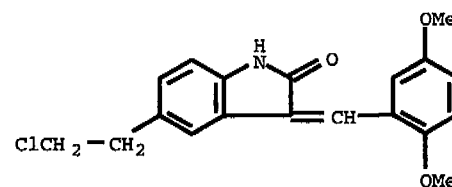
REGISTRY (Copyright (C) 2009 ACS)

Answer 479:



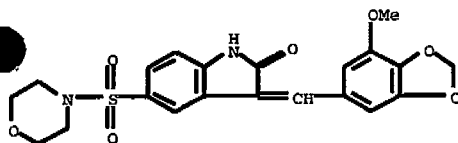
REGISTRY (Copyright (C) 2009 ACS)

Answer 480:



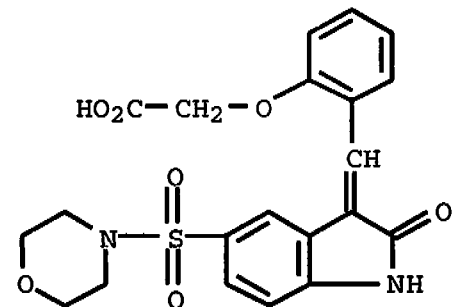
REGISTRY (Copyright (C) 2009 ACS)

Answer 481:



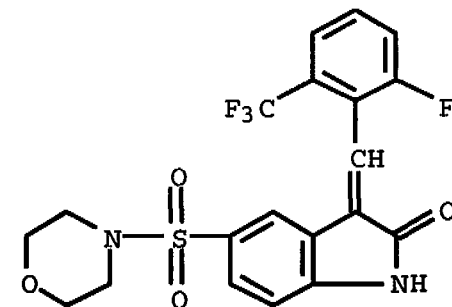
REGISTRY (Copyright (C) 2009 ACS)

Answer 482:



REGISTRY (Copyright (C) 2009 ACS)

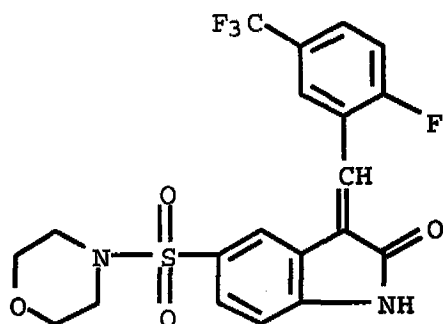
Answer 483:



REGISTRY (Copyright (C) 2009 ACS)

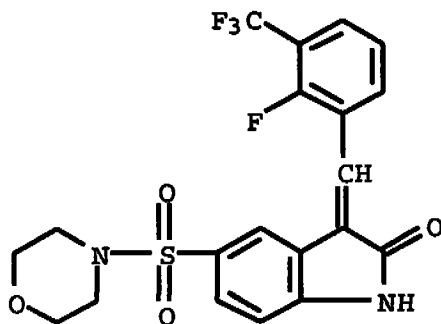
4451
1184

Answer 484:



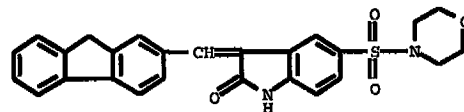
REGISTRY (Copyright (C) 2009 ACS)

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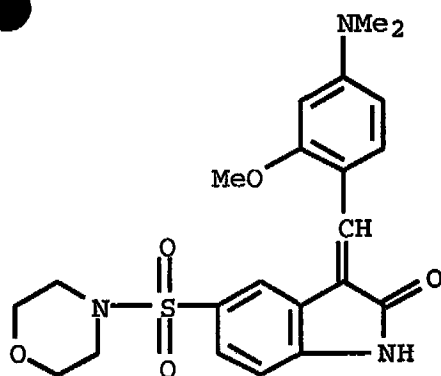
REGISTRY (Copyright (C) 2009 ACS)

Answer 486:



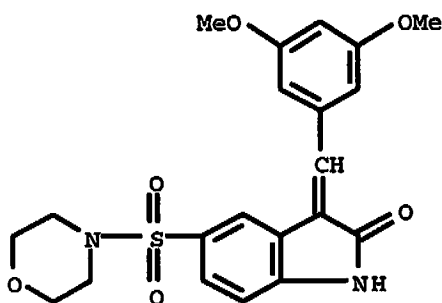
REGISTRY (Copyright (C) 2009 ACS)

Answer 487:



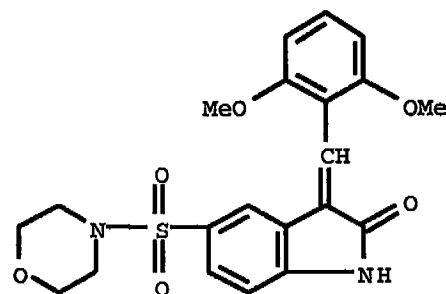
REGISTRY (Copyright (C) 2009 ACS)

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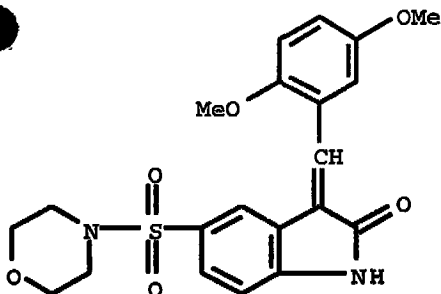
REGISTRY (Copyright (C) 2009 ACS)

Answer 489:



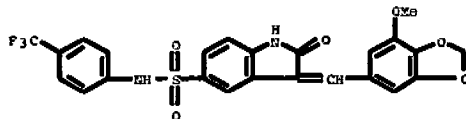
REGISTRY (Copyright (C) 2009 ACS)

Answer 490:



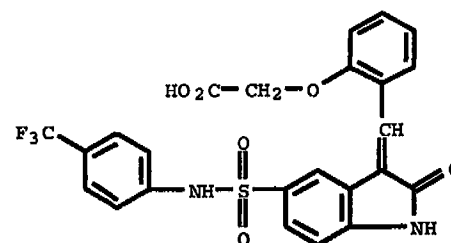
REGISTRY (Copyright (C) 2009 ACS)

Answer 491:



REGISTRY (Copyright (C) 2009 ACS)

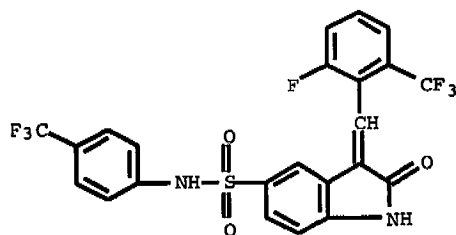
Answer 492:



REGISTRY (Copyright (C) 2009 ACS)

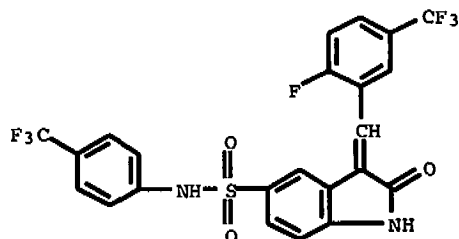
4152
1185

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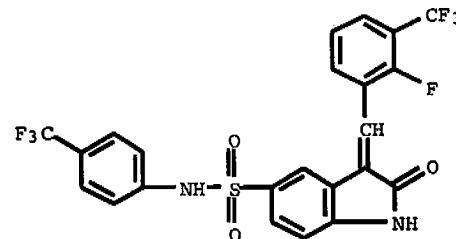
REGISTRY (Copyright (C) 2009 ACS)

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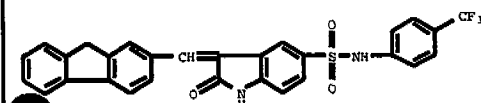
REGISTRY (Copyright (C) 2009 ACS)

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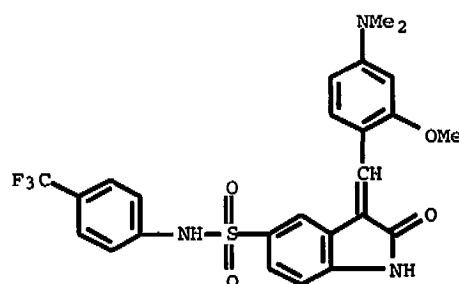
REGISTRY (Copyright (C) 2009 ACS)

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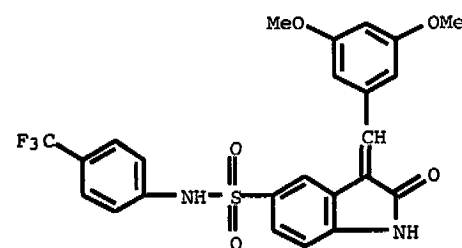
REGISTRY (Copyright (C) 2009 ACS)

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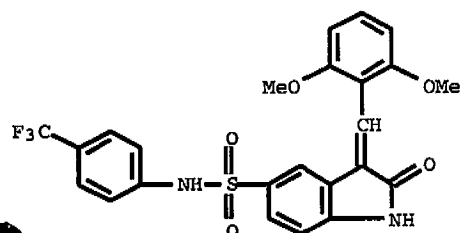
REGISTRY (Copyright (C) 2009 ACS)

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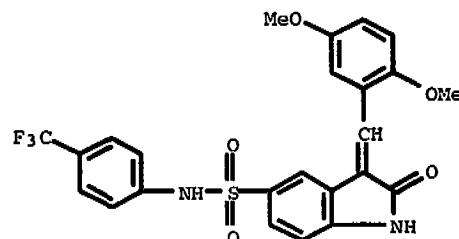
REGISTRY (Copyright (C) 2009 ACS)

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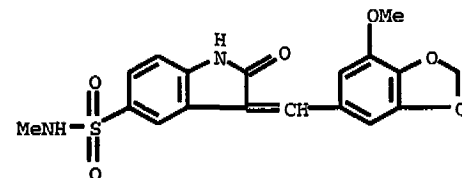
REGISTRY (Copyright (C) 2009 ACS)

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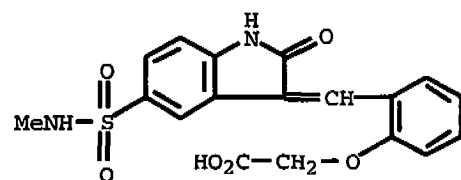
REGISTRY (Copyright (C) 2009 ACS)

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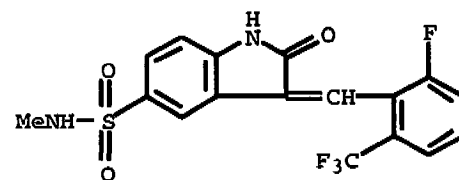
REGISTRY (Copyright (C) 2009 ACS)

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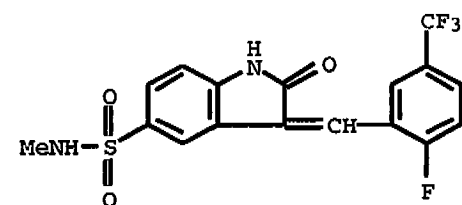
REGISTRY (Copyright (C) 2009 ACS)

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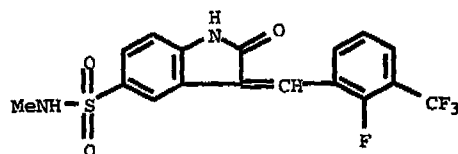
REGISTRY (Copyright (C) 2009 ACS)

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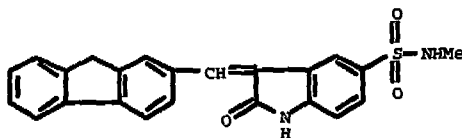
REGISTRY (Copyright (C) 2009 ACS)

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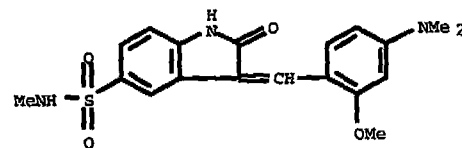
REGISTRY (Copyright (C) 2009 ACS)

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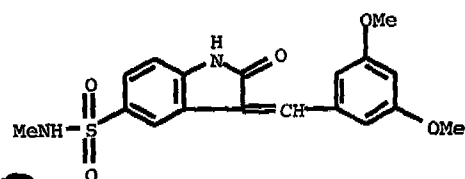
REGISTRY (Copyright (C) 2009 ACS)

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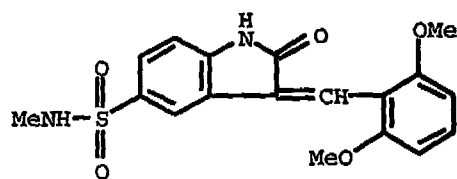
REGISTRY (Copyright (C) 2009 ACS)

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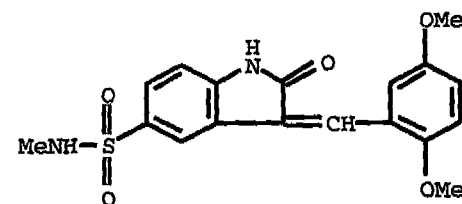
REGISTRY (Copyright (C) 2009 ACS)

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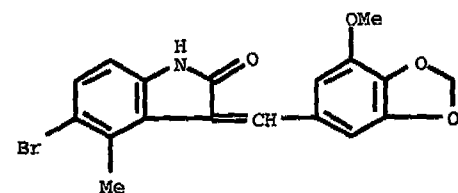
REGISTRY (Copyright (C) 2009 ACS)

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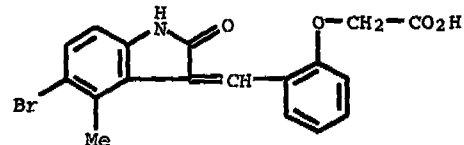
REGISTRY (Copyright (C) 2009 ACS)

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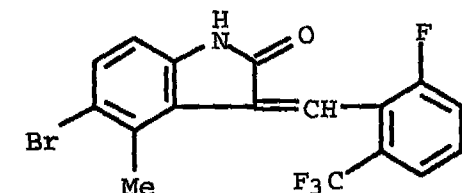
REGISTRY (Copyright (C) 2009 ACS)

Answer 512:



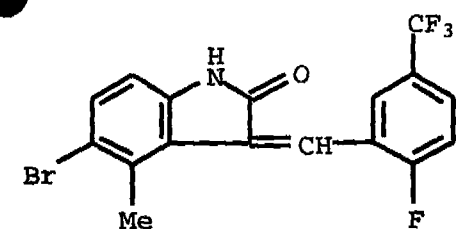
REGISTRY (Copyright (C) 2009 ACS)

Answer 513:



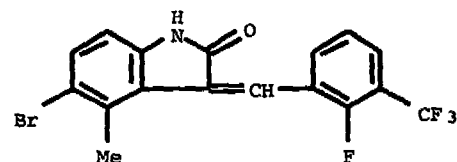
REGISTRY (Copyright (C) 2009 ACS)

Answer 514:



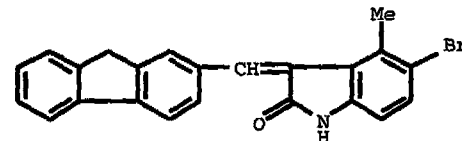
REGISTRY (Copyright (C) 2009 ACS)

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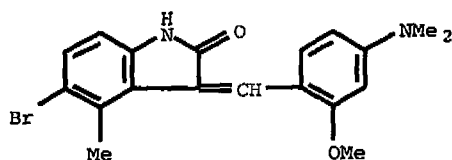
REGISTRY (Copyright (C) 2009 ACS)

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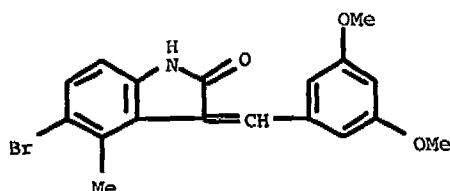
REGISTRY (Copyright (C) 2009 ACS)

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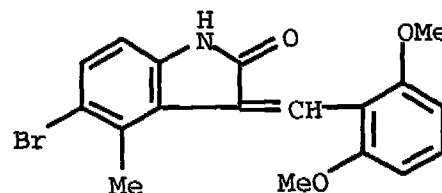
REGISTRY (Copyright (C) 2009 ACS)

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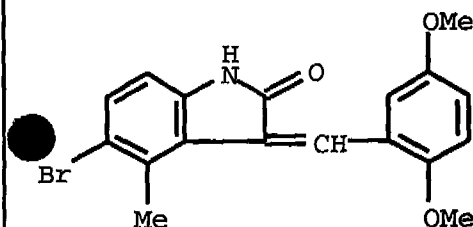
REGISTRY (Copyright (C) 2009 ACS)

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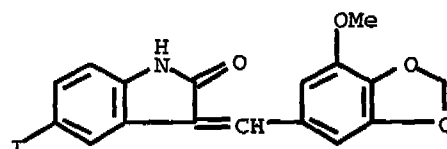
REGISTRY (Copyright (C) 2009 ACS)

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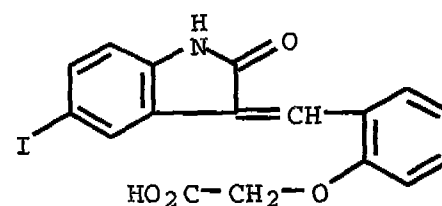
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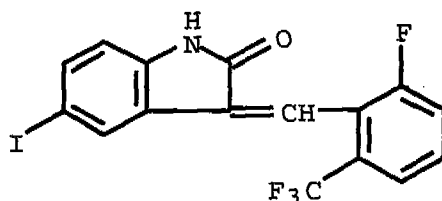
REGISTRY (Copyright (C) 2009 ACS)

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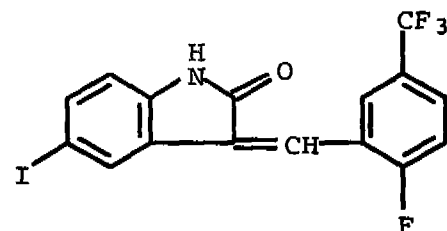
REGISTRY (Copyright (C) 2009 ACS)

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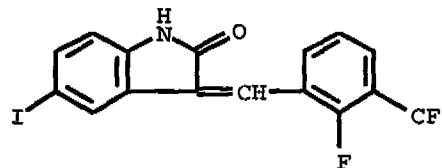
REGISTRY (Copyright (C) 2009 ACS)

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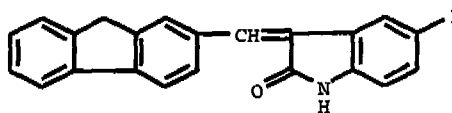
REGISTRY (Copyright (C) 2009 ACS)

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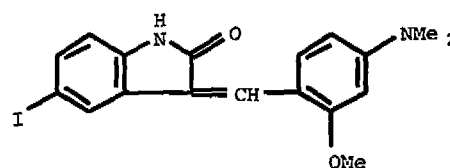
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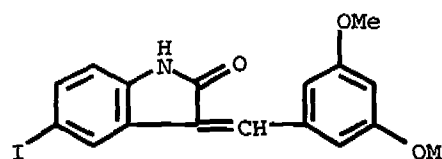
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Answer 527:



REGISTRY (Copyright (C) 2009 ACS)

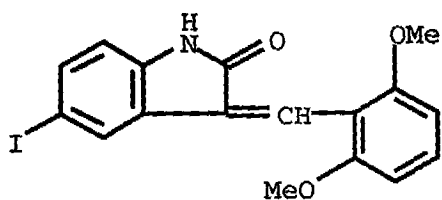
Answer 528:



REGISTRY (Copyright (C) 2009 ACS)

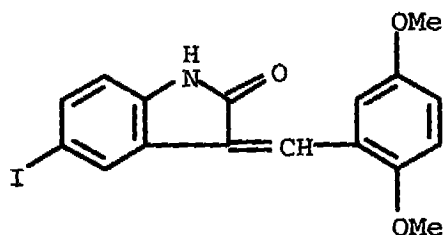
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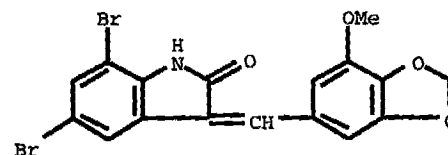
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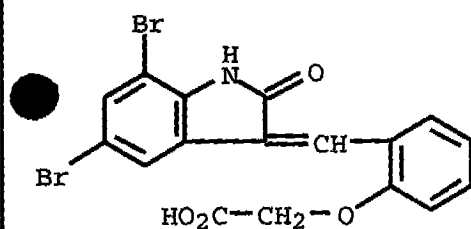
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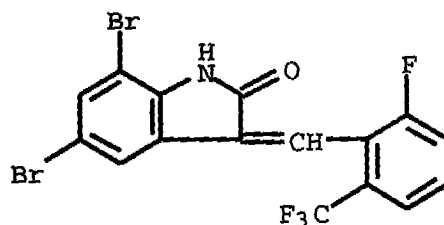
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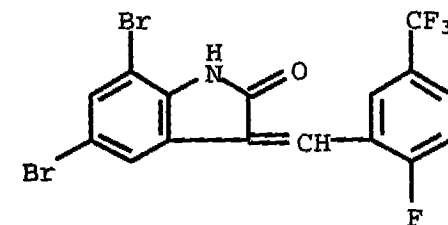
REGISTRY (Copyright (C) 2009 ACS)

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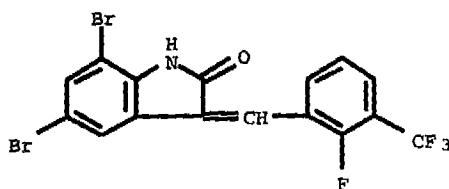
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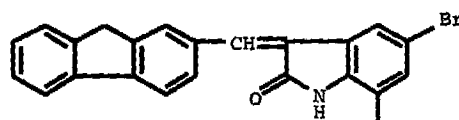
REGISTRY (Copyright (C) 2009 ACS)

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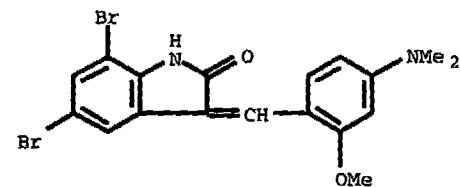
REGISTRY (Copyright (C) 2009 ACS)

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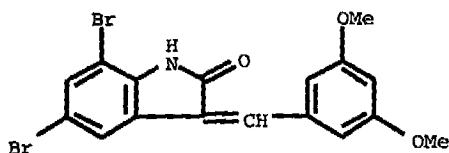
REGISTRY (Copyright (C) 2009 ACS)

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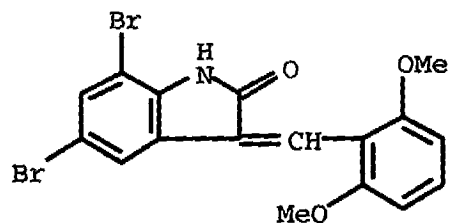
REGISTRY (Copyright (C) 2009 ACS)

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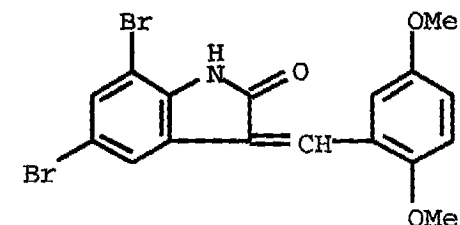
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REGISTRY (Copyright (C) 2009 ACS)

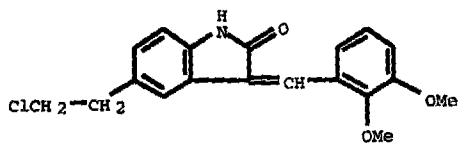
Answer 540:



REGISTRY (Copyright (C) 2009 ACS)

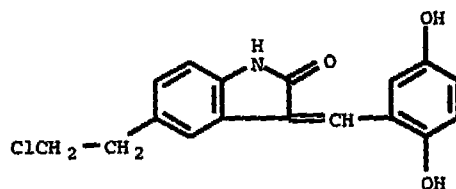
4456
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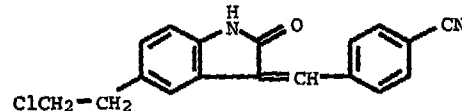
REGISTRY (Copyright (C) 2009 ACS)

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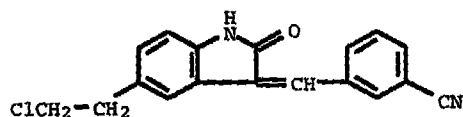
REGISTRY (Copyright (C) 2009 ACS)

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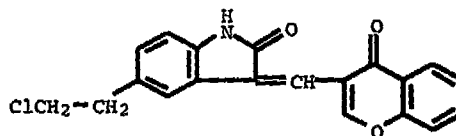
REGISTRY (Copyright (C) 2009 ACS)

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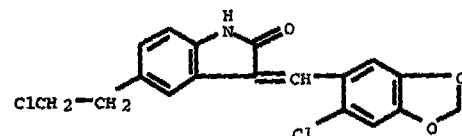
REGISTRY (Copyright (C) 2009 ACS)

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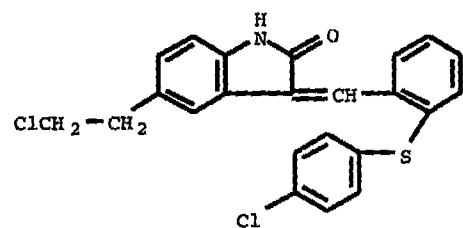
REGISTRY (Copyright (C) 2009 ACS)

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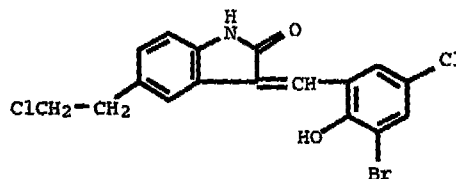
REGISTRY (Copyright (C) 2009 ACS)

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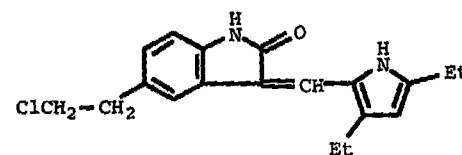
REGISTRY (Copyright (C) 2009 ACS)

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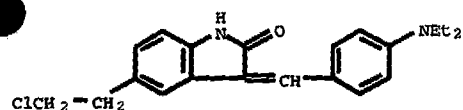
REGISTRY (Copyright (C) 2009 ACS)

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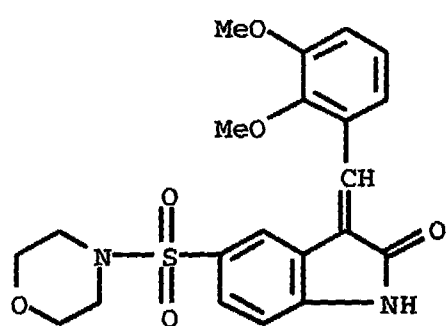
REGISTRY (Copyright (C) 2009 ACS)

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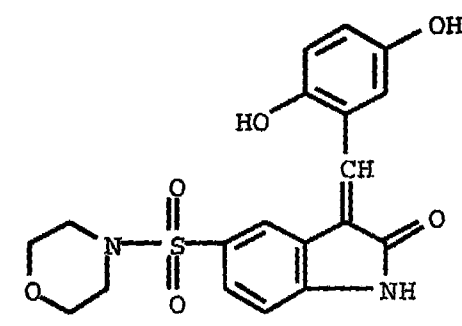
REGISTRY (Copyright (C) 2009 ACS)

Answer 551:



REGISTRY (Copyright (C) 2009 ACS)

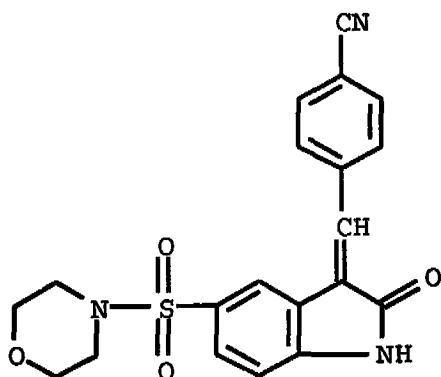
Answer 552:



REGISTRY (Copyright (C) 2009 ACS)

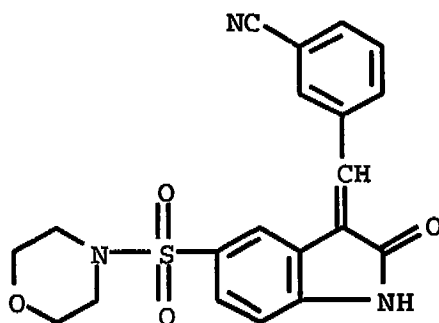
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6/1190

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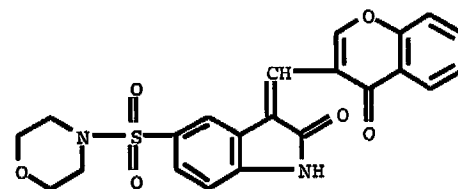
REGISTRY (Copyright (C) 2009 ACS)

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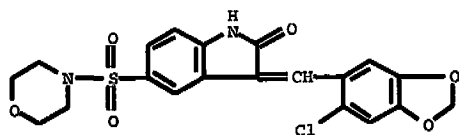
REGISTRY (Copyright (C) 2009 ACS)

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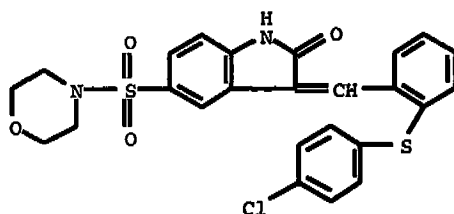
REGISTRY (Copyright (C) 2009 ACS)

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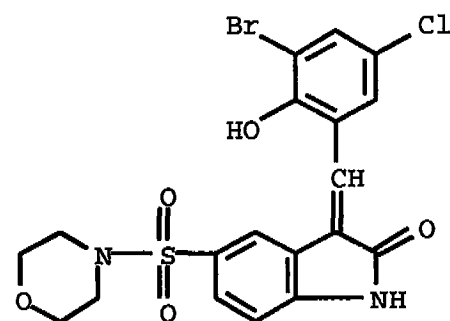
REGISTRY (Copyright (C) 2009 ACS)

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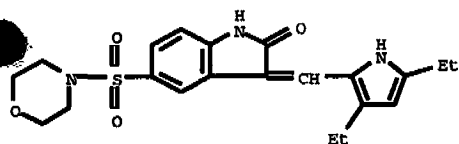
REGISTRY (Copyright (C) 2009 ACS)

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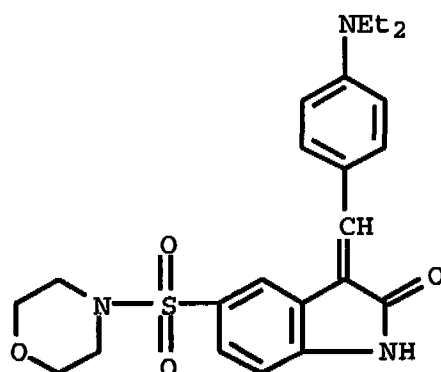
REGISTRY (Copyright (C) 2009 ACS)

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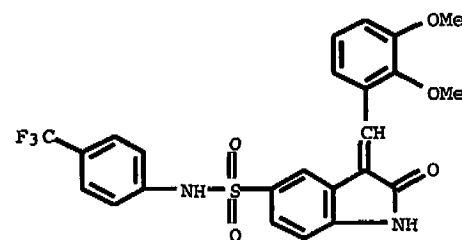
REGISTRY (Copyright (C) 2009 ACS)

Answer 560:



REGISTRY (Copyright (C) 2009 ACS)

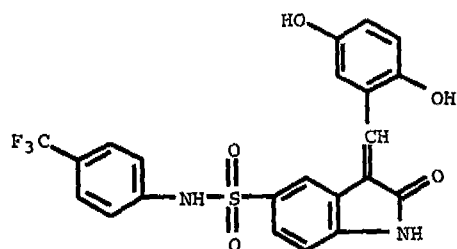
Answer 561:



REGISTRY (Copyright (C) 2009 ACS)

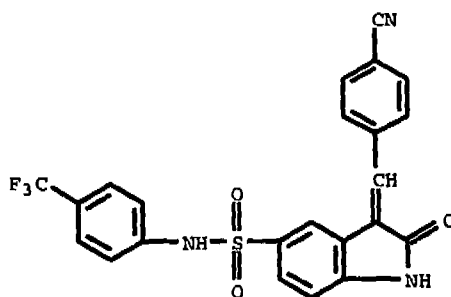
1158
f 1191

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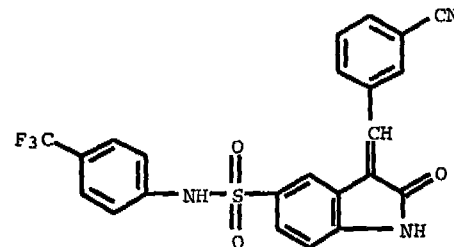
REGISTRY (Copyright (C) 2009 ACS)

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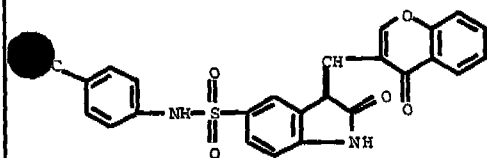
REGISTRY (Copyright (C) 2009 ACS)

Answer 564:



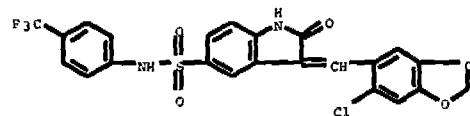
REGISTRY (Copyright (C) 2009 ACS)

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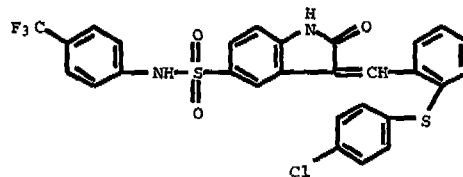
REGISTRY (Copyright (C) 2009 ACS)

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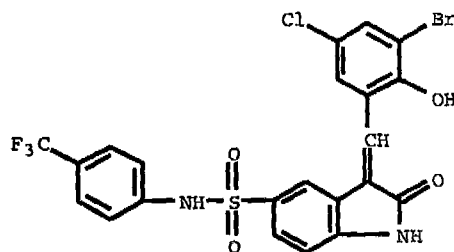
REGISTRY (Copyright (C) 2009 ACS)

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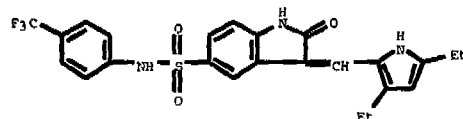
REGISTRY (Copyright (C) 2009 ACS)

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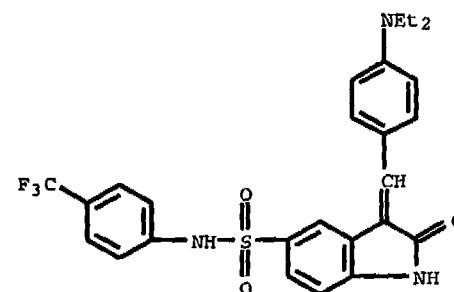
REGISTRY (Copyright (C) 2009 ACS)

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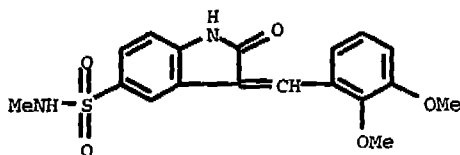
REGISTRY (Copyright (C) 2009 ACS)

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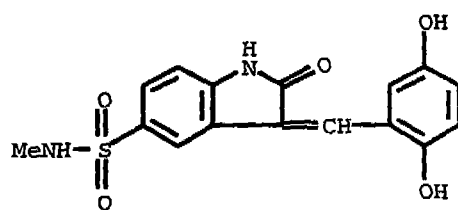
REGISTRY (Copyright (C) 2009 ACS)

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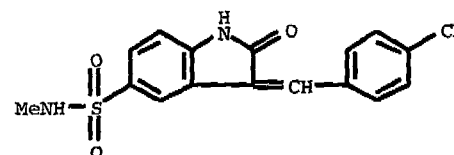
REGISTRY (Copyright (C) 2009 ACS)

Answer 572:



REGISTRY (Copyright (C) 2009 ACS)

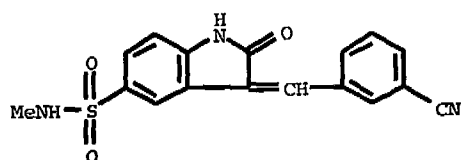
Answer 573:



REGISTRY (Copyright (C) 2009 ACS)

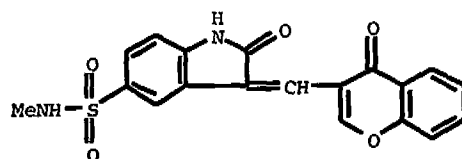
4159
60

Answer 574:



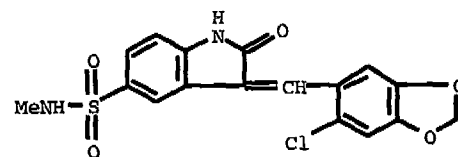
REGISTRY (Copyright (C) 2009 ACS)

Answer 575:



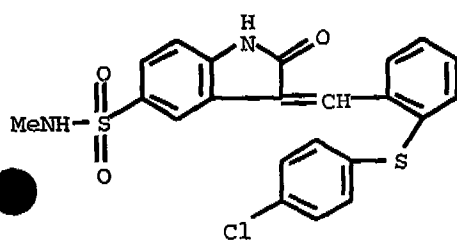
REGISTRY (Copyright (C) 2009 ACS)

Answer 576:



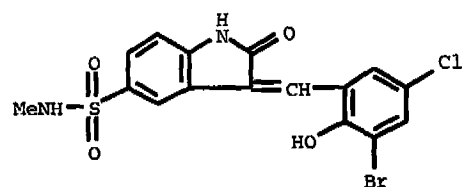
REGISTRY (Copyright (C) 2009 ACS)

Answer 577:



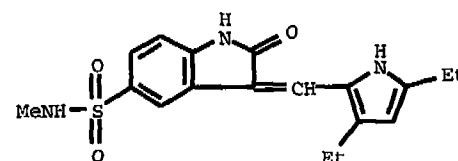
REGISTRY (Copyright (C) 2009 ACS)

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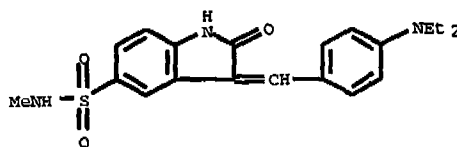
REGISTRY (Copyright (C) 2009 ACS)

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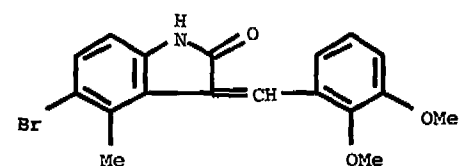
REGISTRY (Copyright (C) 2009 ACS)

Answer 580:



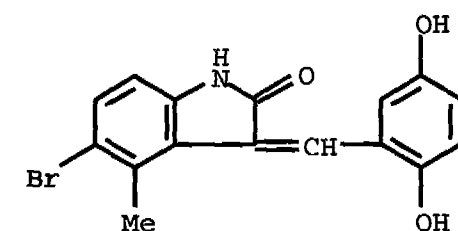
REGISTRY (Copyright (C) 2009 ACS)

Answer 581:



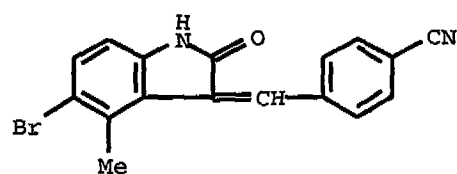
REGISTRY (Copyright (C) 2009 ACS)

Answer 582:



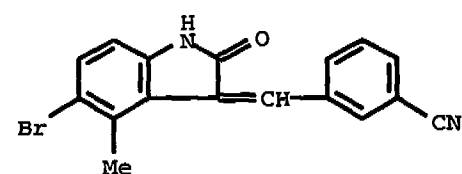
REGISTRY (Copyright (C) 2009 ACS)

Answer 583:



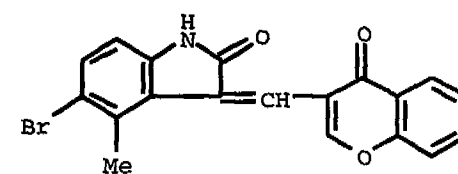
REGISTRY (Copyright (C) 2009 ACS)

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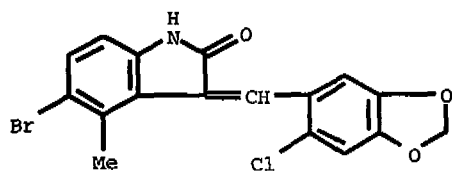
REGISTRY (Copyright (C) 2009 ACS)

Answer 585:



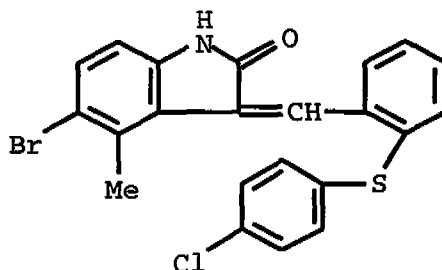
REGISTRY (Copyright (C) 2009 ACS)

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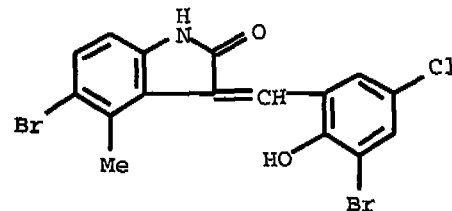
REGISTRY (Copyright (C) 2009 ACS)

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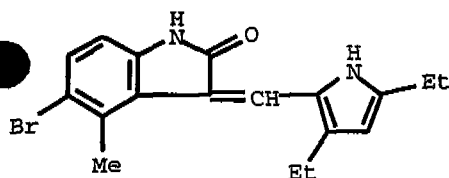
REGISTRY (Copyright (C) 2009 ACS)

Answer 588:



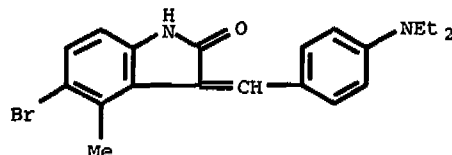
REGISTRY (Copyright (C) 2009 ACS)

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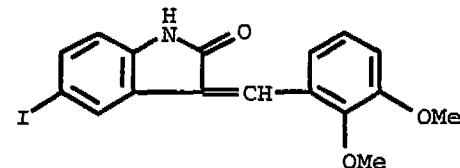
REGISTRY (Copyright (C) 2009 ACS)

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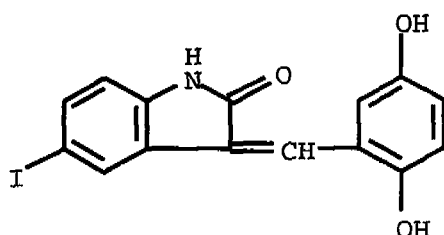
REGISTRY (Copyright (C) 2009 ACS)

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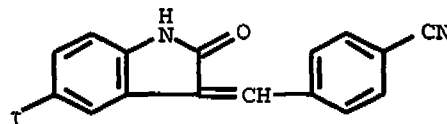
REGISTRY (Copyright (C) 2009 ACS)

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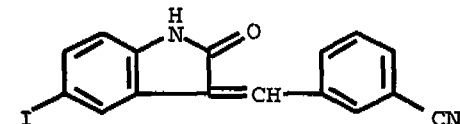
REGISTRY (Copyright (C) 2009 ACS)

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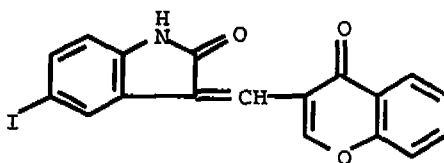
REGISTRY (Copyright (C) 2009 ACS)

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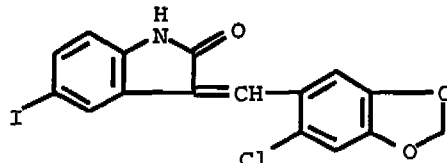
REGISTRY (Copyright (C) 2009 ACS)

Answer 595:



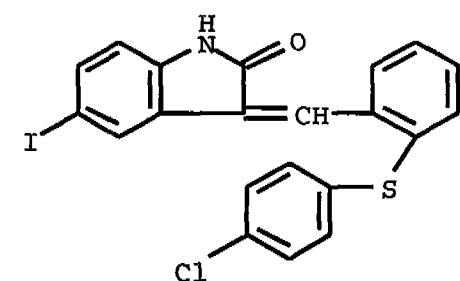
REGISTRY (Copyright (C) 2009 ACS)

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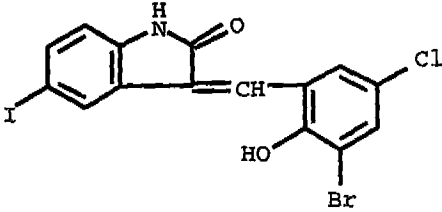
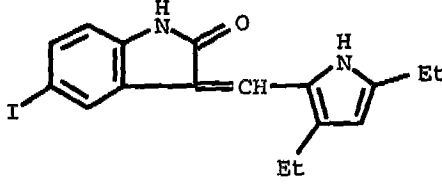
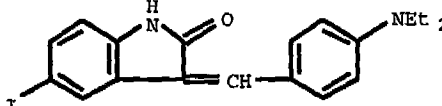
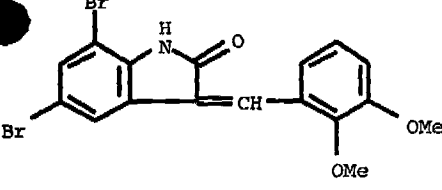
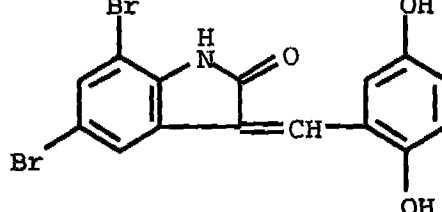
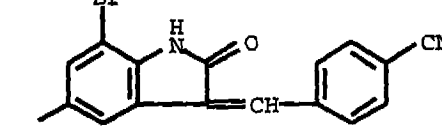
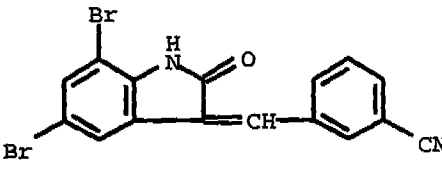
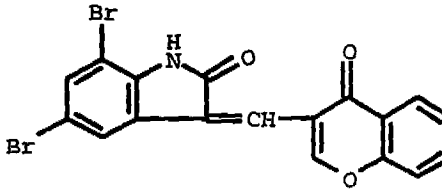
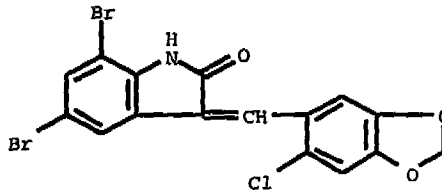
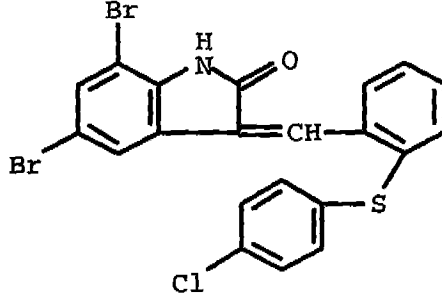
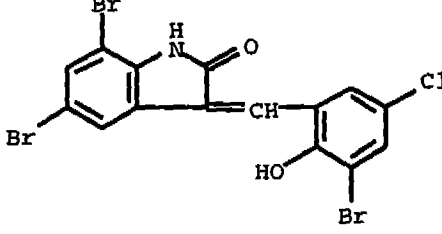
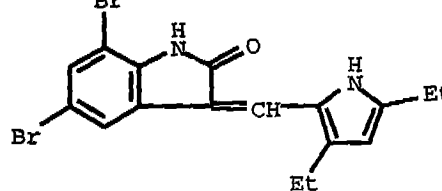


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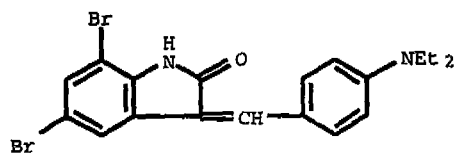
4468
671194

Answer 598:  REGISTRY (Copyright (C) 2009 ACS)	Answer 599:  REGISTRY (Copyright (C) 2009 ACS)	Answer 600:  REGISTRY (Copyright (C) 2009 ACS)
Answer 601:  REGISTRY (Copyright (C) 2009 ACS)	Answer 602:  REGISTRY (Copyright (C) 2009 ACS)	Answer 603:  REGISTRY (Copyright (C) 2009 ACS)
Answer 604:  REGISTRY (Copyright (C) 2009 ACS)	Answer 605:  REGISTRY (Copyright (C) 2009 ACS)	Answer 606:  REGISTRY (Copyright (C) 2009 ACS)
Answer 607:  REGISTRY (Copyright (C) 2009 ACS)	Answer 608:  REGISTRY (Copyright (C) 2009 ACS)	Answer 609:  REGISTRY (Copyright (C) 2009 ACS)

4462
66
1195

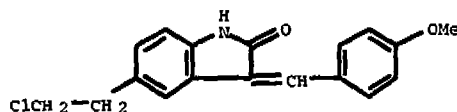
REGISTRY (Copyright (C) 2009 ACS)

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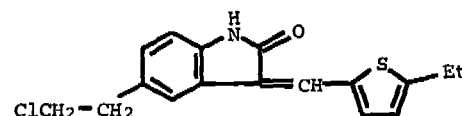
REGISTRY (Copyright (C) 2009 ACS)

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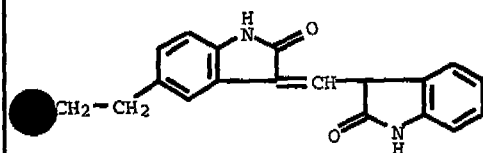
REGISTRY (Copyright (C) 2009 ACS)

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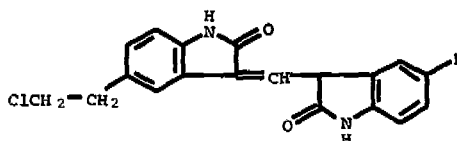
REGISTRY (Copyright (C) 2009 ACS)

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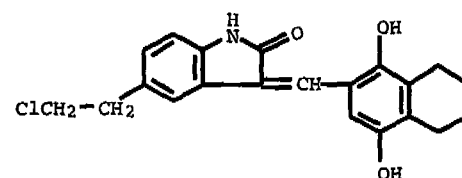
REGISTRY (Copyright (C) 2009 ACS)

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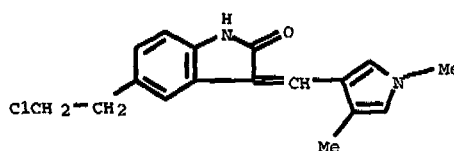
REGISTRY (Copyright (C) 2009 ACS)

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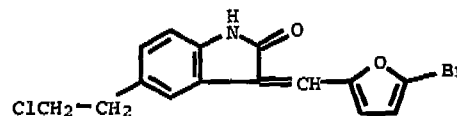
REGISTRY (Copyright (C) 2009 ACS)

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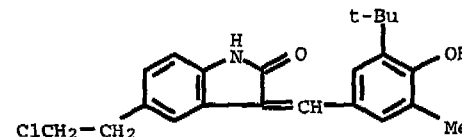
REGISTRY (Copyright (C) 2009 ACS)

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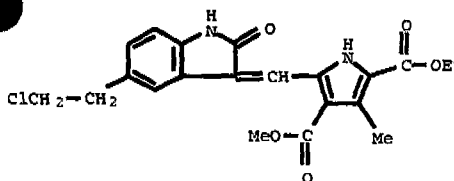
REGISTRY (Copyright (C) 2009 ACS)

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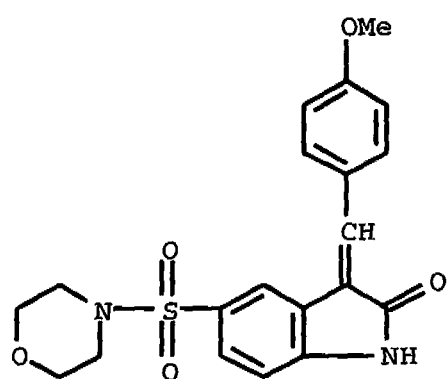
REGISTRY (Copyright (C) 2009 ACS)

Answer 619:



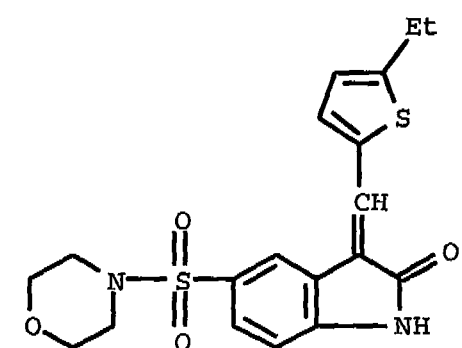
REGISTRY (Copyright (C) 2009 ACS)

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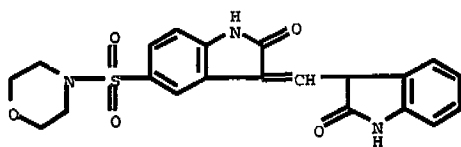
REGISTRY (Copyright (C) 2009 ACS)

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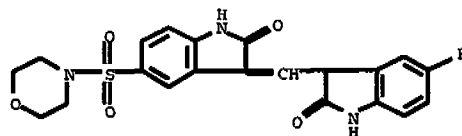
REGISTRY (Copyright (C) 2009 ACS)

Answer 622:



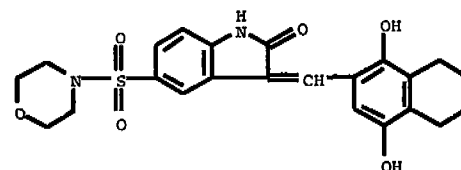
REGISTRY (Copyright (C) 2009 ACS)

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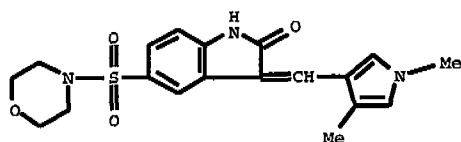
REGISTRY (Copyright (C) 2009 ACS)

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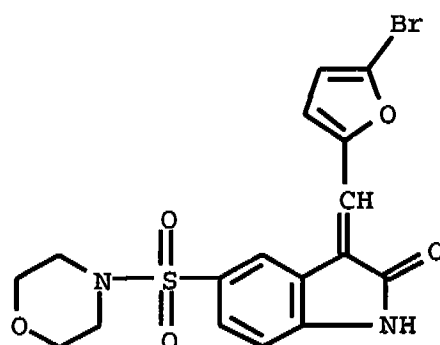
REGISTRY (Copyright (C) 2009 ACS)

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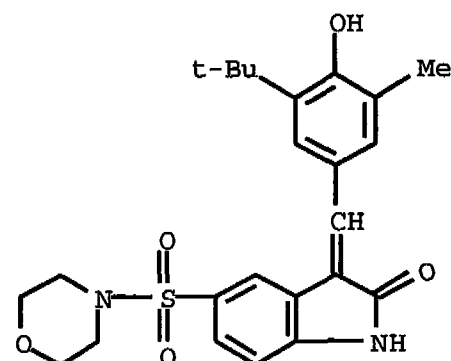
REGISTRY (Copyright (C) 2009 ACS)

Answer 626:



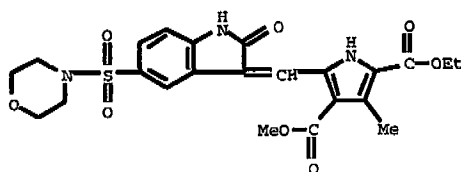
REGISTRY (Copyright (C) 2009 ACS)

Answer 627:



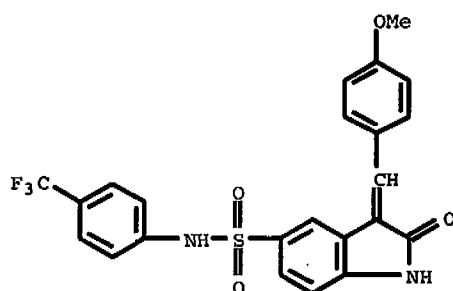
REGISTRY (Copyright (C) 2009 ACS)

Answer 628:



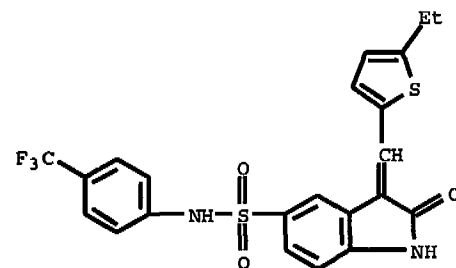
REGISTRY (Copyright (C) 2009 ACS)

Answer 629:



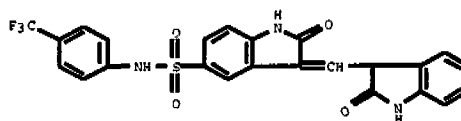
REGISTRY (Copyright (C) 2009 ACS)

Answer 630:



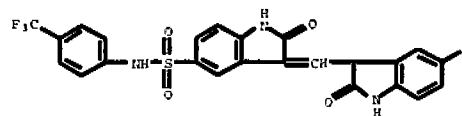
REGISTRY (Copyright (C) 2009 ACS)

Answer 631:



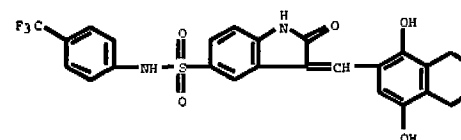
REGISTRY (Copyright (C) 2009 ACS)

Answer 632:



REGISTRY (Copyright (C) 2009 ACS)

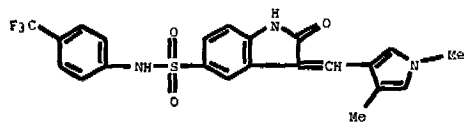
Answer 633:



REGISTRY (Copyright (C) 2009 ACS)

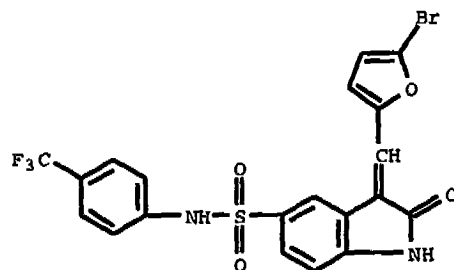
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Answer 634:



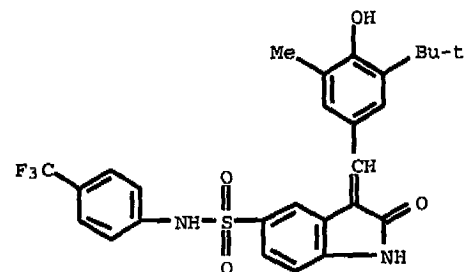
REGISTRY (Copyright (C) 2009 ACS)

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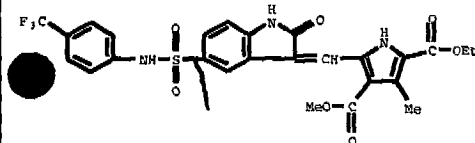
REGISTRY (Copyright (C) 2009 ACS)

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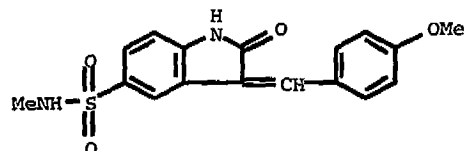
REGISTRY (Copyright (C) 2009 ACS)

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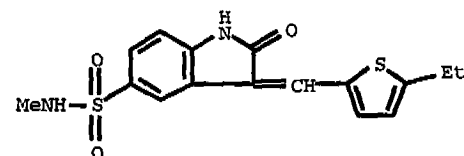
REGISTRY (Copyright (C) 2009 ACS)

Answer 638:



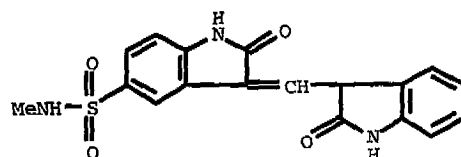
REGISTRY (Copyright (C) 2009 ACS)

Answer 639:



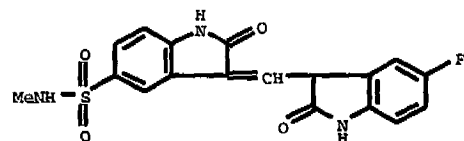
REGISTRY (Copyright (C) 2009 ACS)

Answer 640:



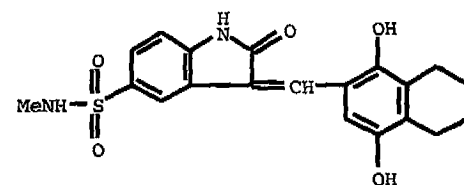
REGISTRY (Copyright (C) 2009 ACS)

Answer 641:



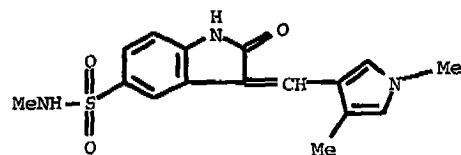
REGISTRY (Copyright (C) 2009 ACS)

Answer 642:



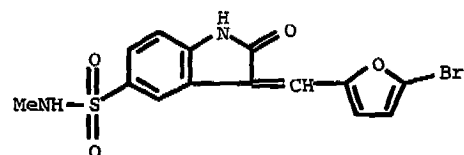
REGISTRY (Copyright (C) 2009 ACS)

Answer 643:



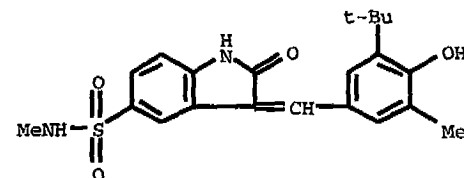
REGISTRY (Copyright (C) 2009 ACS)

Answer 644:



REGISTRY (Copyright (C) 2009 ACS)

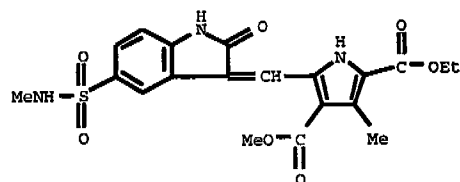
Answer 645:



REGISTRY (Copyright (C) 2009 ACS)

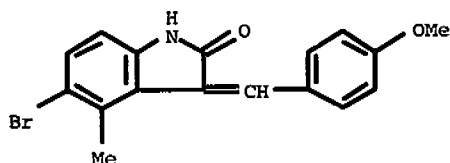
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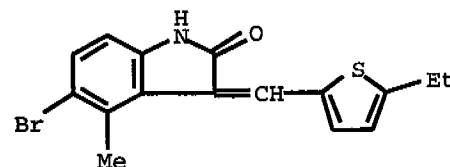
REGISTRY (Copyright (C) 2009 ACS)

Answer 647:



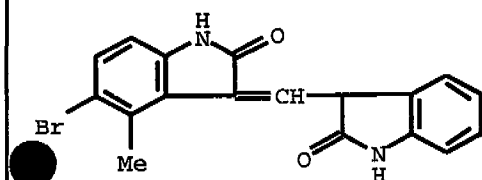
REGISTRY (Copyright (C) 2009 ACS)

Answer 648:



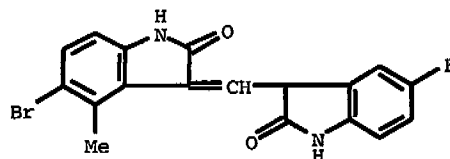
REGISTRY (Copyright (C) 2009 ACS)

Answer 649:



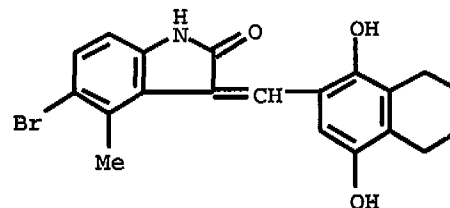
REGISTRY (Copyright (C) 2009 ACS)

Answer 650:



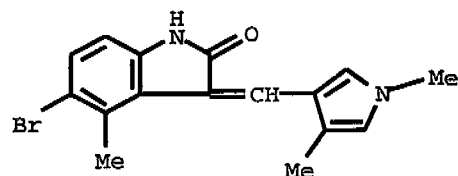
REGISTRY (Copyright (C) 2009 ACS)

Answer 651:



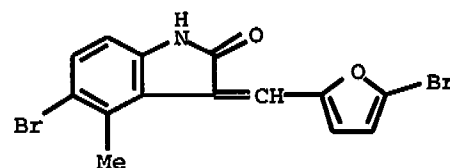
REGISTRY (Copyright (C) 2009 ACS)

Answer 652:



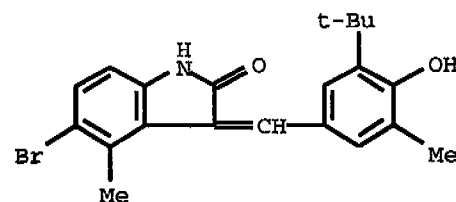
REGISTRY (Copyright (C) 2009 ACS)

Answer 653:



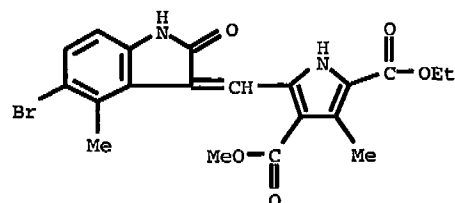
REGISTRY (Copyright (C) 2009 ACS)

Answer 654:



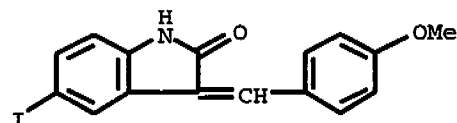
REGISTRY (Copyright (C) 2009 ACS)

Answer 655:



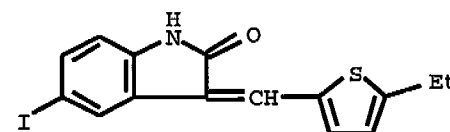
REGISTRY (Copyright (C) 2009 ACS)

Answer 656:



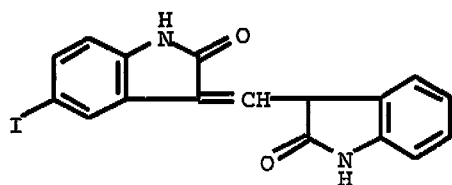
REGISTRY (Copyright (C) 2009 ACS)

Answer 657:



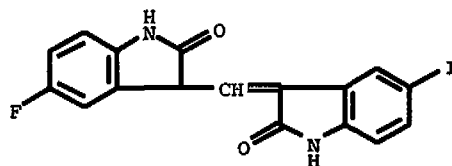
REGISTRY (Copyright (C) 2009 ACS)

Answer 658:



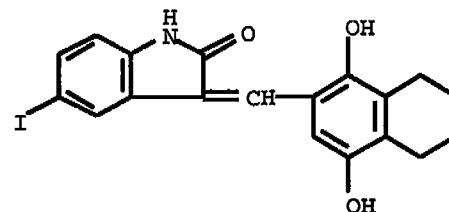
REGISTRY (Copyright (C) 2009 ACS)

Answer 659:



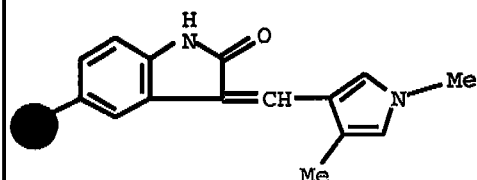
REGISTRY (Copyright (C) 2009 ACS)

Answer 660:



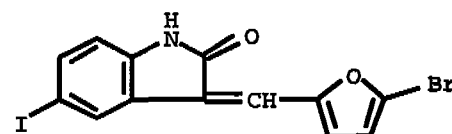
REGISTRY (Copyright (C) 2009 ACS)

Answer 661:



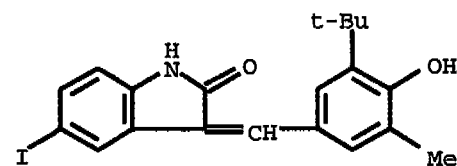
REGISTRY (Copyright (C) 2009 ACS)

Answer 662:



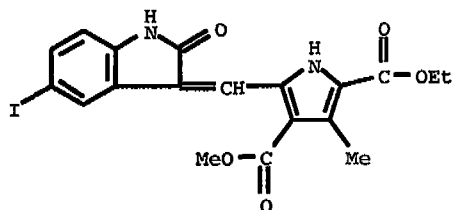
REGISTRY (Copyright (C) 2009 ACS)

Answer 663:



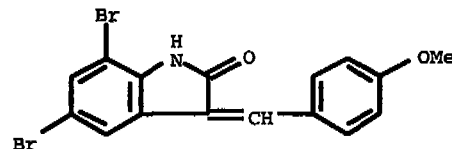
REGISTRY (Copyright (C) 2009 ACS)

Answer 664:



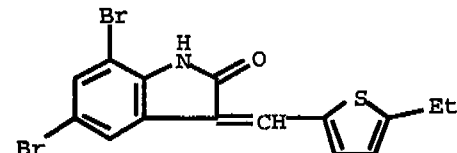
REGISTRY (Copyright (C) 2009 ACS)

Answer 665:



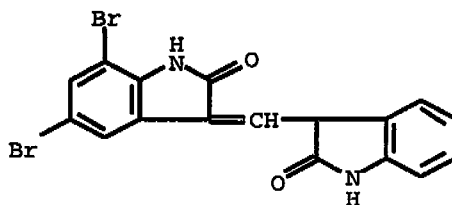
REGISTRY (Copyright (C) 2009 ACS)

Answer 666:



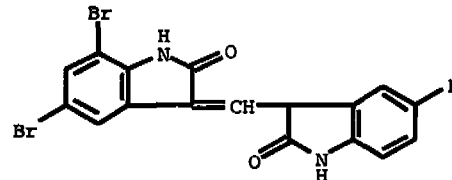
REGISTRY (Copyright (C) 2009 ACS)

Answer 667:



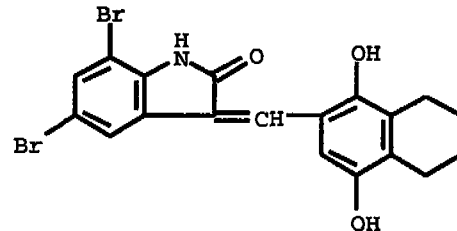
REGISTRY (Copyright (C) 2009 ACS)

Answer 668:



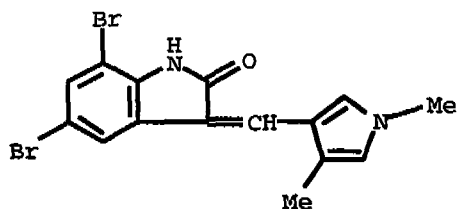
REGISTRY (Copyright (C) 2009 ACS)

Answer 669:



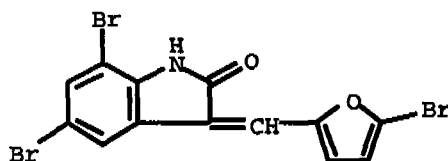
REGISTRY (Copyright (C) 2009 ACS)

Answer 670:



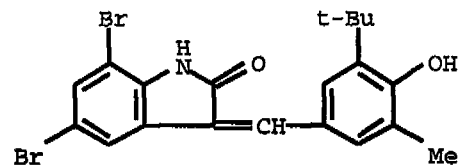
REGISTRY (Copyright (C) 2009 ACS)

Answer 671:



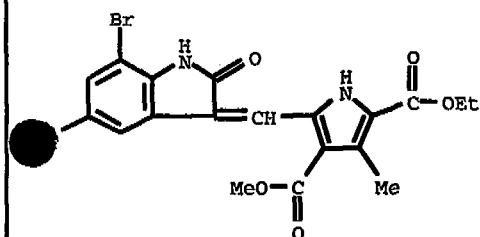
REGISTRY (Copyright (C) 2009 ACS)

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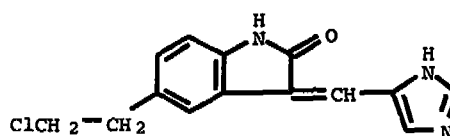
REGISTRY (Copyright (C) 2009 ACS)

Answer 673:



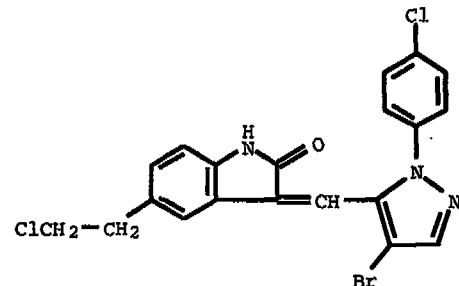
REGISTRY (Copyright (C) 2009 ACS)

Answer 674:



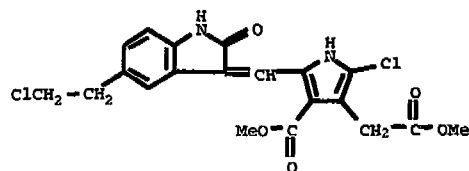
REGISTRY (Copyright (C) 2009 ACS)

Answer 675:



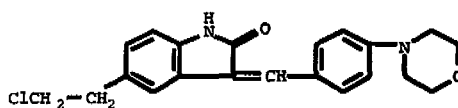
REGISTRY (Copyright (C) 2009 ACS)

Answer 676:



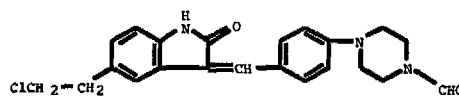
REGISTRY (Copyright (C) 2009 ACS)

Answer 677:



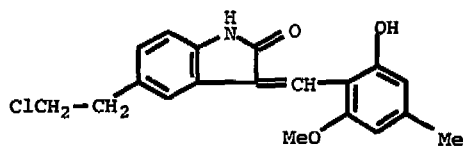
REGISTRY (Copyright (C) 2009 ACS)

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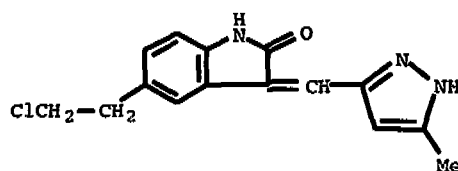
REGISTRY (Copyright (C) 2009 ACS)

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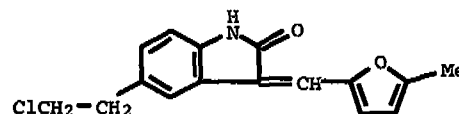
REGISTRY (Copyright (C) 2009 ACS)

Answer 680:



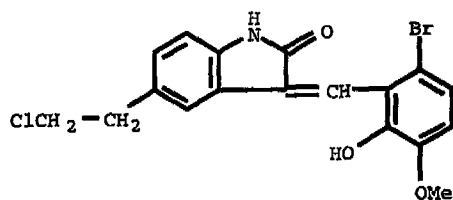
REGISTRY (Copyright (C) 2009 ACS)

Answer 681:



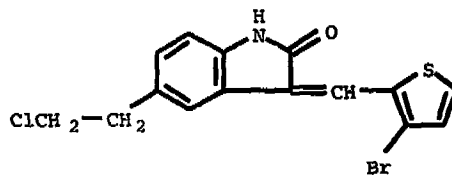
REGISTRY (Copyright (C) 2009 ACS)

Answer 682:



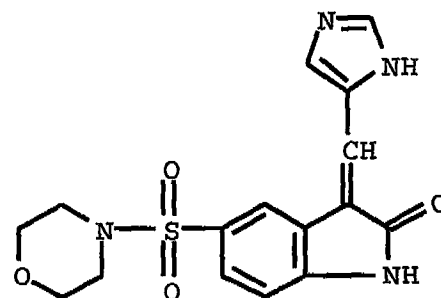
REGISTRY (Copyright (C) 2009 ACS)

Answer 683:



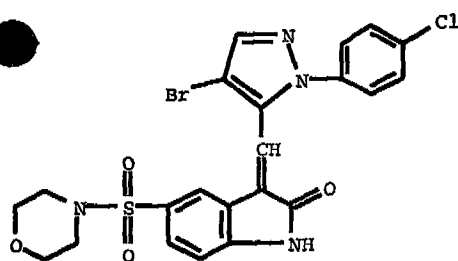
REGISTRY (Copyright (C) 2009 ACS)

Answer 684:



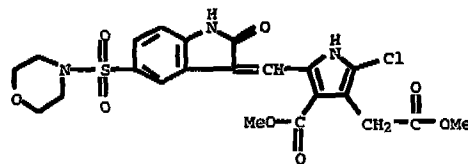
REGISTRY (Copyright (C) 2009 ACS)

Answer 685:



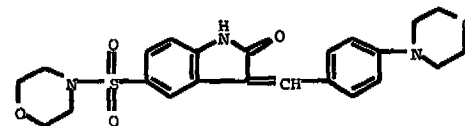
REGISTRY (Copyright (C) 2009 ACS)

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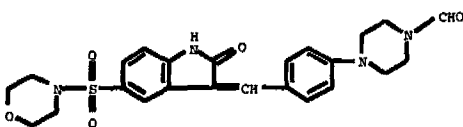
REGISTRY (Copyright (C) 2009 ACS)

Answer 687:



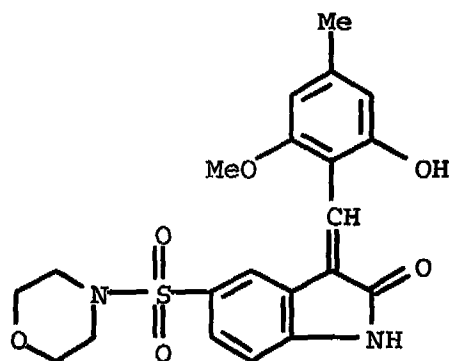
REGISTRY (Copyright (C) 2009 ACS)

Answer 688:



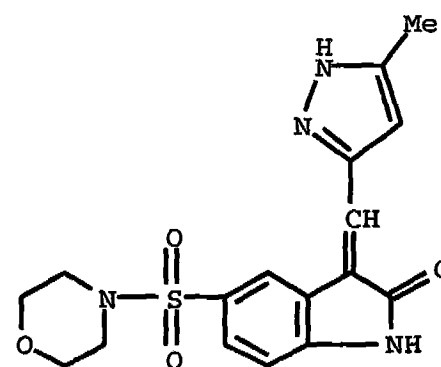
REGISTRY (Copyright (C) 2009 ACS)

Answer 689:



REGISTRY (Copyright (C) 2009 ACS)

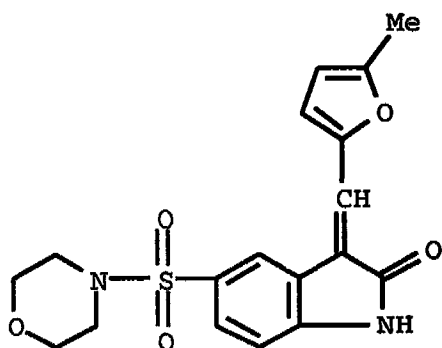
Answer 690:



REGISTRY (Copyright (C) 2009 ACS)

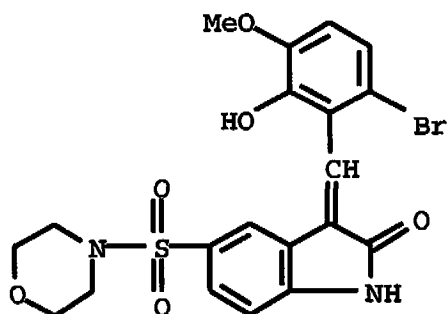
4169
2/2 1202

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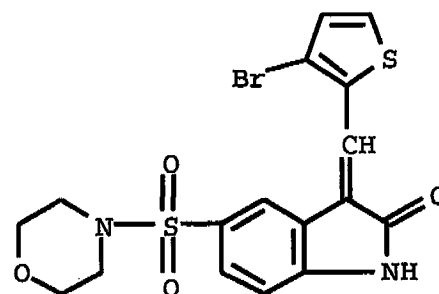
REGISTRY (Copyright (C) 2009 ACS)

Answer 692:



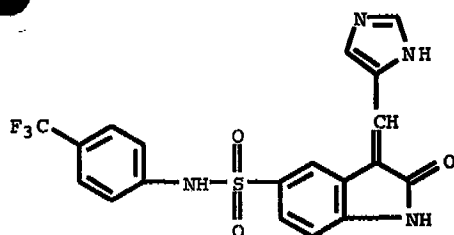
REGISTRY (Copyright (C) 2009 ACS)

Answer 693:



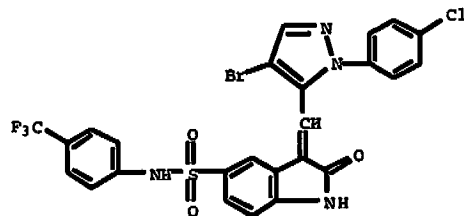
REGISTRY (Copyright (C) 2009 ACS)

Answer 694:



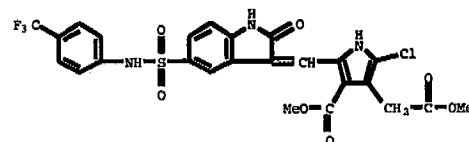
REGISTRY (Copyright (C) 2009 ACS)

Answer 695:



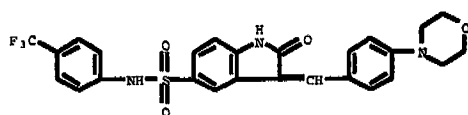
REGISTRY (Copyright (C) 2009 ACS)

Answer 696:



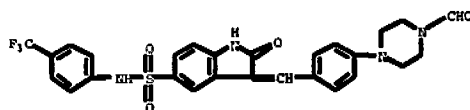
REGISTRY (Copyright (C) 2009 ACS)

Answer 697:



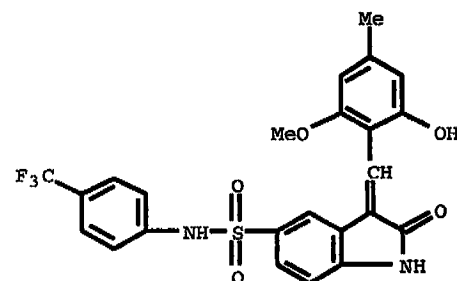
REGISTRY (Copyright (C) 2009 ACS)

Answer 698:



REGISTRY (Copyright (C) 2009 ACS)

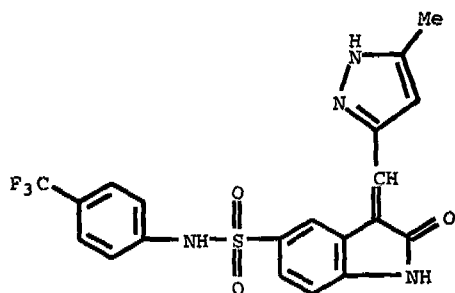
Answer 699:



REGISTRY (Copyright (C) 2009 ACS)

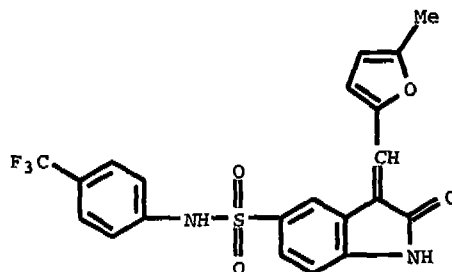
1170
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1203

Answer 700:



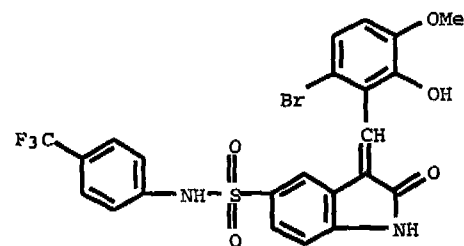
REGISTRY (Copyright (C) 2009 ACS)

Answer 701:



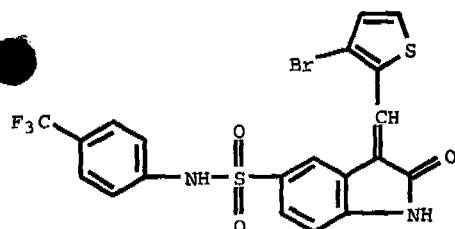
REGISTRY (Copyright (C) 2009 ACS)

Answer 702:



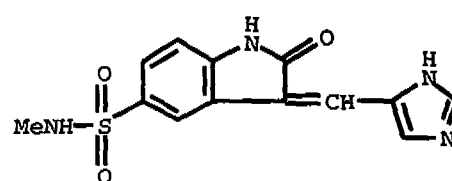
REGISTRY (Copyright (C) 2009 ACS)

Answer 703:



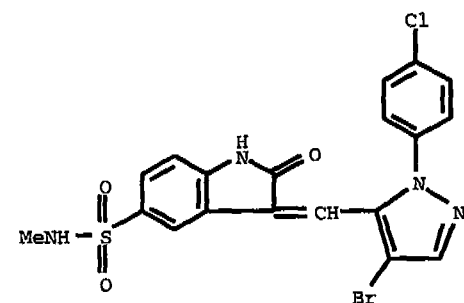
REGISTRY (Copyright (C) 2009 ACS)

Answer 704:



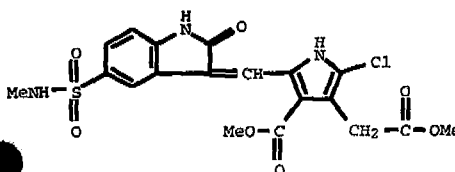
REGISTRY (Copyright (C) 2009 ACS)

Answer 705:



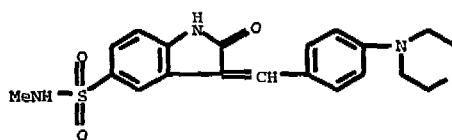
REGISTRY (Copyright (C) 2009 ACS)

Answer 706:



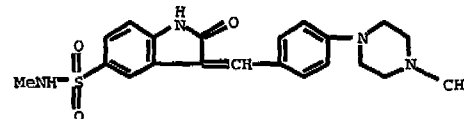
REGISTRY (Copyright (C) 2009 ACS)

Answer 707:



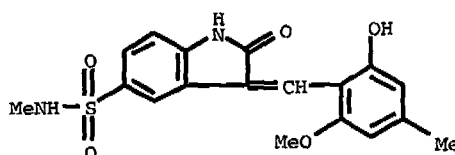
REGISTRY (Copyright (C) 2009 ACS)

Answer 708:



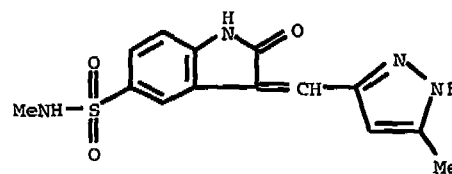
REGISTRY (Copyright (C) 2009 ACS)

Answer 709:



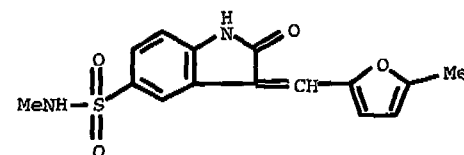
REGISTRY (Copyright (C) 2009 ACS)

Answer 710:



REGISTRY (Copyright (C) 2009 ACS)

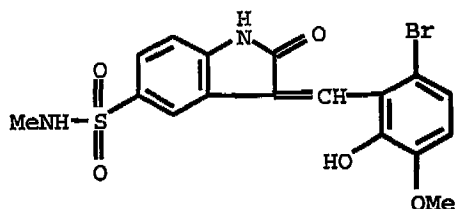
Answer 711:



REGISTRY (Copyright (C) 2009 ACS)

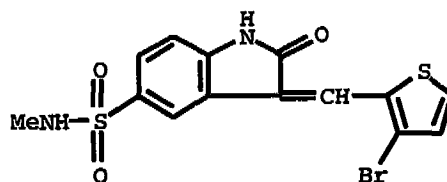
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1204

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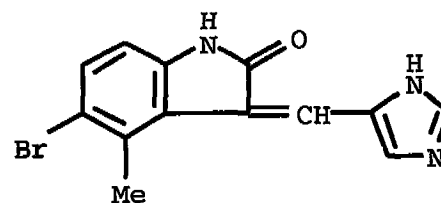
REGISTRY (Copyright (C) 2009 ACS)

Answer 713:



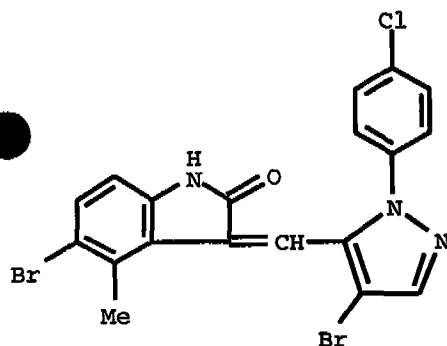
REGISTRY (Copyright (C) 2009 ACS)

Answer 714:



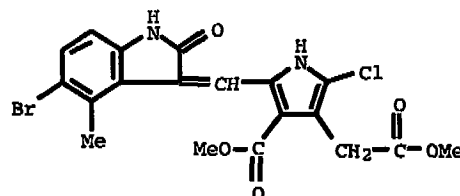
REGISTRY (Copyright (C) 2009 ACS)

Answer 715:



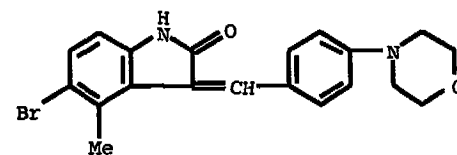
REGISTRY (Copyright (C) 2009 ACS)

Answer 716:



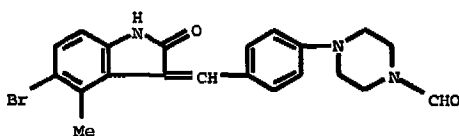
REGISTRY (Copyright (C) 2009 ACS)

Answer 717:



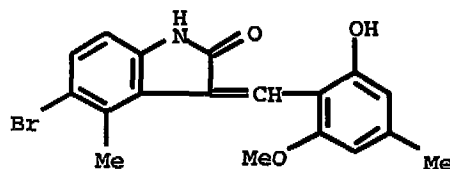
REGISTRY (Copyright (C) 2009 ACS)

Answer 718:



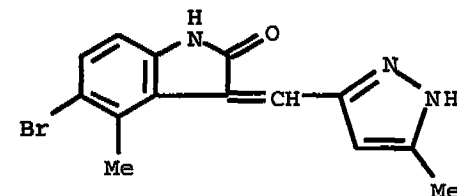
REGISTRY (Copyright (C) 2009 ACS)

Answer 719:



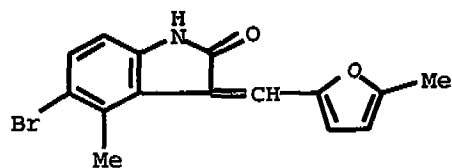
REGISTRY (Copyright (C) 2009 ACS)

Answer 720:



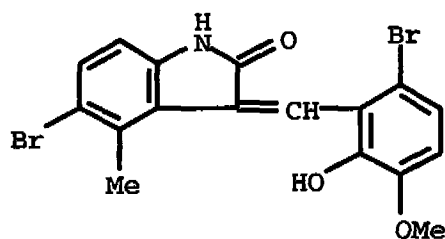
REGISTRY (Copyright (C) 2009 ACS)

Answer 721:

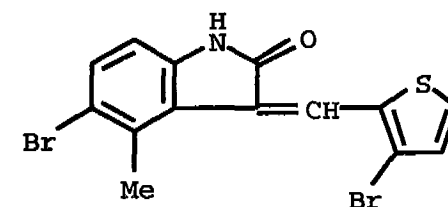


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Answer 722:



Answer 723:

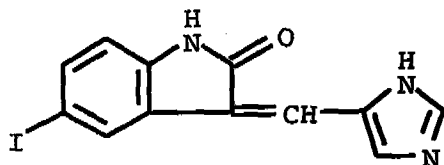


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44726
205

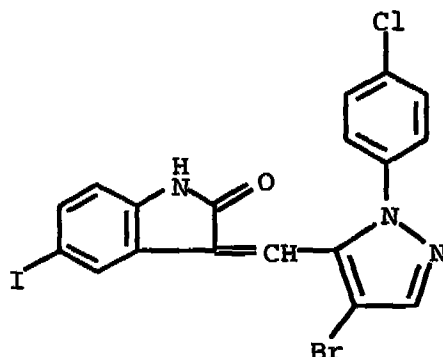
REGISTRY (Copyright (C) 2009 ACS)

Answer 724:



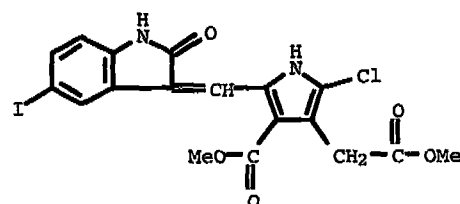
REGISTRY (Copyright (C) 2009 ACS)

Answer 725:



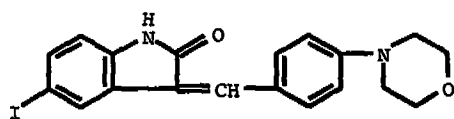
REGISTRY (Copyright (C) 2009 ACS)

Answer 726:



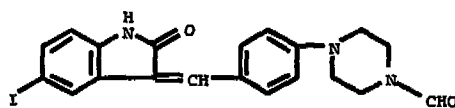
REGISTRY (Copyright (C) 2009 ACS)

Answer 727:



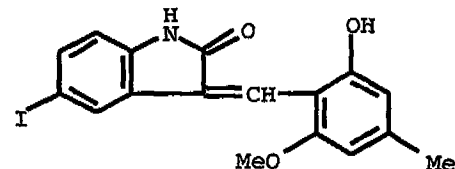
REGISTRY (Copyright (C) 2009 ACS)

Answer 728:



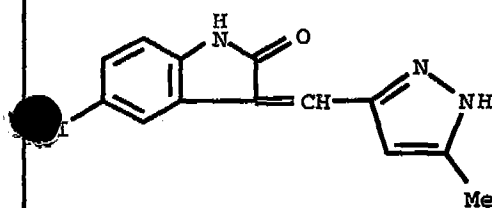
REGISTRY (Copyright (C) 2009 ACS)

Answer 729:



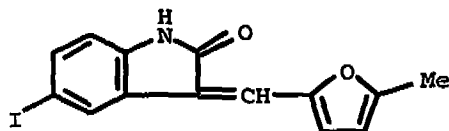
REGISTRY (Copyright (C) 2009 ACS)

Answer 730:



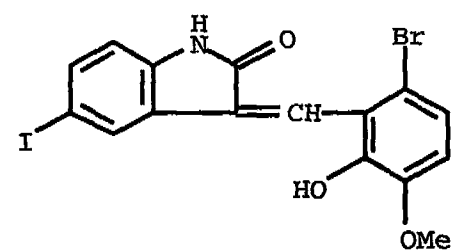
REGISTRY (Copyright (C) 2009 ACS)

Answer 731:



REGISTRY (Copyright (C) 2009 ACS)

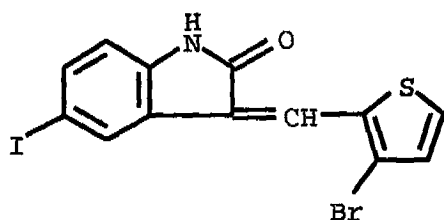
Answer 732:



REGISTRY (Copyright (C) 2009 ACS)

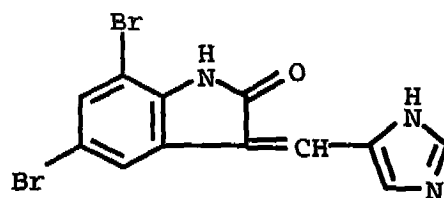
1473
1206

Answer 733:



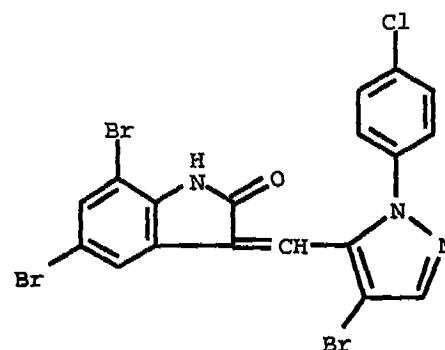
REGISTRY (Copyright (C) 2009 ACS)

Answer 734:



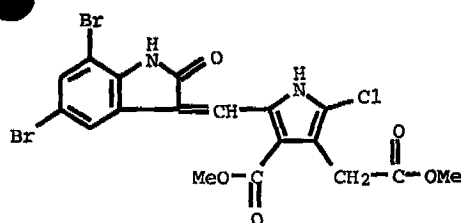
REGISTRY (Copyright (C) 2009 ACS)

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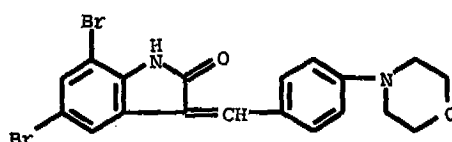
REGISTRY (Copyright (C) 2009 ACS)

Answer 736:



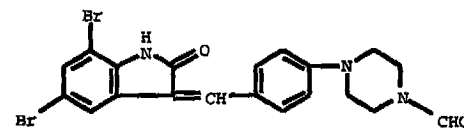
REGISTRY (Copyright (C) 2009 ACS)

Answer 737:



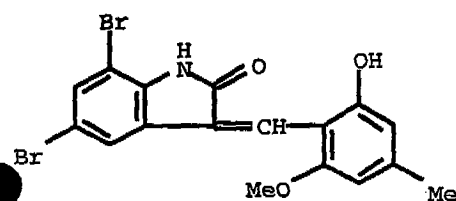
REGISTRY (Copyright (C) 2009 ACS)

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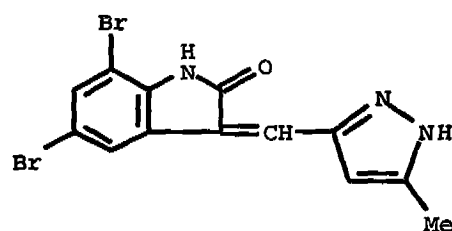
REGISTRY (Copyright (C) 2009 ACS)

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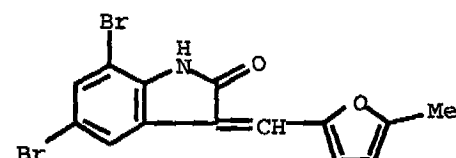
REGISTRY (Copyright (C) 2009 ACS)

Answer 740:



REGISTRY (Copyright (C) 2009 ACS)

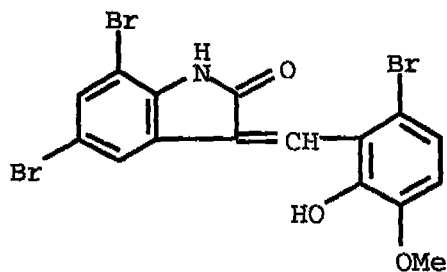
Answer 741:



REGISTRY (Copyright (C) 2009 ACS)

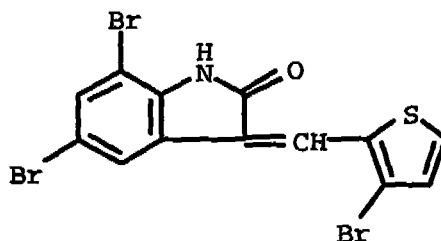
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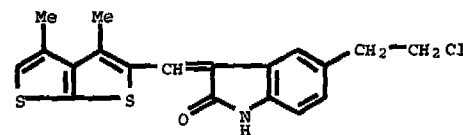
REGISTRY (Copyright (C) 2009 ACS)

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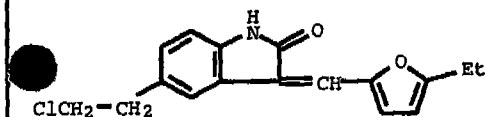
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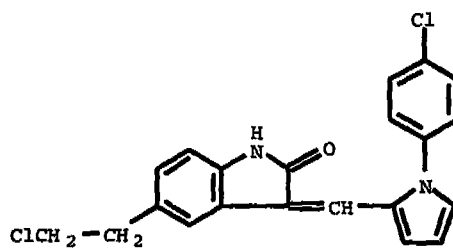
REGISTRY (Copyright (C) 2009 ACS)

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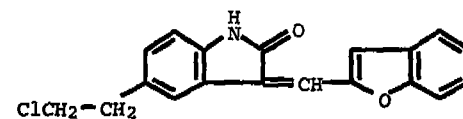
REGISTRY (Copyright (C) 2009 ACS)

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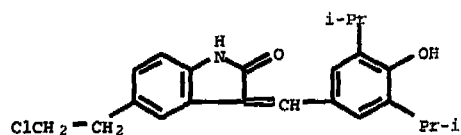
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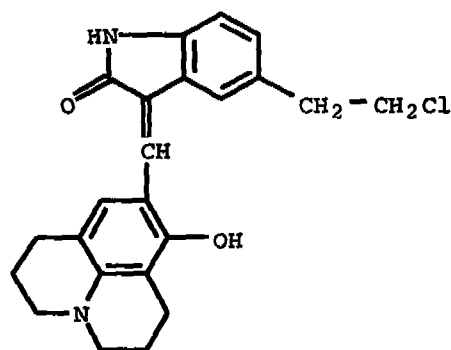
REGISTRY (Copyright (C) 2009 ACS)

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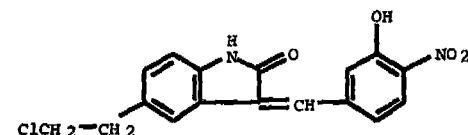
REGISTRY (Copyright (C) 2009 ACS)

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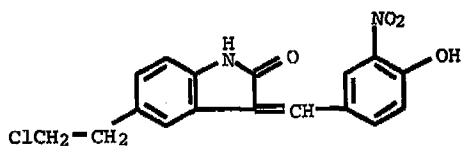
REGISTRY (Copyright (C) 2009 ACS)

Answer 750:



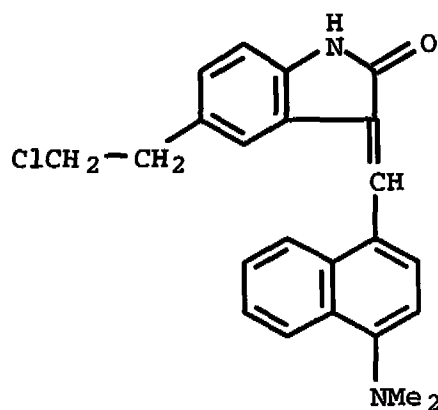
REGISTRY (Copyright (C) 2009 ACS)

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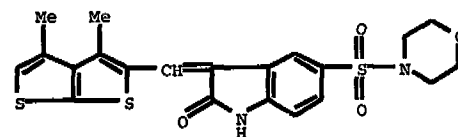
REGISTRY (Copyright (C) 2009 ACS)

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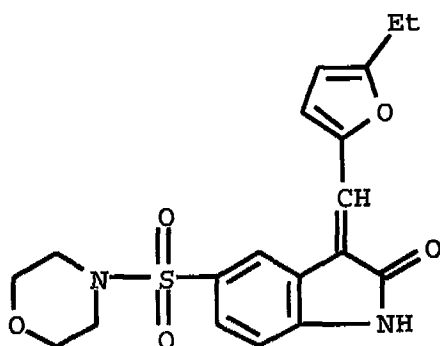
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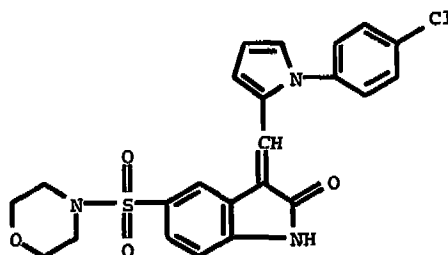
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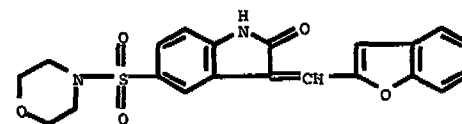
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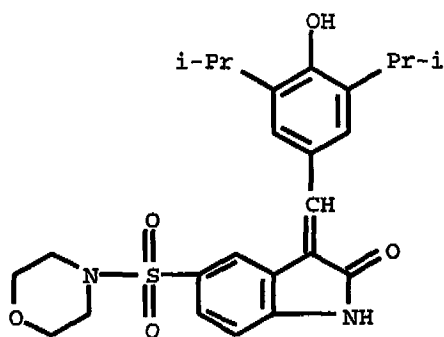
REGISTRY (Copyright (C) 2009 ACS)

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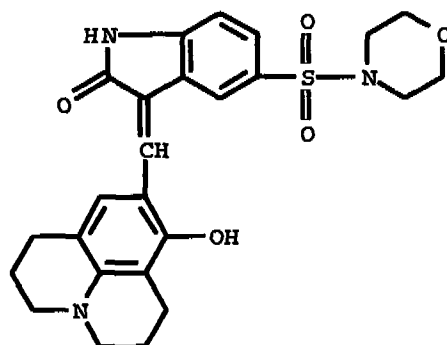
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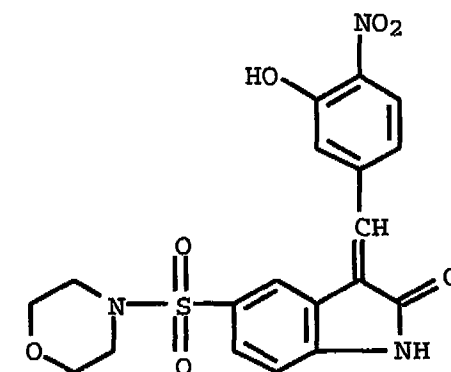
REGISTRY (Copyright (C) 2009 ACS)

Answer 758:



REGISTRY (Copyright (C) 2009 ACS)

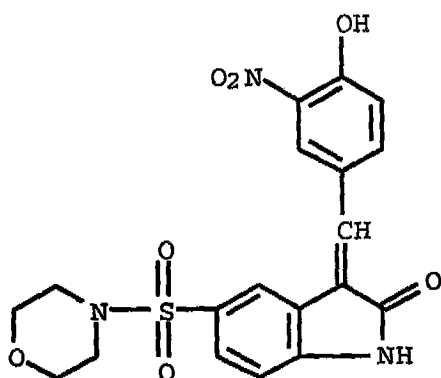
Answer 759:



REGISTRY (Copyright (C) 2009 ACS)

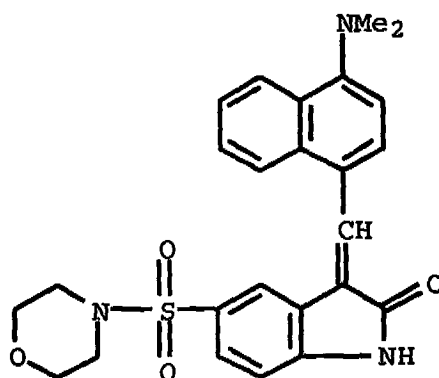
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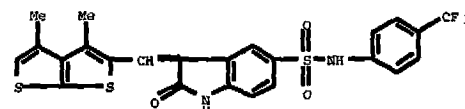
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Answer 761:



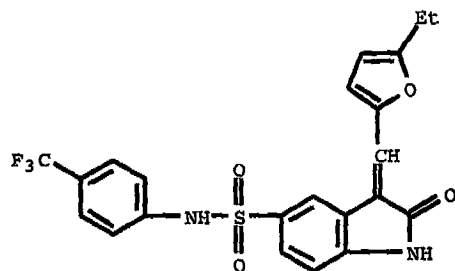
REGISTRY (Copyright (C) 2009 ACS)

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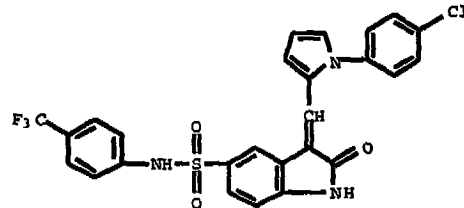
REGISTRY (Copyright (C) 2009 ACS)

Answer 763:



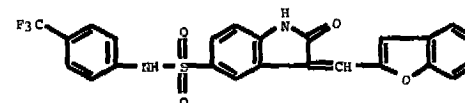
REGISTRY (Copyright (C) 2009 ACS)

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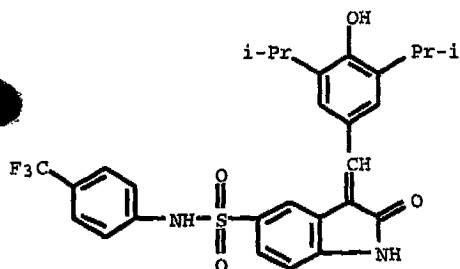
REGISTRY (Copyright (C) 2009 ACS)

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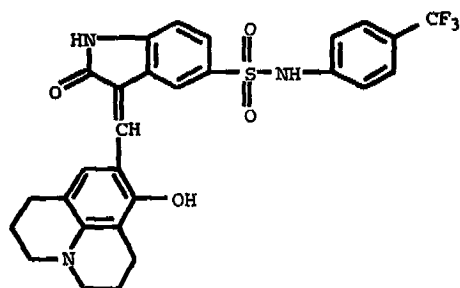
REGISTRY (Copyright (C) 2009 ACS)

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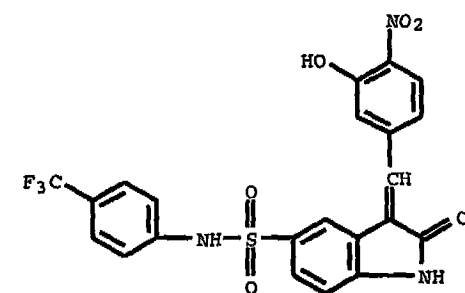
REGISTRY (Copyright (C) 2009 ACS)

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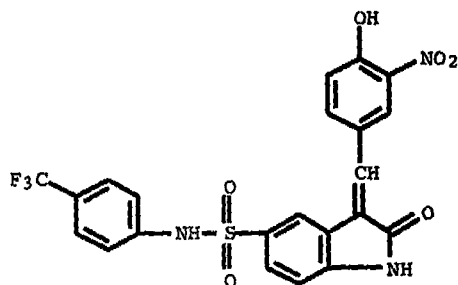
REGISTRY (Copyright (C) 2009 ACS)

Answer 768:



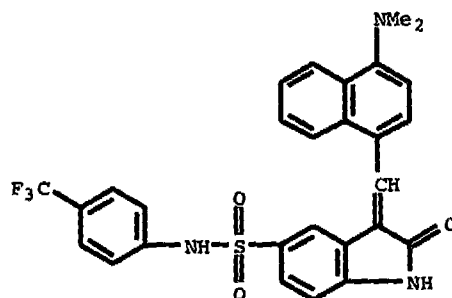
REGISTRY (Copyright (C) 2009 ACS)

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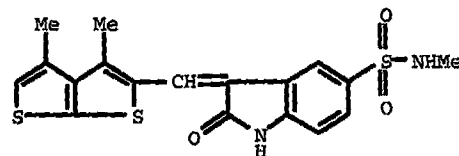
REGISTRY (Copyright (C) 2009 ACS)

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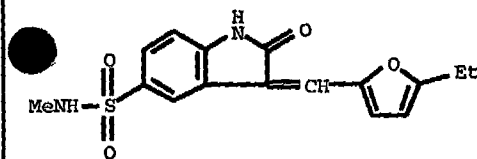
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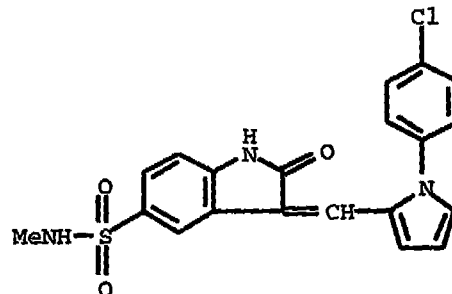
REGISTRY (Copyright (C) 2009 ACS)

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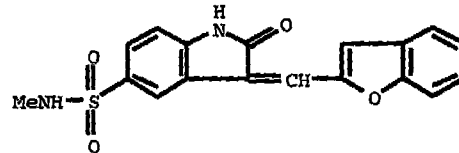
REGISTRY (Copyright (C) 2009 ACS)

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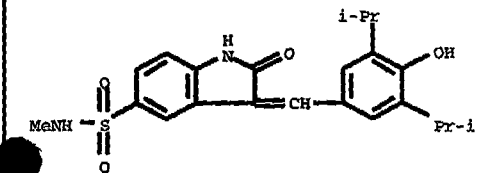
REGISTRY (Copyright (C) 2009 ACS)

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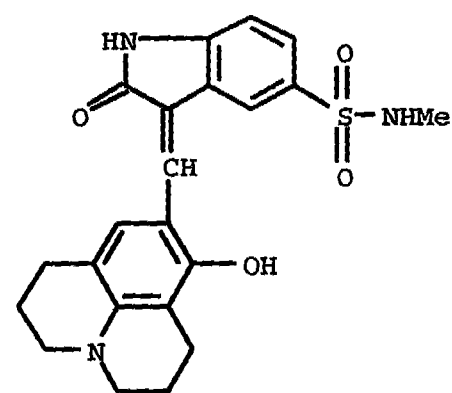
REGISTRY (Copyright (C) 2009 ACS)

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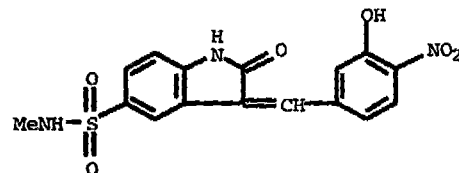
REGISTRY (Copyright (C) 2009 ACS)

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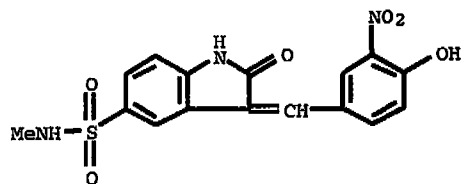
REGISTRY (Copyright (C) 2009 ACS)

Answer 777:



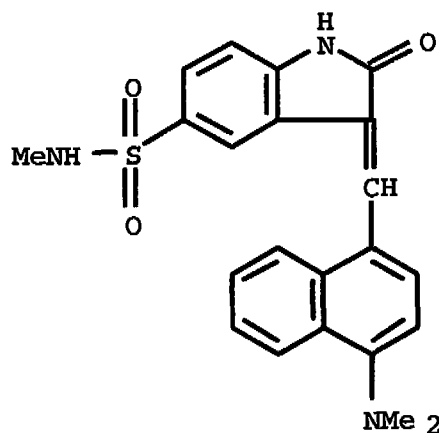
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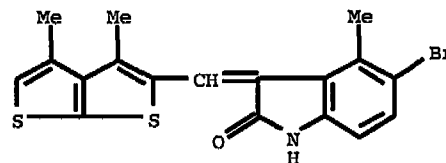
REGISTRY (Copyright (C) 2009 ACS)

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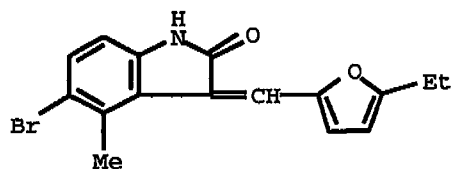
REGISTRY (Copyright (C) 2009 ACS)

Answer 780:



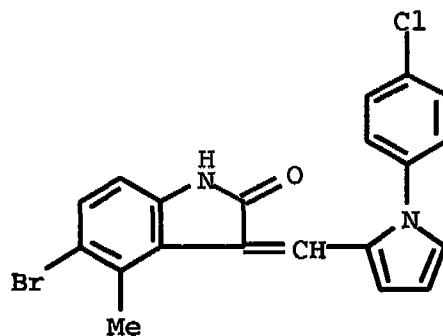
REGISTRY (Copyright (C) 2009 ACS)

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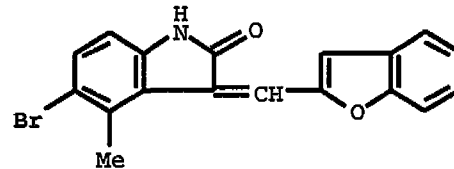
REGISTRY (Copyright (C) 2009 ACS)

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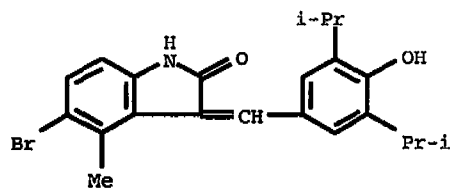
REGISTRY (Copyright (C) 2009 ACS)

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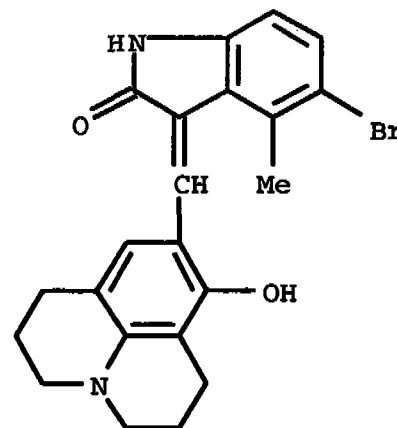
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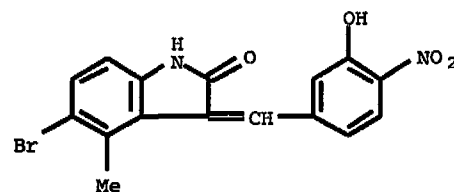


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Answer 785:



Answer 786:

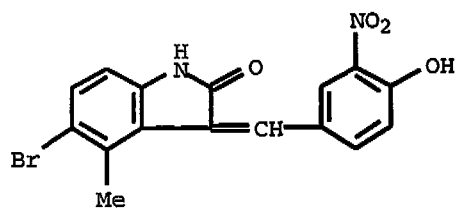


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1212

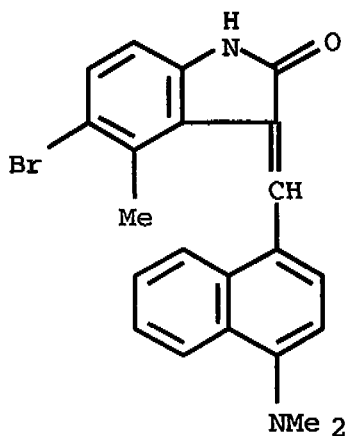
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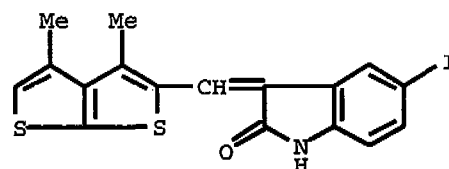
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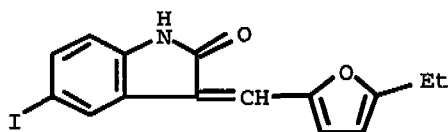
REGISTRY (Copyright (C) 2009 ACS)

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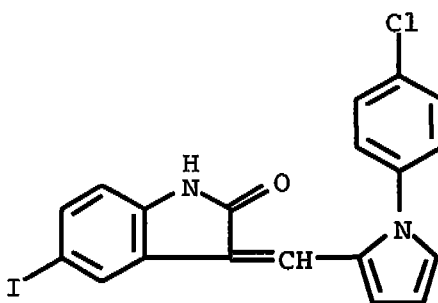
REGISTRY (Copyright (C) 2009 ACS)

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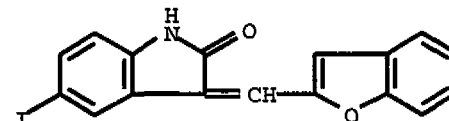
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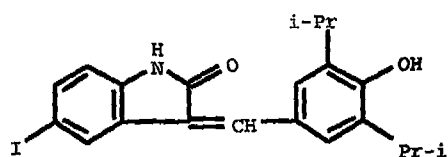
REGISTRY (Copyright (C) 2009 ACS)

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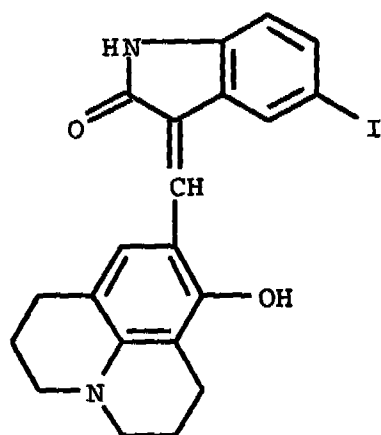
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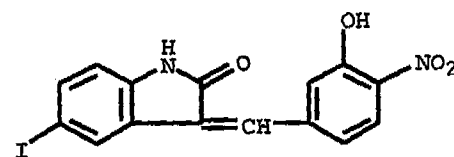
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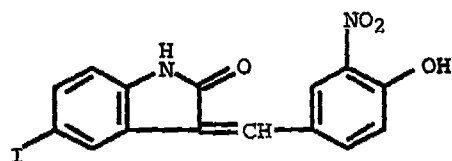
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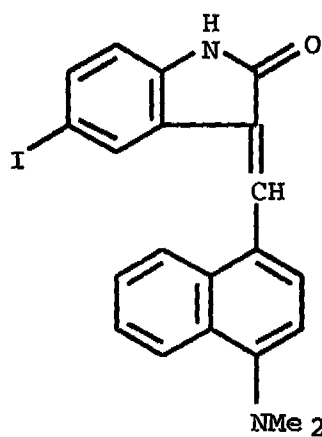
REGISTRY (Copyright (C) 2009 ACS)

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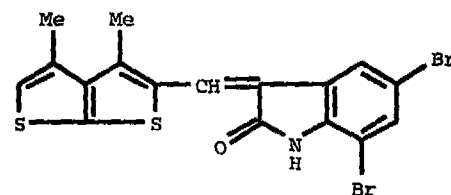
REGISTRY (Copyright (C) 2009 ACS)

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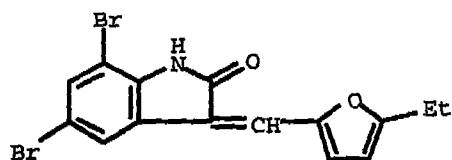
REGISTRY (Copyright (C) 2009 ACS)

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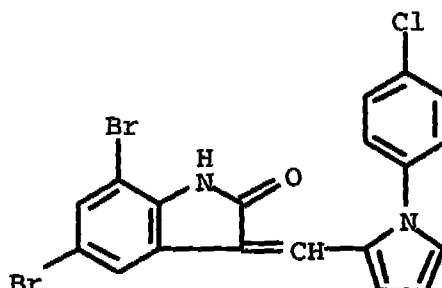
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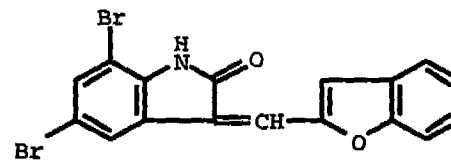


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Answer 800:



Answer 801:

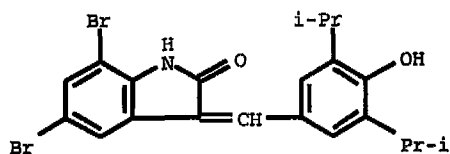


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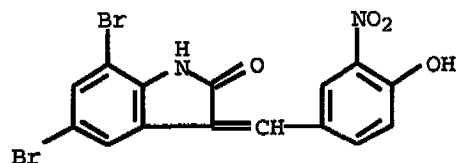
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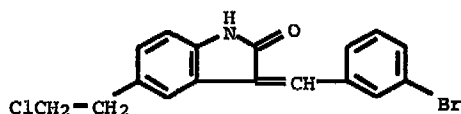
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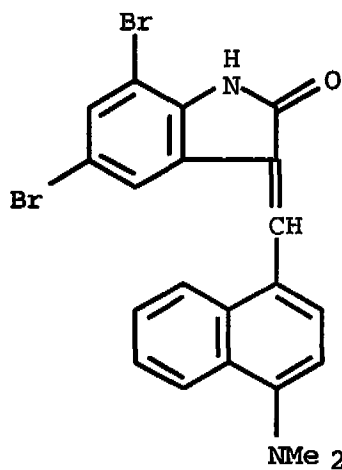
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Answer 808:



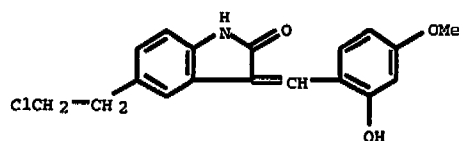
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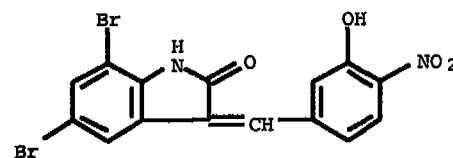


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Answer 809:

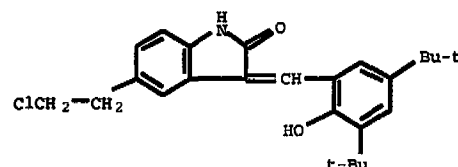


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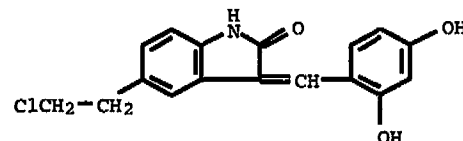
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Answer 807:



REGISTRY (Copyright (C) 2009 ACS)

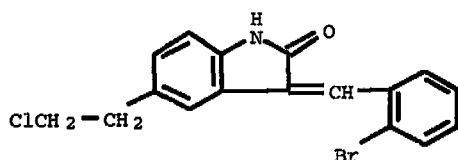
Answer 810:



4182
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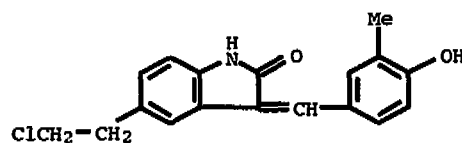
Answer 811:



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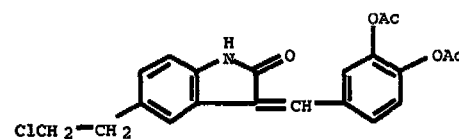
Answer 812:



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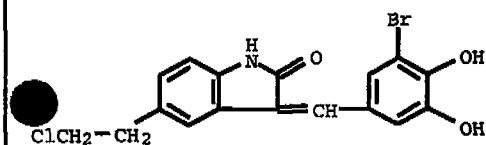
REGISTRY (Copyright (C) 2009 ACS)

Answer 813:



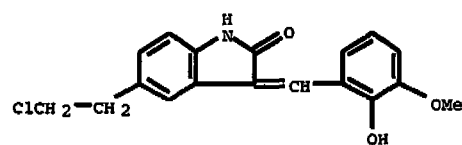
REGISTRY (Copyright (C) 2009 ACS)

Answer 814:



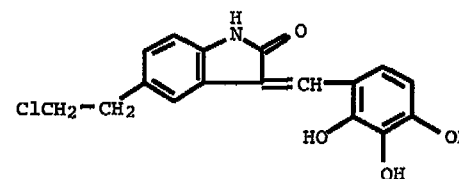
REGISTRY (Copyright (C) 2009 ACS)

Answer 815:



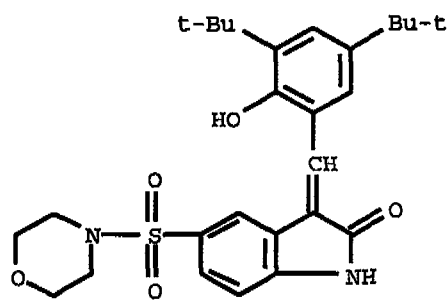
REGISTRY (Copyright (C) 2009 ACS)

Answer 816:



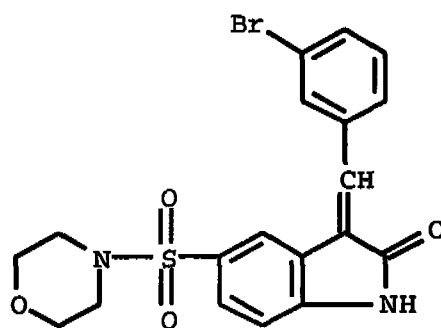
REGISTRY (Copyright (C) 2009 ACS)

Answer 817:



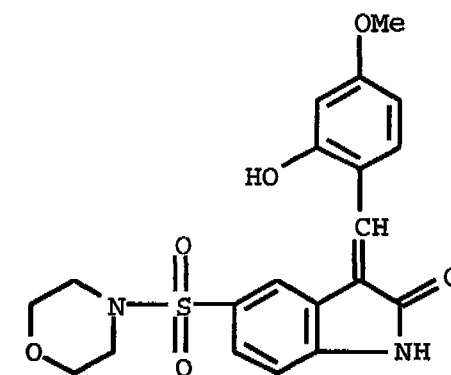
REGISTRY (Copyright (C) 2009 ACS)

Answer 818:



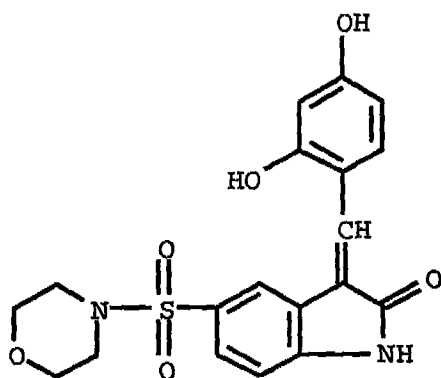
REGISTRY (Copyright (C) 2009 ACS)

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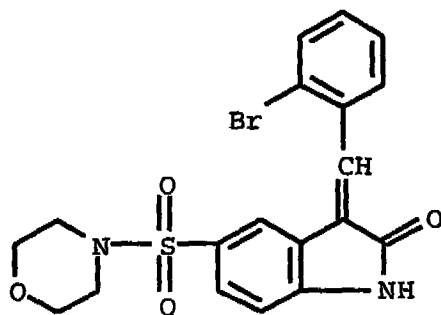
REGISTRY (Copyright (C) 2009 ACS)

Answer 820:



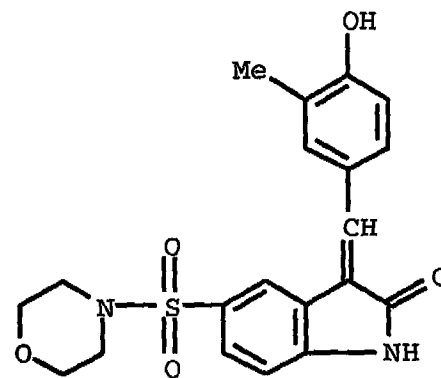
REGISTRY (Copyright (C) 2009 ACS)

Answer 821:



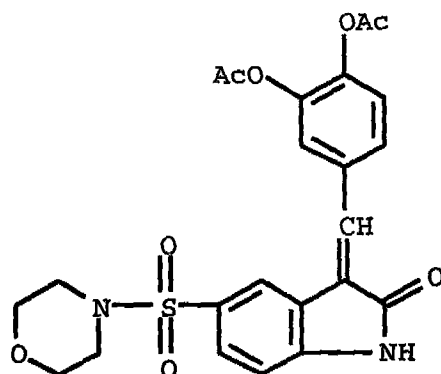
REGISTRY (Copyright (C) 2009 ACS)

Answer 822:



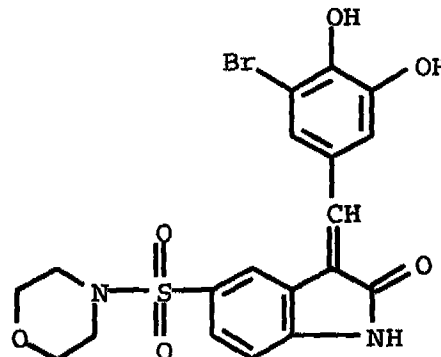
REGISTRY (Copyright (C) 2009 ACS)

Answer 823:



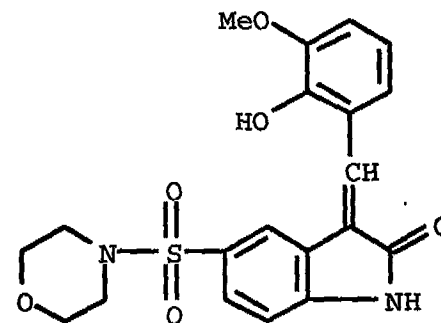
REGISTRY (Copyright (C) 2009 ACS)

Answer 824:



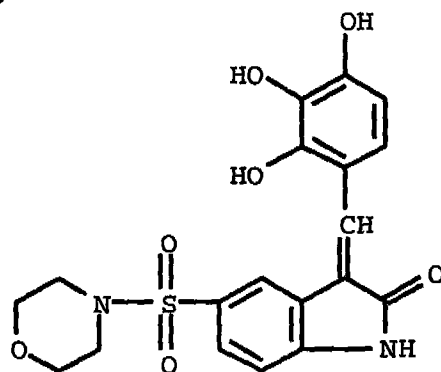
REGISTRY (Copyright (C) 2009 ACS)

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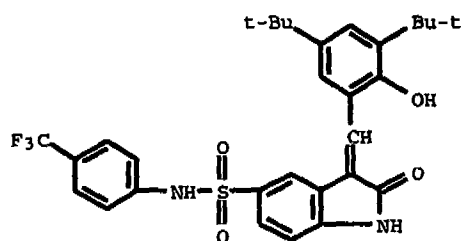
REGISTRY (Copyright (C) 2009 ACS)

Answer 826:



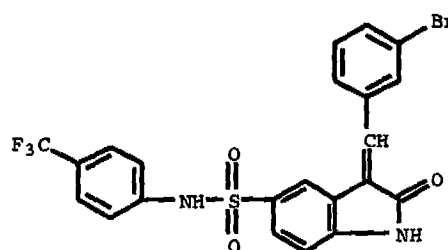
REGISTRY (Copyright (C) 2009 ACS)

Answer 827:



REGISTRY (Copyright (C) 2009 ACS)

Answer 828:

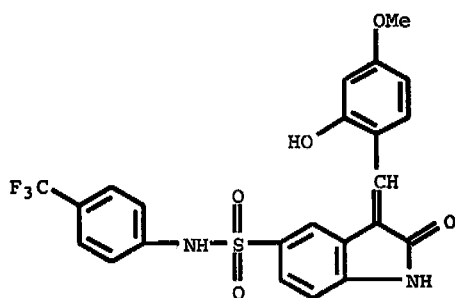


REGISTRY (Copyright (C) 2009 ACS)

1184

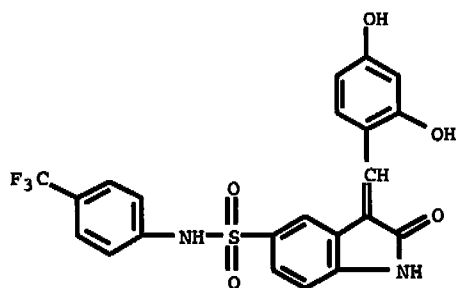
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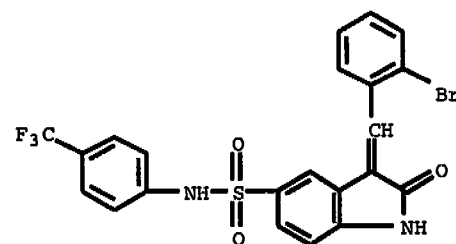
REGISTRY (Copyright (C) 2009 ACS)

Answer 830:



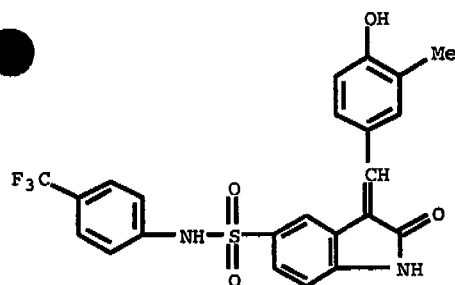
REGISTRY (Copyright (C) 2009 ACS)

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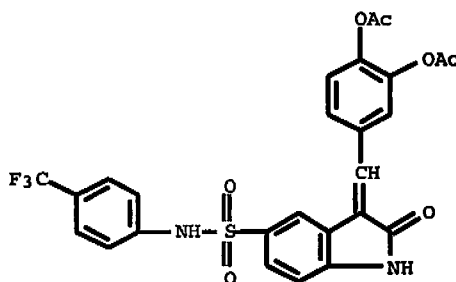
REGISTRY (Copyright (C) 2009 ACS)

Answer 832:



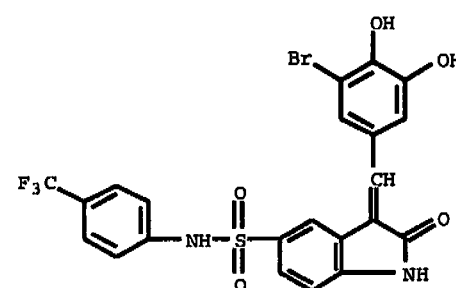
REGISTRY (Copyright (C) 2009 ACS)

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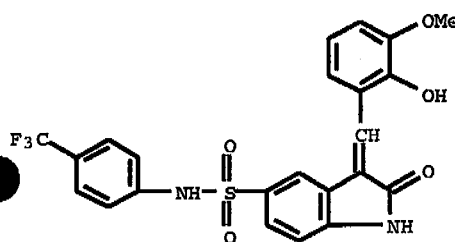
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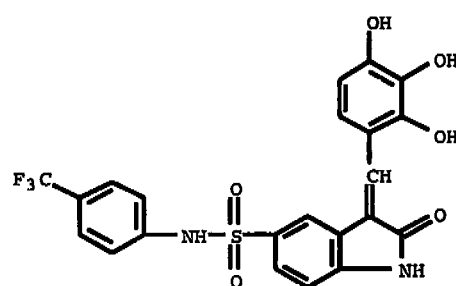
REGISTRY (Copyright (C) 2009 ACS)

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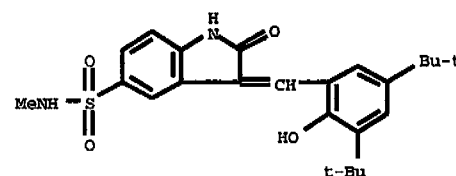
REGISTRY (Copyright (C) 2009 ACS)

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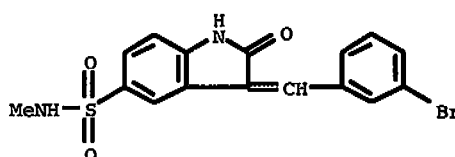
REGISTRY (Copyright (C) 2009 ACS)

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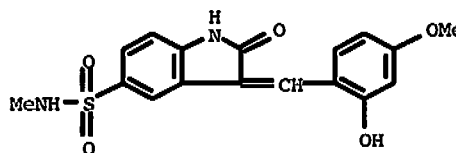


REGISTRY (Copyright (C) 2009 ACS)

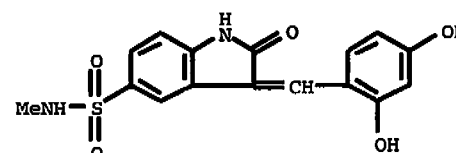
Answer 838:



Answer 839:

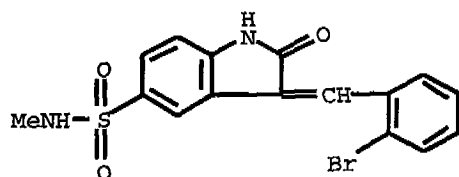


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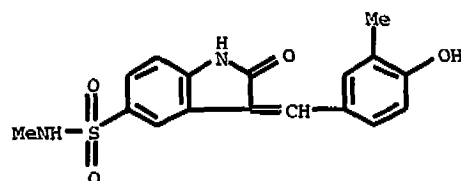
REGISTRY (Copyright (C) 2009 ACS)

Answer 841:



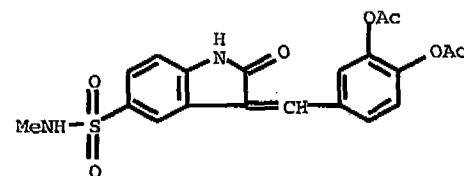
REGISTRY (Copyright (C) 2009 ACS)

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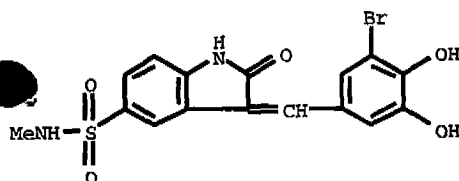
REGISTRY (Copyright (C) 2009 ACS)

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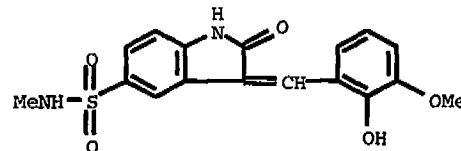
REGISTRY (Copyright (C) 2009 ACS)

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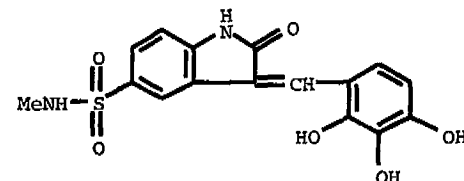
REGISTRY (Copyright (C) 2009 ACS)

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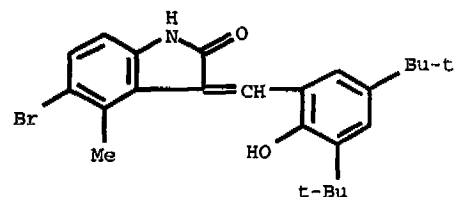
REGISTRY (Copyright (C) 2009 ACS)

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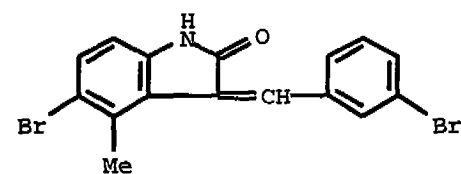
REGISTRY (Copyright (C) 2009 ACS)

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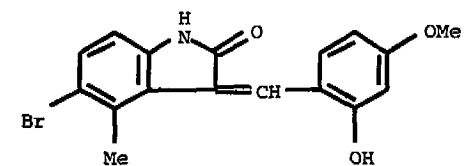
REGISTRY (Copyright (C) 2009 ACS)

Answer 848:



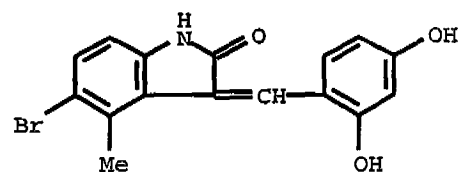
REGISTRY (Copyright (C) 2009 ACS)

Answer 849:



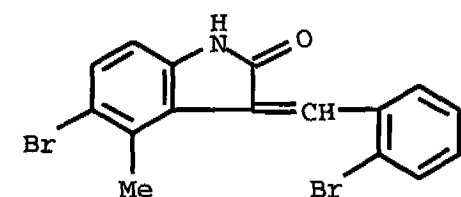
REGISTRY (Copyright (C) 2009 ACS)

Answer 850:



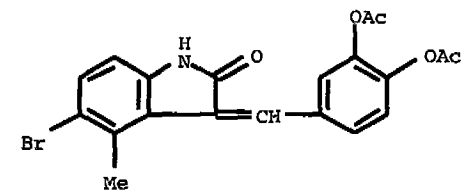
REGISTRY (Copyright (C) 2009 ACS)

Answer 851:



REGISTRY (Copyright (C) 2009 ACS)

Answer 852:



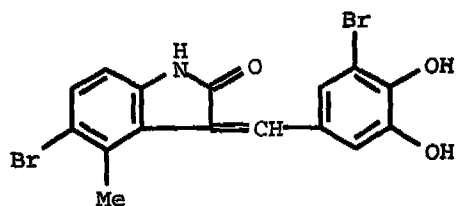
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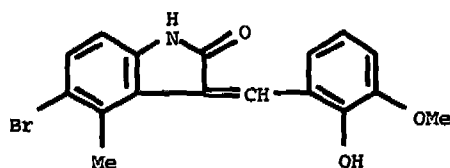
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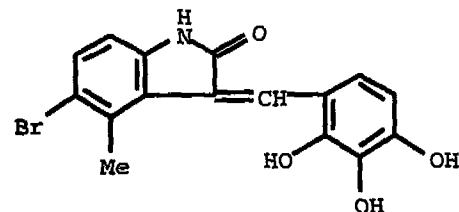
REGISTRY (Copyright (C) 2009 ACS)

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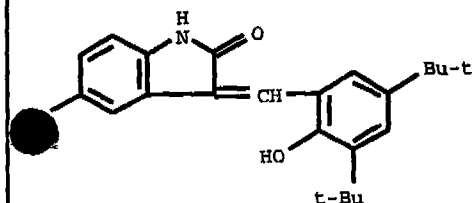
REGISTRY (Copyright (C) 2009 ACS)

Answer 855:



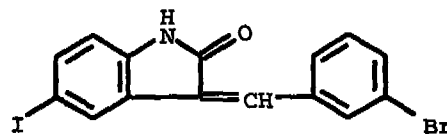
REGISTRY (Copyright (C) 2009 ACS)

Answer 856:



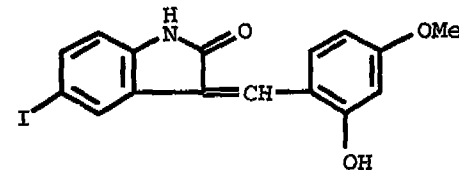
REGISTRY (Copyright (C) 2009 ACS)

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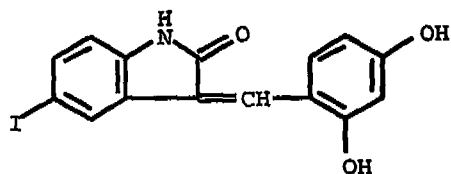
REGISTRY (Copyright (C) 2009 ACS)

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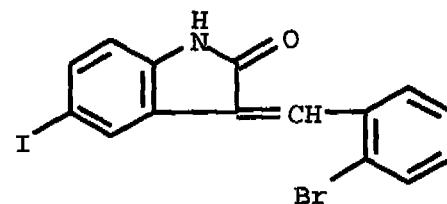
REGISTRY (Copyright (C) 2009 ACS)

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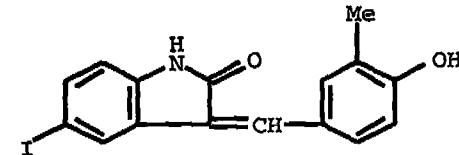
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Answer 860:



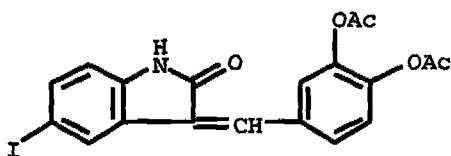
REGISTRY (Copyright (C) 2009 ACS)

Answer 861:



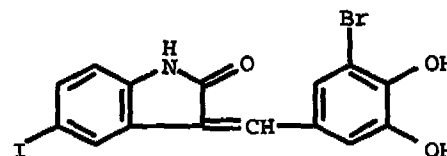
REGISTRY (Copyright (C) 2009 ACS)

Answer 862:



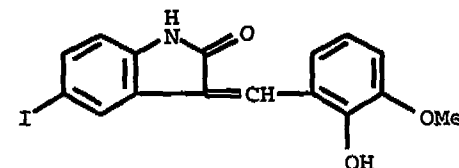
REGISTRY (Copyright (C) 2009 ACS)

Answer 863:



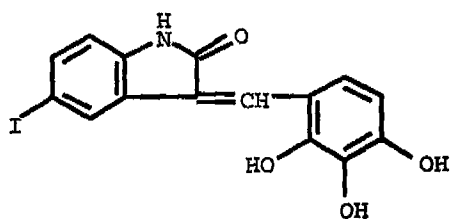
REGISTRY (Copyright (C) 2009 ACS)

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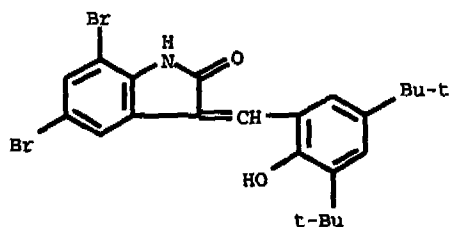
REGISTRY (Copyright (C) 2009 ACS)

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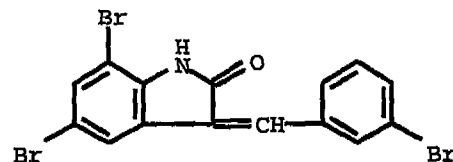
REGISTRY (Copyright (C) 2009 ACS)

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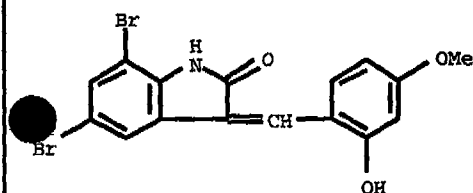
REGISTRY (Copyright (C) 2009 ACS)

Answer 867:



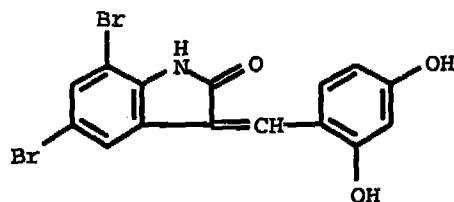
REGISTRY (Copyright (C) 2009 ACS)

Answer 868:



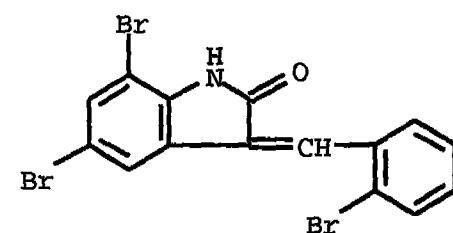
REGISTRY (Copyright (C) 2009 ACS)

Answer 869:



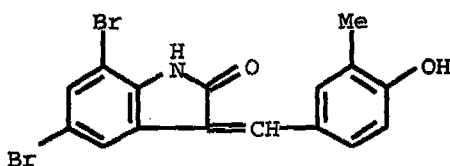
REGISTRY (Copyright (C) 2009 ACS)

Answer 870:



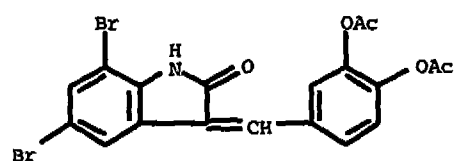
REGISTRY (Copyright (C) 2009 ACS)

Answer 871:



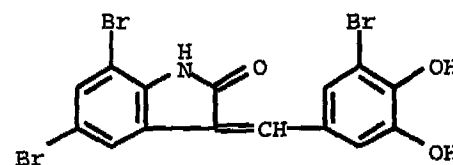
REGISTRY (Copyright (C) 2009 ACS)

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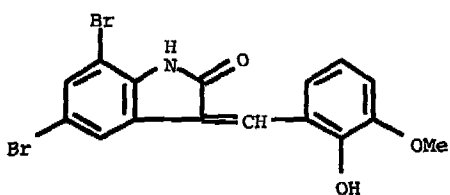
REGISTRY (Copyright (C) 2009 ACS)

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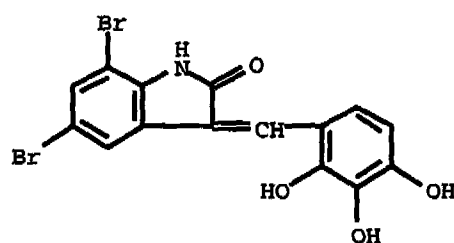
REGISTRY (Copyright (C) 2009 ACS)

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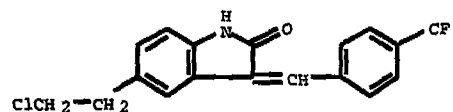
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Answer 875:



REGISTRY (Copyright (C) 2009 ACS)

Answer 876:

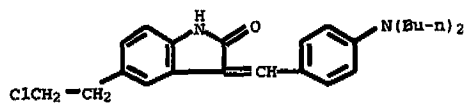


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1188
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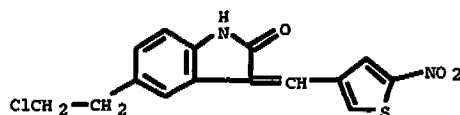
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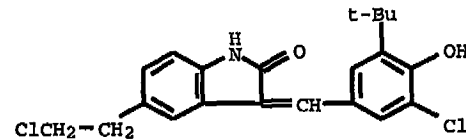
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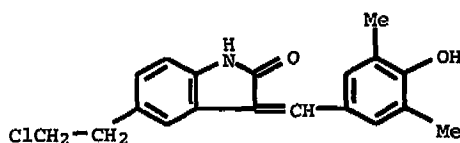
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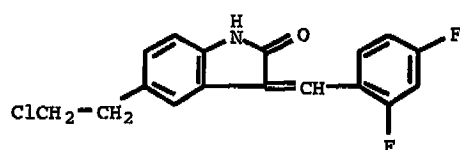
REGISTRY (Copyright (C) 2009 ACS)

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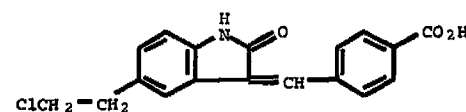
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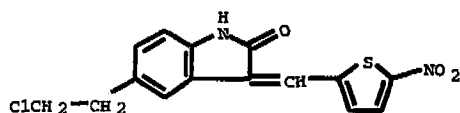
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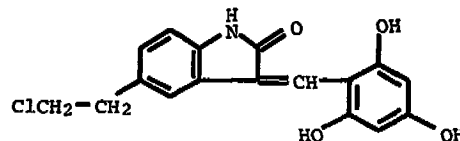
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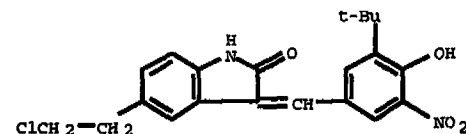
REGISTRY (Copyright (C) 2009 ACS)

Answer 884:



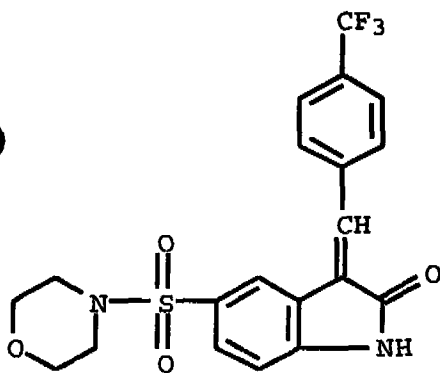
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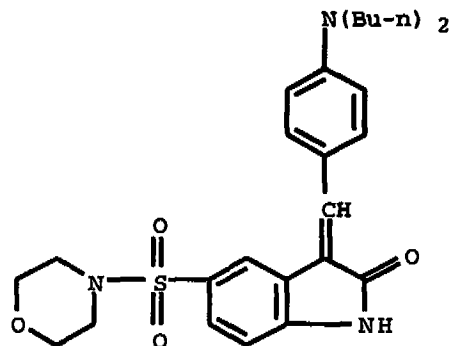
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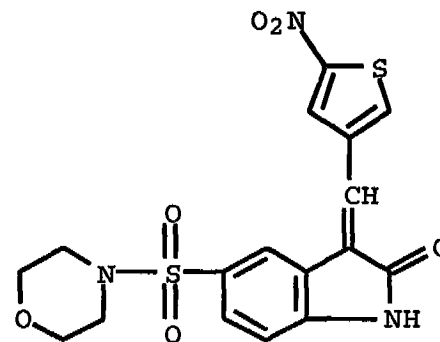
REGISTRY (Copyright (C) 2009 ACS)

Answer 887:



REGISTRY (Copyright (C) 2009 ACS)

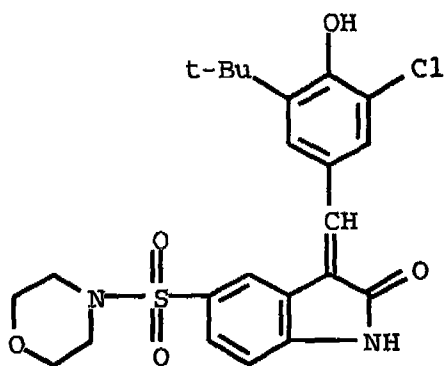
Answer 888:



REGISTRY (Copyright (C) 2009 ACS)

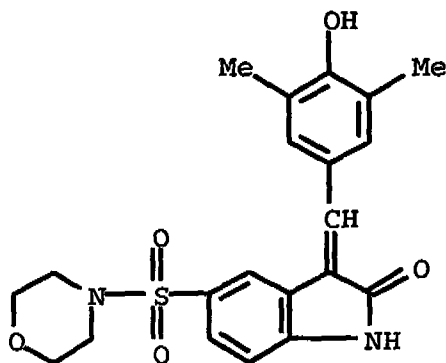
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Answer 889:



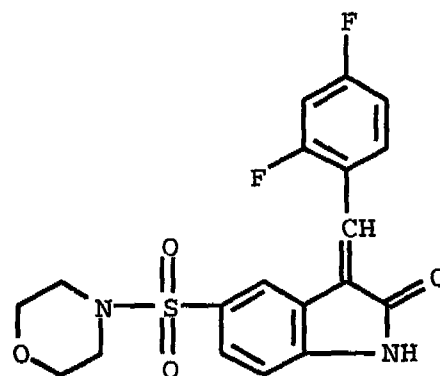
REGISTRY (Copyright (C) 2009 ACS)

Answer 890:



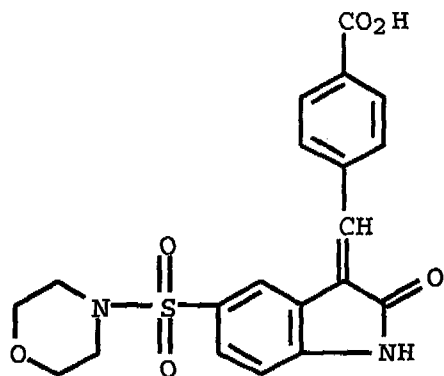
REGISTRY (Copyright (C) 2009 ACS)

Answer 891:



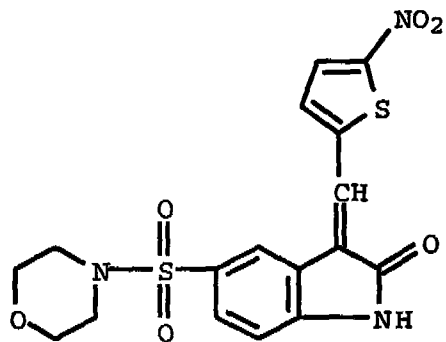
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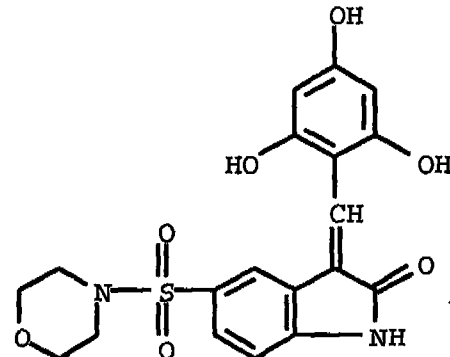
REGISTRY (Copyright (C) 2009 ACS)

Answer 893:



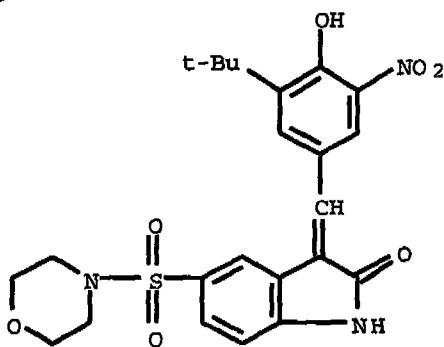
REGISTRY (Copyright (C) 2009 ACS)

Answer 894:



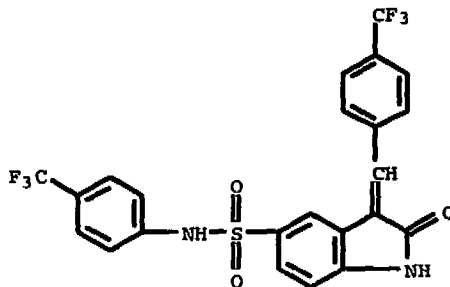
REGISTRY (Copyright (C) 2009 ACS)

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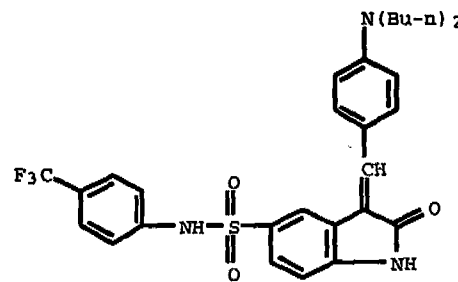
REGISTRY (Copyright (C) 2009 ACS)

Answer 896:



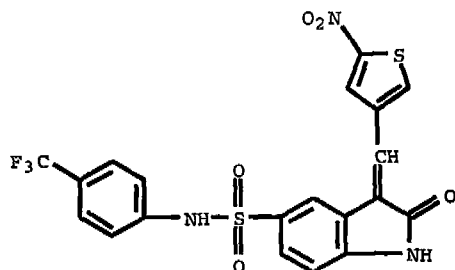
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Answer 897:



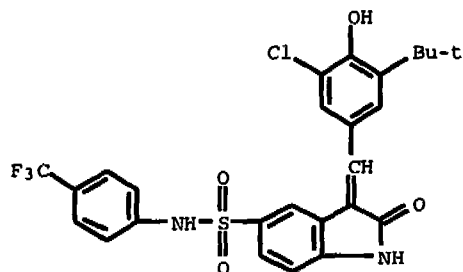
REGISTRY (Copyright (C) 2009 ACS)

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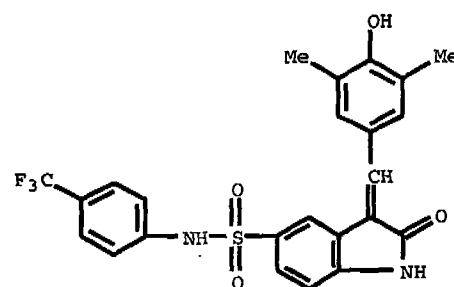
REGISTRY (Copyright (C) 2009 ACS)

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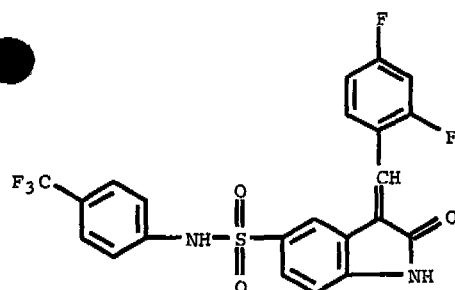
REGISTRY (Copyright (C) 2009 ACS)

Answer 900:



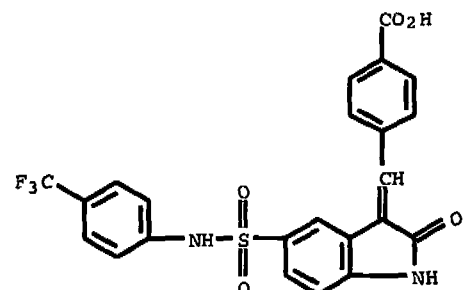
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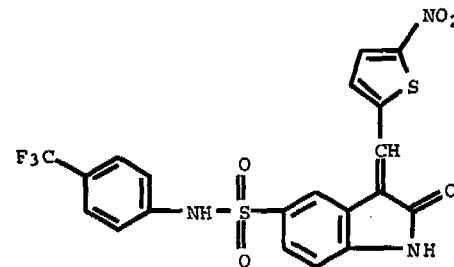
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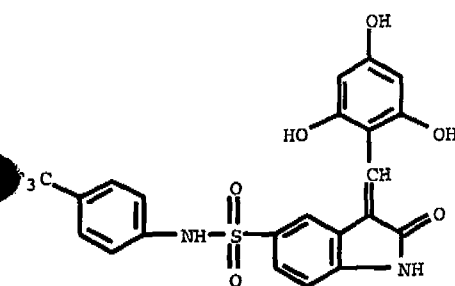
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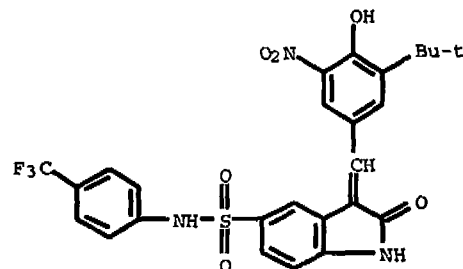
REGISTRY (Copyright (C) 2009 ACS)

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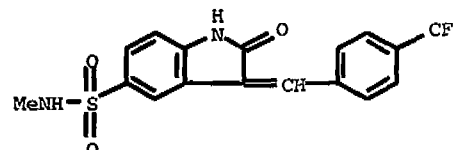
REGISTRY (Copyright (C) 2009 ACS)

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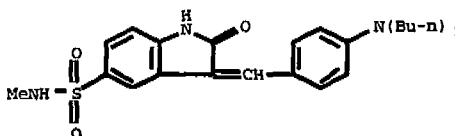
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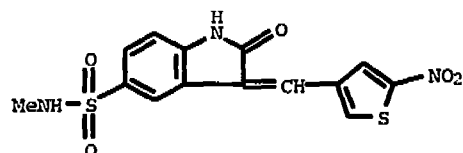
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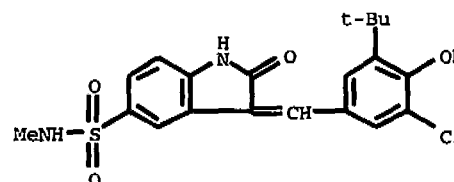


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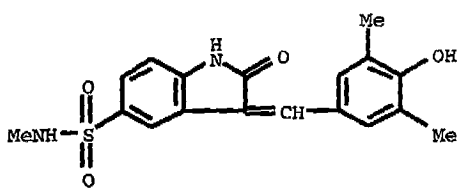
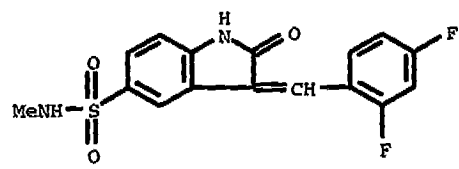
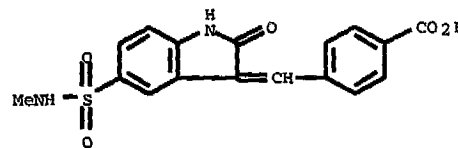
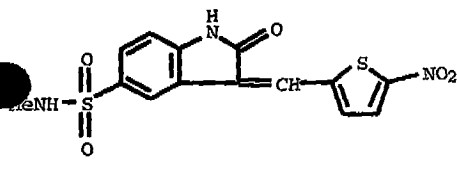
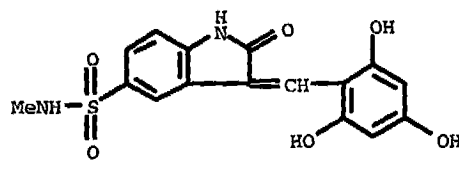
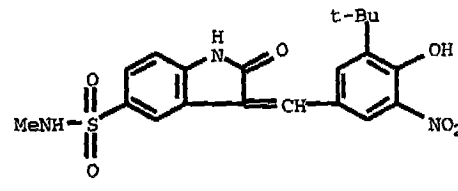
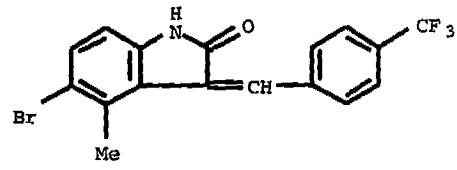
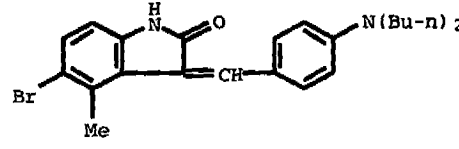
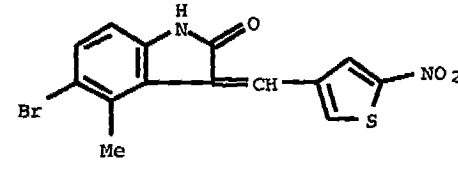
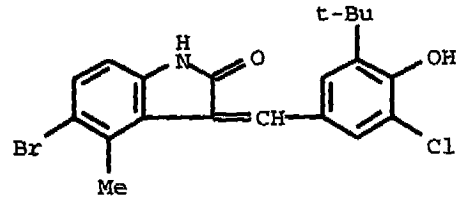
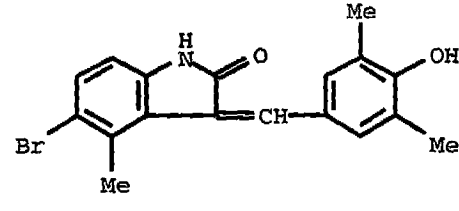
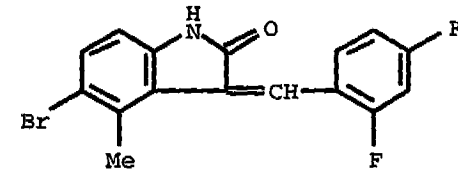
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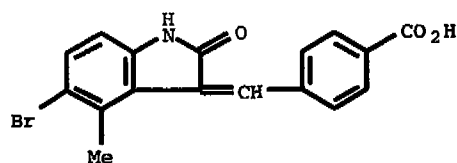
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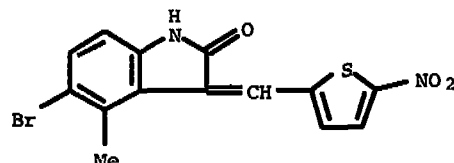
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REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 913: 	Answer 914: 	Answer 915: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 916: 	Answer 917: 	Answer 918: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 919: 	Answer 920: 	Answer 921: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)

Answer 922:



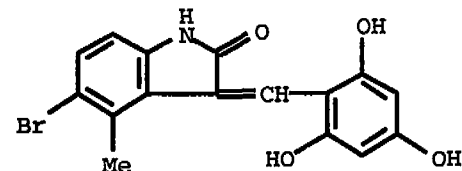
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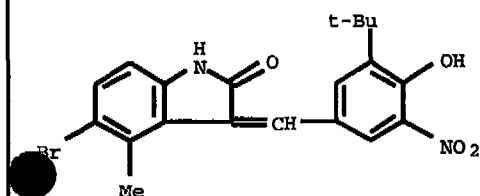
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Answer 924:



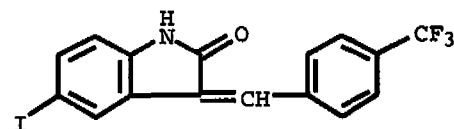
REGISTRY (Copyright (C) 2009 ACS)

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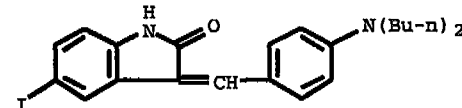
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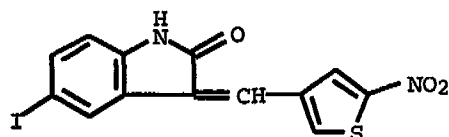
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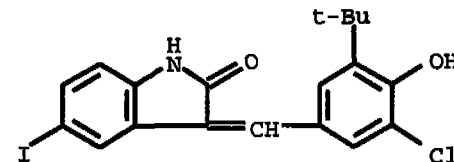
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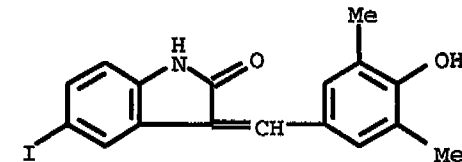
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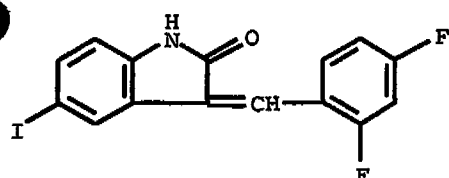
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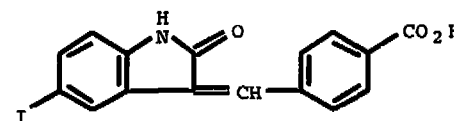
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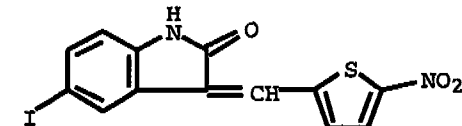
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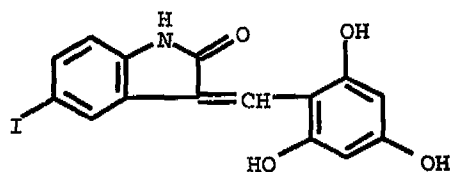
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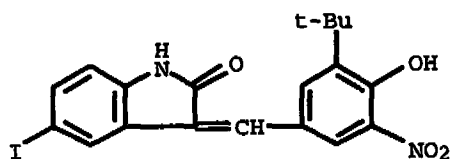
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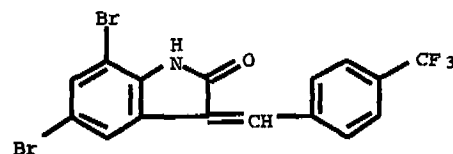
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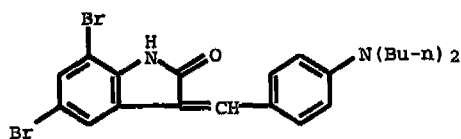
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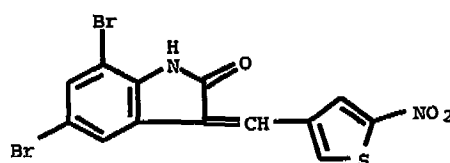
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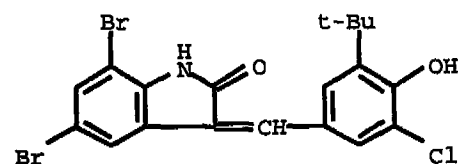
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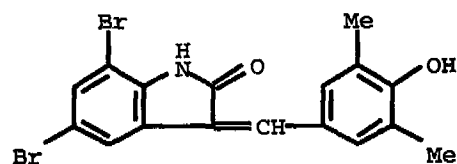
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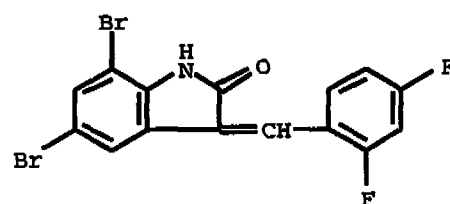
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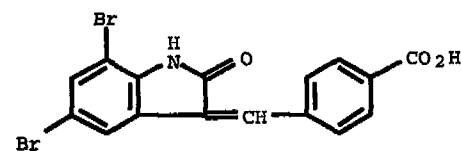
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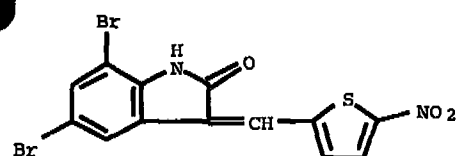
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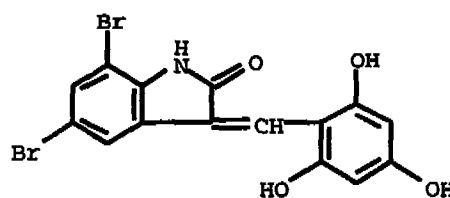
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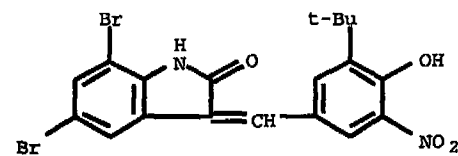
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Answer 944:



REGISTRY (Copyright (C) 2009 ACS)

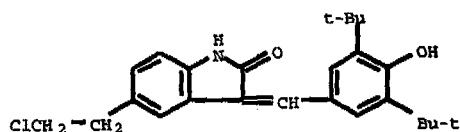
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REGISTRY (Copyright (C) 2009 ACS)

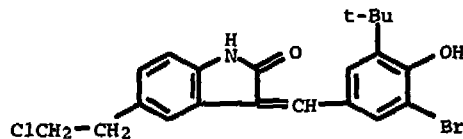
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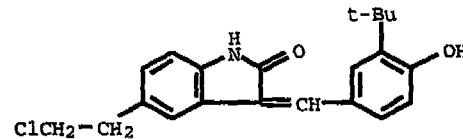
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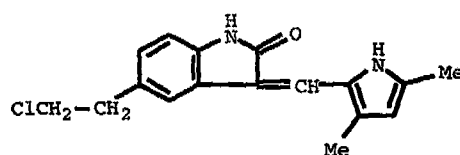
REGISTRY (Copyright (C) 2009 ACS)

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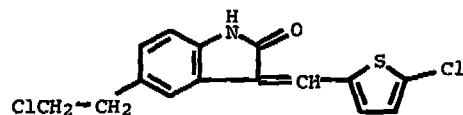
REGISTRY (Copyright (C) 2009 ACS)

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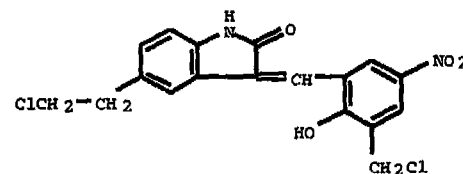
REGISTRY (Copyright (C) 2009 ACS)

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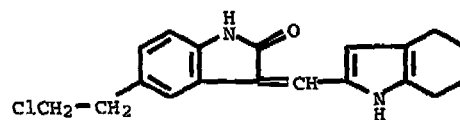
REGISTRY (Copyright (C) 2009 ACS)

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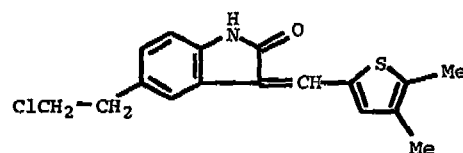
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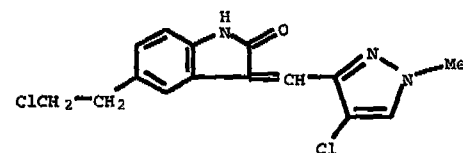
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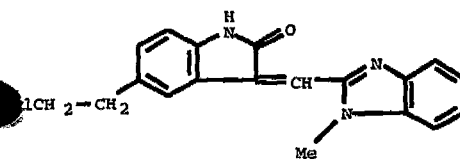
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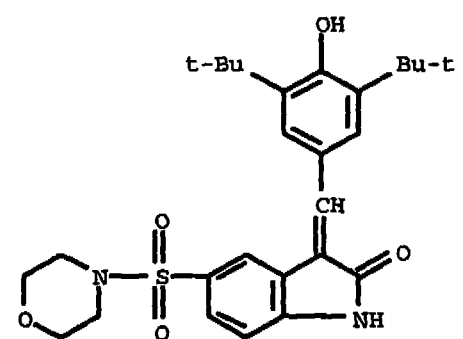
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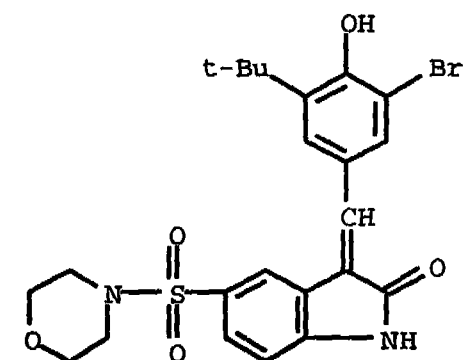
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Answer 956:



REGISTRY (Copyright (C) 2009 ACS)

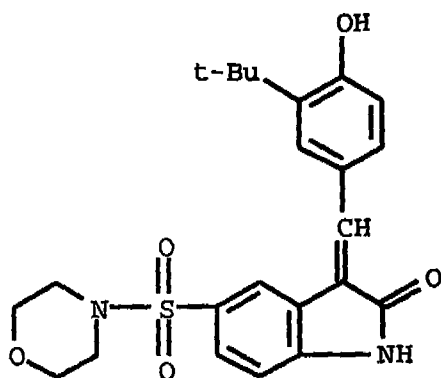
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REGISTRY (Copyright (C) 2009 ACS)

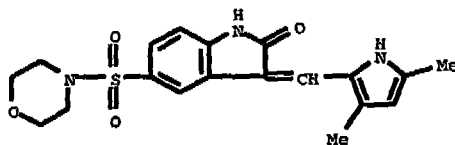
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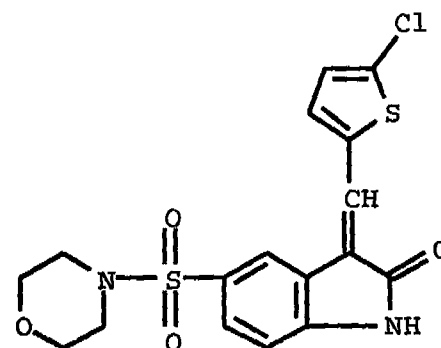
REGISTRY (Copyright (C) 2009 ACS)

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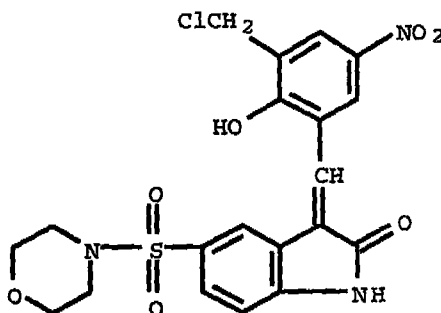
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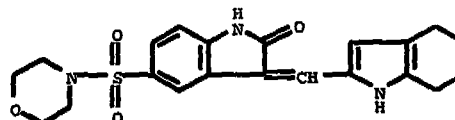
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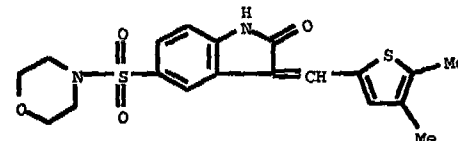
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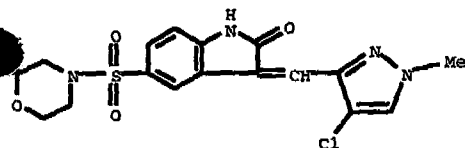
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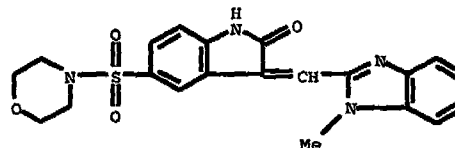
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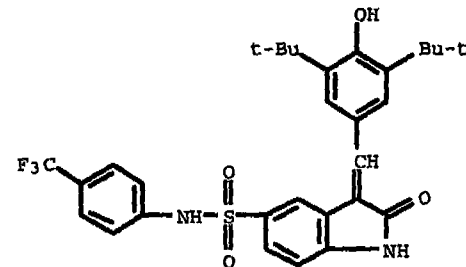
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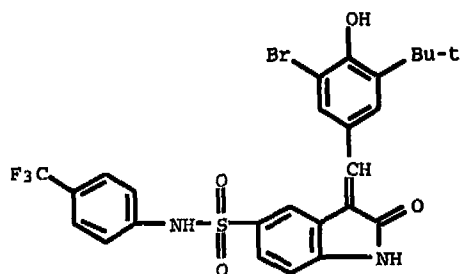
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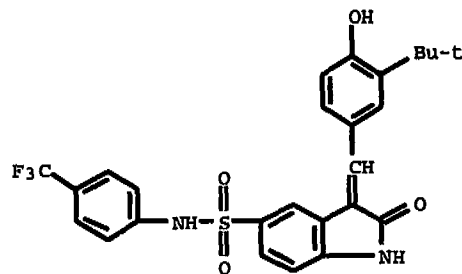
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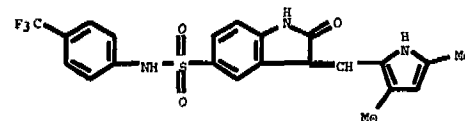
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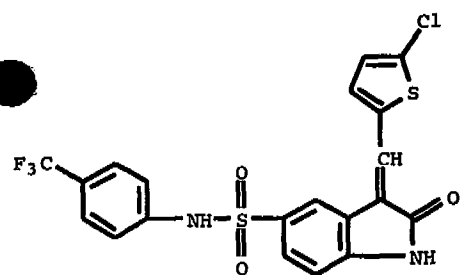
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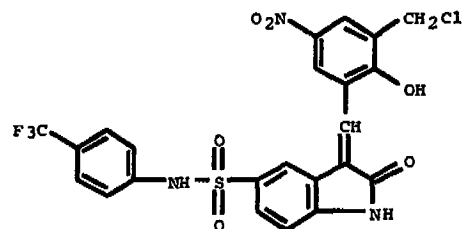
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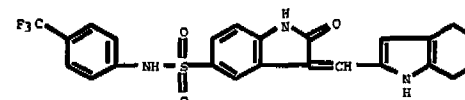
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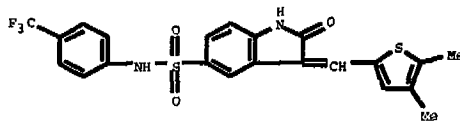
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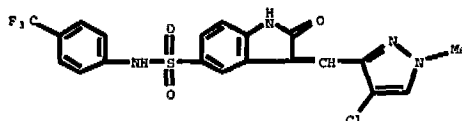
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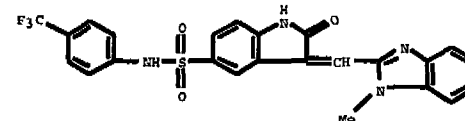
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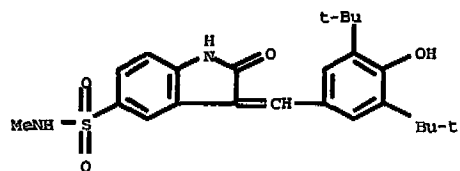
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Answer 975:



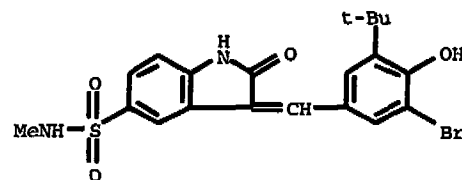
REGISTRY (Copyright (C) 2009 ACS)

Answer 976:



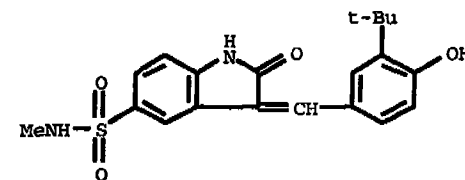
REGISTRY (Copyright (C) 2009 ACS)

Answer 977:



REGISTRY (Copyright (C) 2009 ACS)

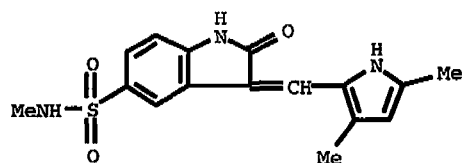
Answer 978:



REGISTRY (Copyright (C) 2009 ACS)

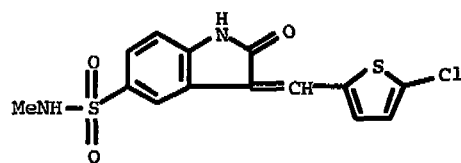
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Answer 979:



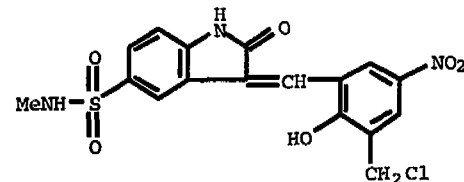
REGISTRY (Copyright (C) 2009 ACS)

Answer 980:



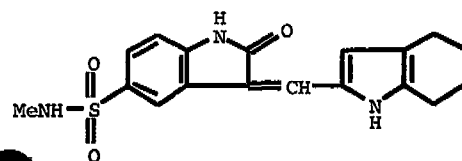
REGISTRY (Copyright (C) 2009 ACS)

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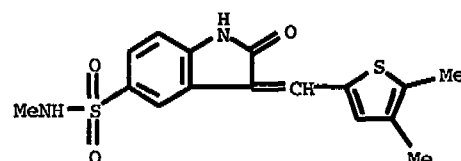
REGISTRY (Copyright (C) 2009 ACS)

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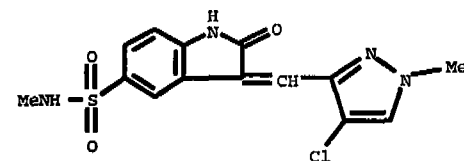
REGISTRY (Copyright (C) 2009 ACS)

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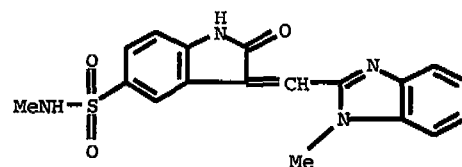
REGISTRY (Copyright (C) 2009 ACS)

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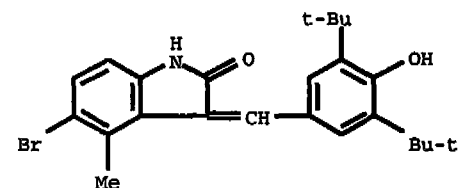
REGISTRY (Copyright (C) 2009 ACS)

Answer 985:



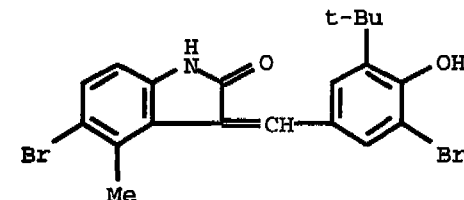
REGISTRY (Copyright (C) 2009 ACS)

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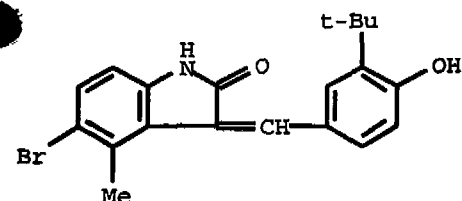
REGISTRY (Copyright (C) 2009 ACS)

Answer 987:



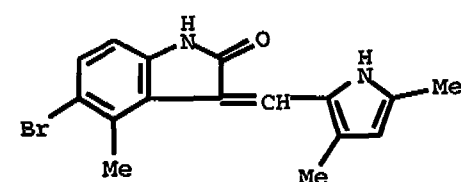
REGISTRY (Copyright (C) 2009 ACS)

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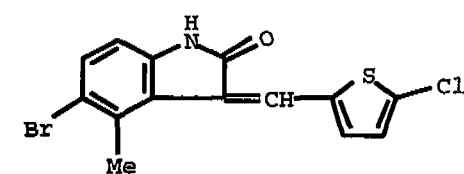
REGISTRY (Copyright (C) 2009 ACS)

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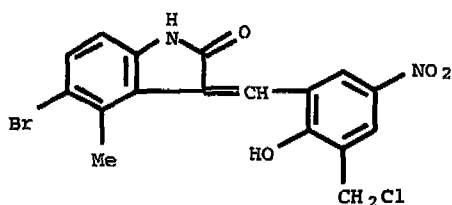
REGISTRY (Copyright (C) 2009 ACS)

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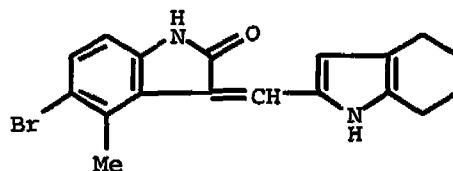
REGISTRY (Copyright (C) 2009 ACS)

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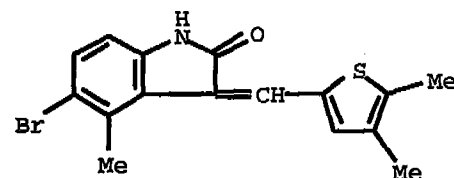
REGISTRY (Copyright (C) 2009 ACS)

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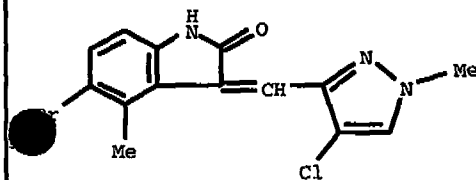
REGISTRY (Copyright (C) 2009 ACS)

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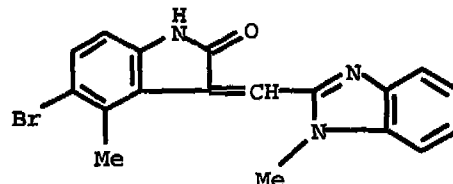
REGISTRY (Copyright (C) 2009 ACS)

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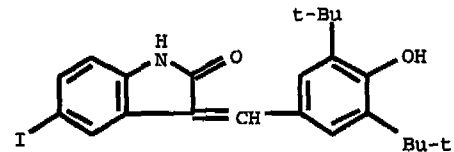
REGISTRY (Copyright (C) 2009 ACS)

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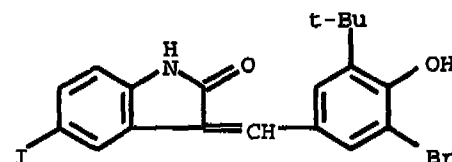
REGISTRY (Copyright (C) 2009 ACS)

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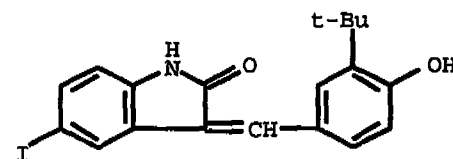
REGISTRY (Copyright (C) 2009 ACS)

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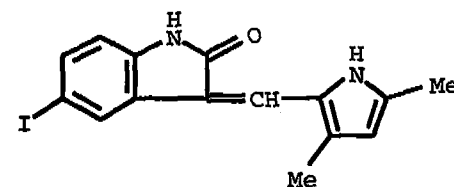
REGISTRY (Copyright (C) 2009 ACS)

Answer 998:



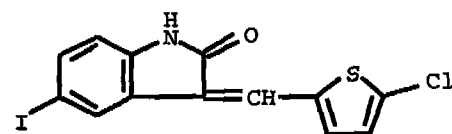
REGISTRY (Copyright (C) 2009 ACS)

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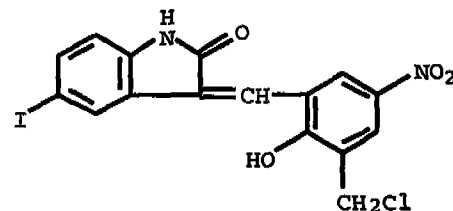
REGISTRY (Copyright (C) 2009 ACS)

Answer 1000:



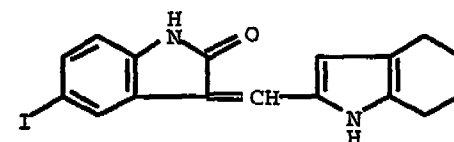
REGISTRY (Copyright (C) 2009 ACS)

Answer 1001:



REGISTRY (Copyright (C) 2009 ACS)

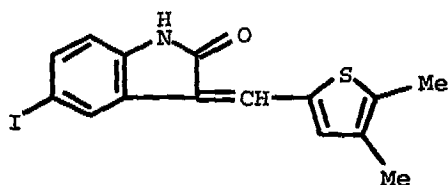
Answer 1002:



REGISTRY (Copyright (C) 2009 ACS)

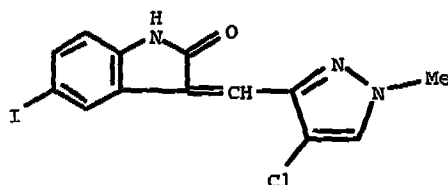
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Answer 1003:



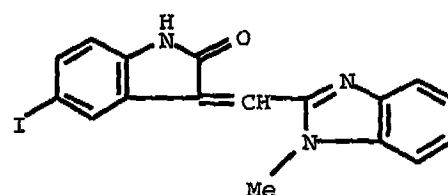
REGISTRY (Copyright (C) 2009 ACS)

Answer 1004:



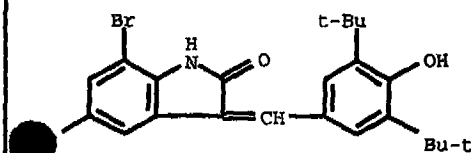
REGISTRY (Copyright (C) 2009 ACS)

Answer 1005:



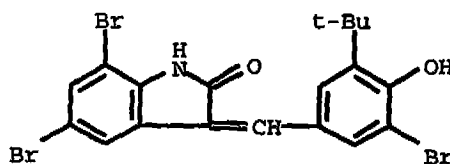
REGISTRY (Copyright (C) 2009 ACS)

Answer 1006:



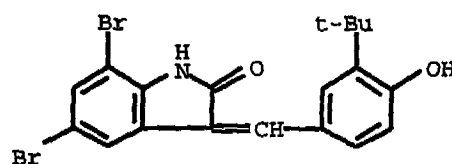
REGISTRY (Copyright (C) 2009 ACS)

Answer 1007:



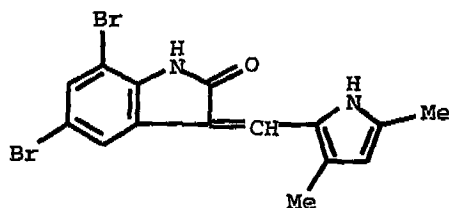
REGISTRY (Copyright (C) 2009 ACS)

Answer 1008:



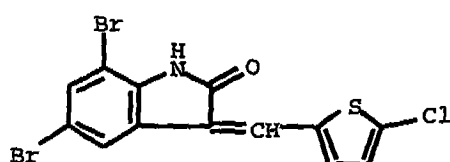
REGISTRY (Copyright (C) 2009 ACS)

Answer 1009:



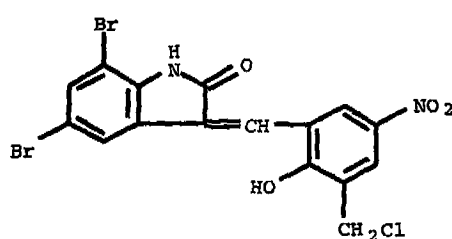
REGISTRY (Copyright (C) 2009 ACS)

Answer 1010:



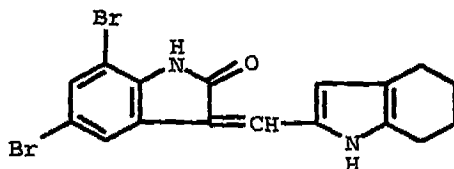
REGISTRY (Copyright (C) 2009 ACS)

Answer 1011:



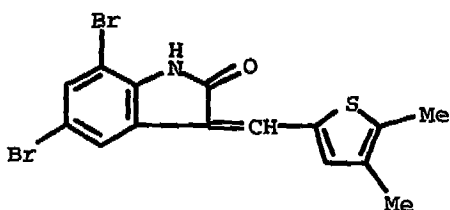
REGISTRY (Copyright (C) 2009 ACS)

Answer 1012:



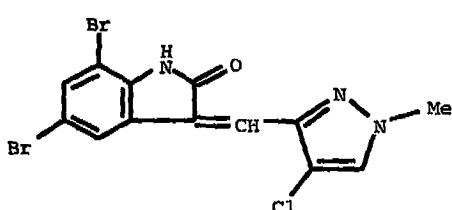
REGISTRY (Copyright (C) 2009 ACS)

Answer 1013:



REGISTRY (Copyright (C) 2009 ACS)

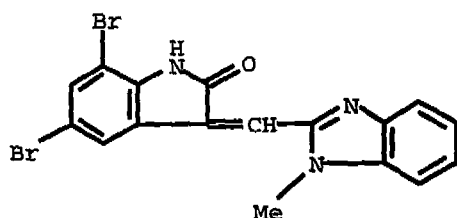
Answer 1014:



REGISTRY (Copyright (C) 2009 ACS)

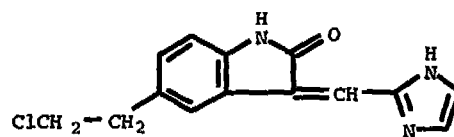
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Answer 1015:



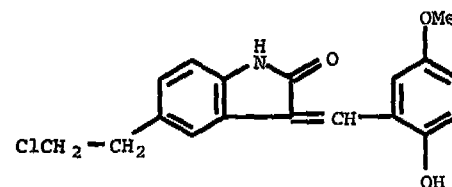
REGISTRY (Copyright (C) 2009 ACS)

Answer 1016:



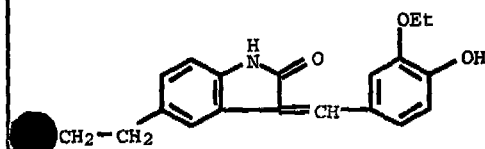
REGISTRY (Copyright (C) 2009 ACS)

Answer 1017:



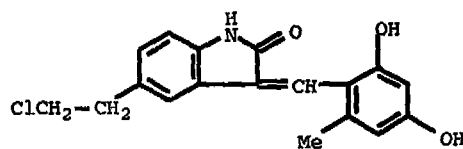
REGISTRY (Copyright (C) 2009 ACS)

Answer 1018:



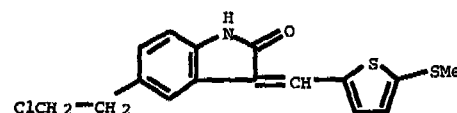
REGISTRY (Copyright (C) 2009 ACS)

Answer 1019:



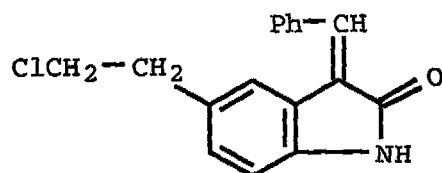
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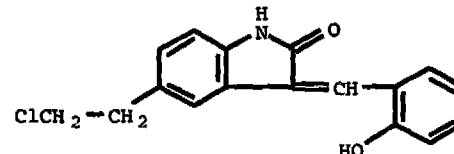
REGISTRY (Copyright (C) 2009 ACS)

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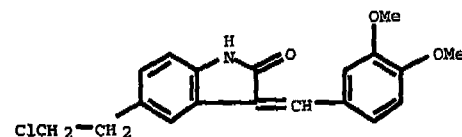
REGISTRY (Copyright (C) 2009 ACS)

Answer 1022:



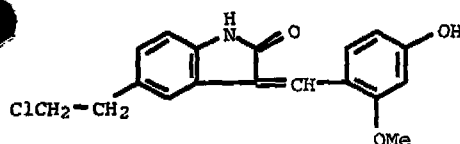
REGISTRY (Copyright (C) 2009 ACS)

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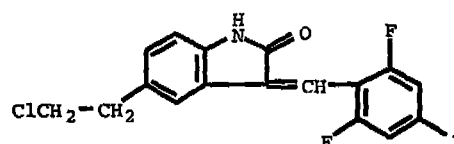
REGISTRY (Copyright (C) 2009 ACS)

Answer 1024:



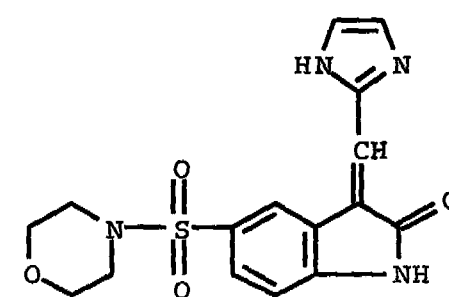
REGISTRY (Copyright (C) 2009 ACS)

Answer 1025:



REGISTRY (Copyright (C) 2009 ACS)

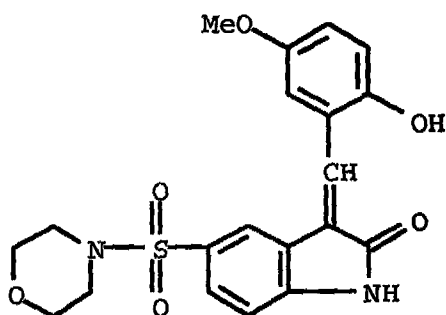
Answer 1026:



REGISTRY (Copyright (C) 2009 ACS)

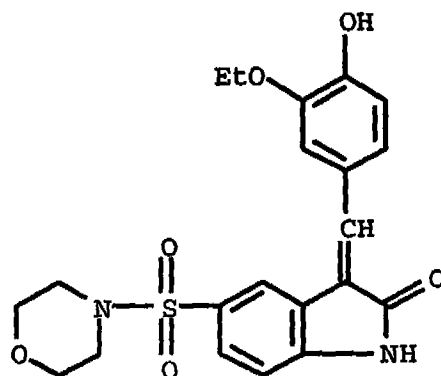
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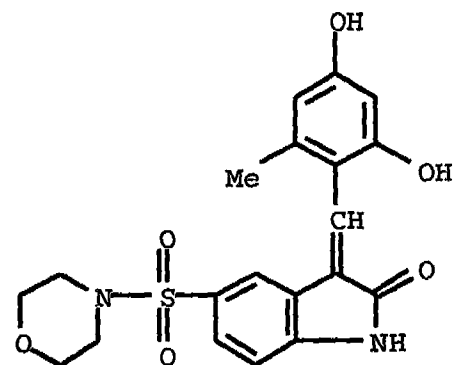
REGISTRY (Copyright (C) 2009 ACS)

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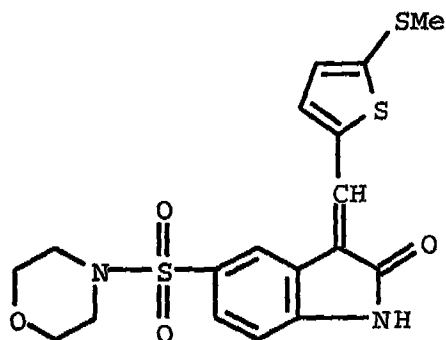
REGISTRY (Copyright (C) 2009 ACS)

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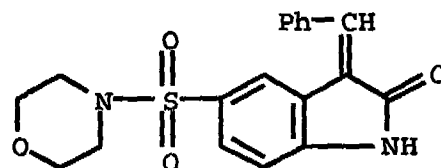
REGISTRY (Copyright (C) 2009 ACS)

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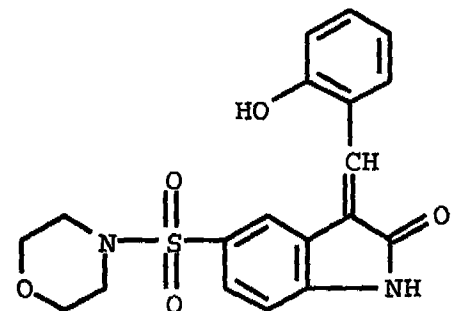
REGISTRY (Copyright (C) 2009 ACS)

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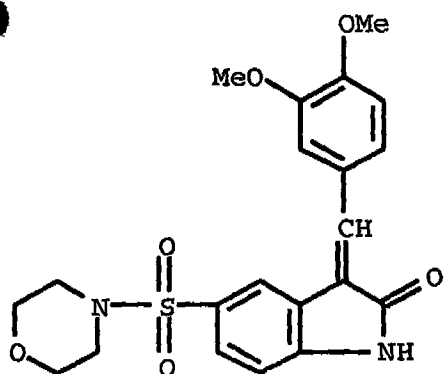
REGISTRY (Copyright (C) 2009 ACS)

Answer 1032:



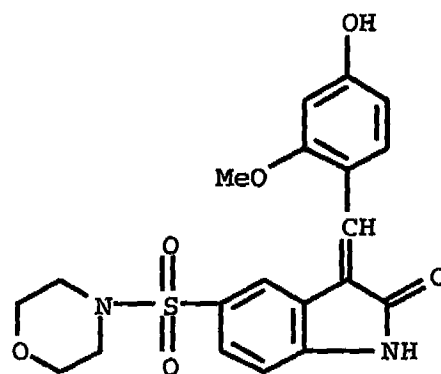
REGISTRY (Copyright (C) 2009 ACS)

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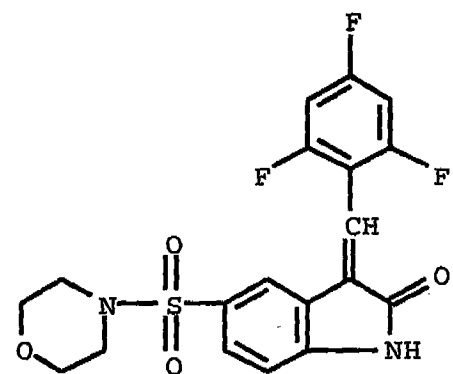
REGISTRY (Copyright (C) 2009 ACS)

Answer 1034:



REGISTRY (Copyright (C) 2009 ACS)

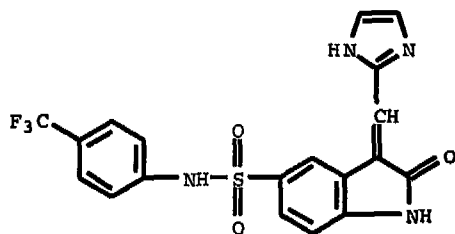
Answer 1035:



REGISTRY (Copyright (C) 2009 ACS)

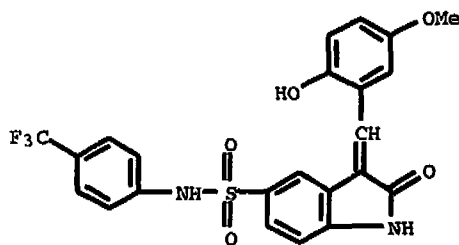
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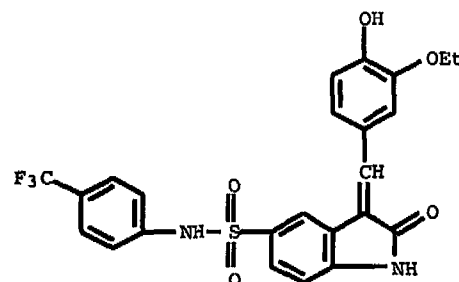
REGISTRY (Copyright (C) 2009 ACS)

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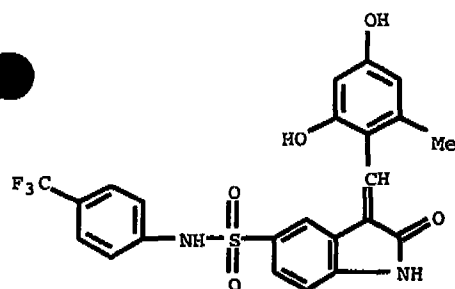
REGISTRY (Copyright (C) 2009 ACS)

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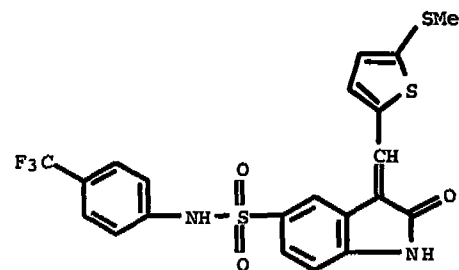
REGISTRY (Copyright (C) 2009 ACS)

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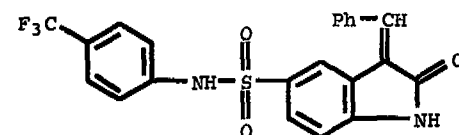
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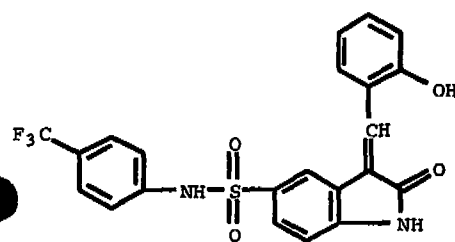
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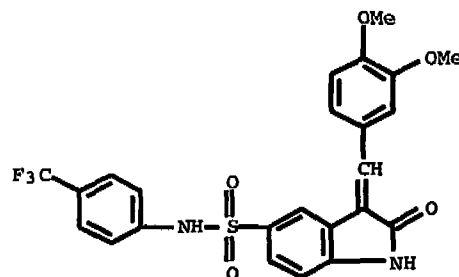
REGISTRY (Copyright (C) 2009 ACS)

Answer 1042:



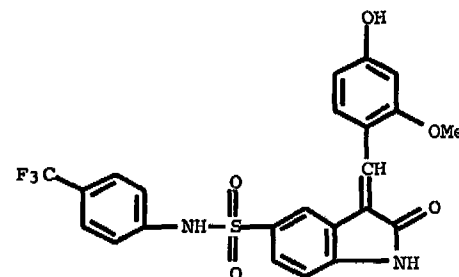
REGISTRY (Copyright (C) 2009 ACS)

Answer 1043:



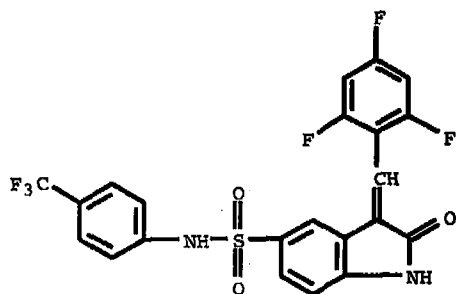
REGISTRY (Copyright (C) 2009 ACS)

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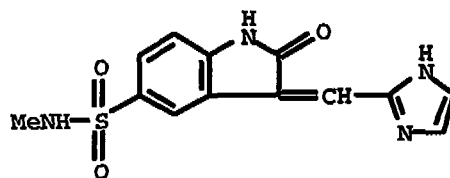
REGISTRY (Copyright (C) 2009 ACS)

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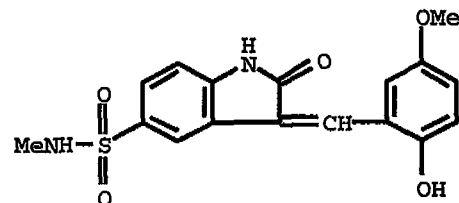
REGISTRY (Copyright (C) 2009 ACS)

Answer 1046:



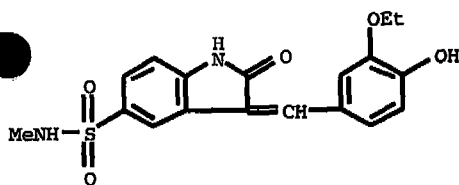
REGISTRY (Copyright (C) 2009 ACS)

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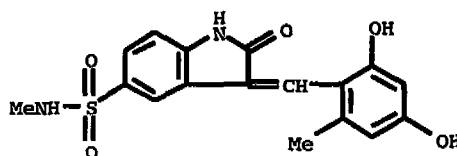
REGISTRY (Copyright (C) 2009 ACS)

Answer 1048:



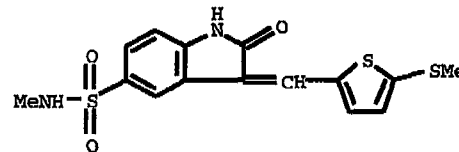
REGISTRY (Copyright (C) 2009 ACS)

Answer 1049:



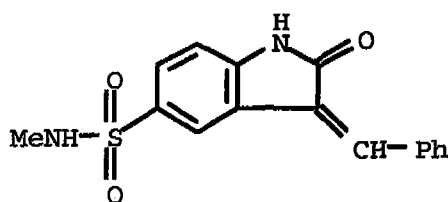
REGISTRY (Copyright (C) 2009 ACS)

Answer 1050:



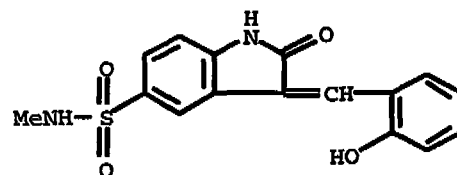
REGISTRY (Copyright (C) 2009 ACS)

Answer 1051:



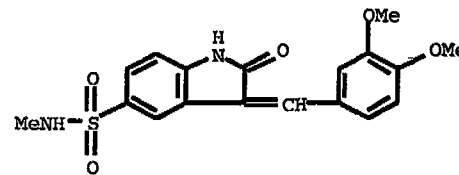
REGISTRY (Copyright (C) 2009 ACS)

Answer 1052:



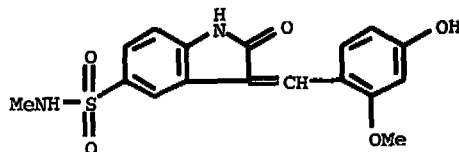
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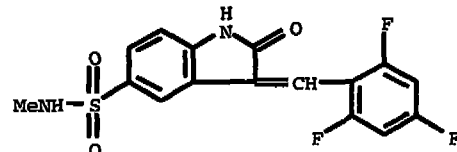
REGISTRY (Copyright (C) 2009 ACS)

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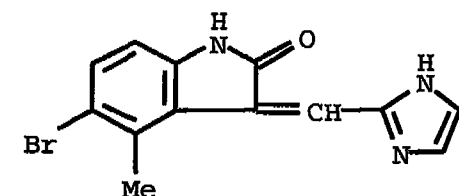
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Answer 1055:



REGISTRY (Copyright (C) 2009 ACS)

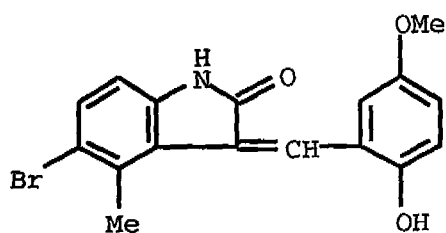
Answer 1056:



REGISTRY (Copyright (C) 2009 ACS)

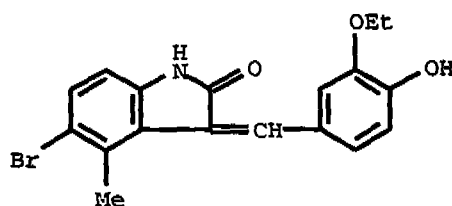
1204
108
1237

Answer 1057:



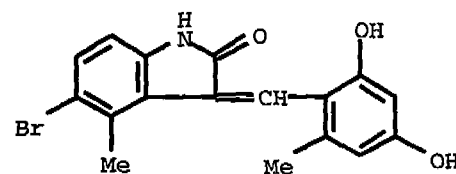
REGISTRY (Copyright (C) 2009 ACS)

Answer 1058:



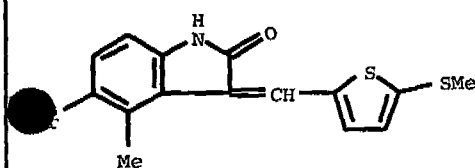
REGISTRY (Copyright (C) 2009 ACS)

Answer 1059:



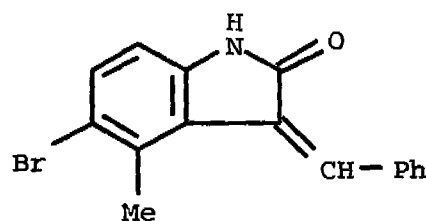
REGISTRY (Copyright (C) 2009 ACS)

Answer 1060:



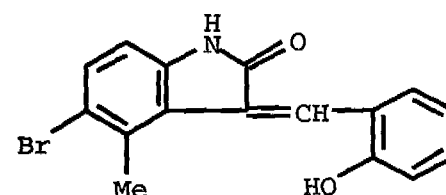
REGISTRY (Copyright (C) 2009 ACS)

Answer 1061:



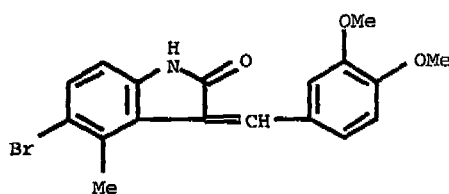
REGISTRY (Copyright (C) 2009 ACS)

Answer 1062:



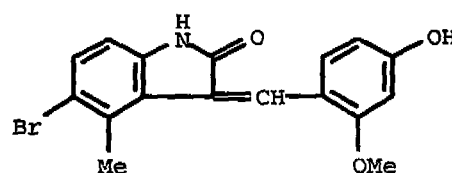
REGISTRY (Copyright (C) 2009 ACS)

Answer 1063:



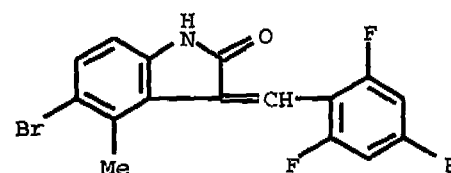
REGISTRY (Copyright (C) 2009 ACS)

Answer 1064:



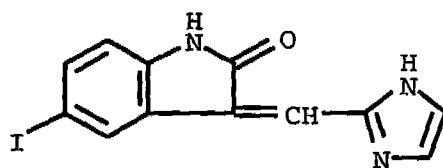
REGISTRY (Copyright (C) 2009 ACS)

Answer 1065:



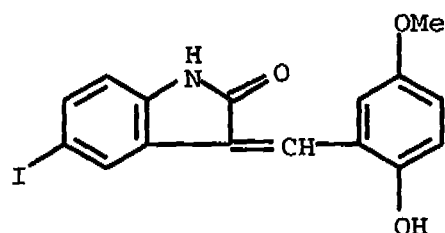
REGISTRY (Copyright (C) 2009 ACS)

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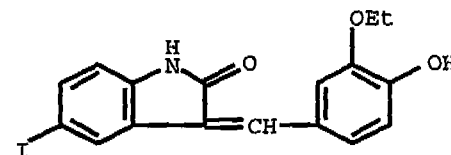
REGISTRY (Copyright (C) 2009 ACS)

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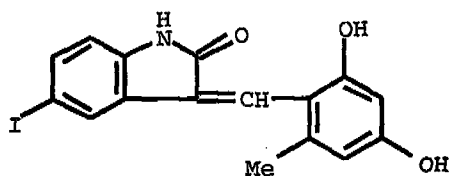
REGISTRY (Copyright (C) 2009 ACS)

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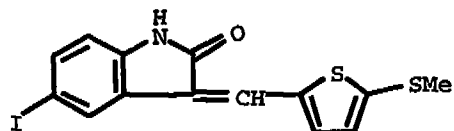
REGISTRY (Copyright (C) 2009 ACS)

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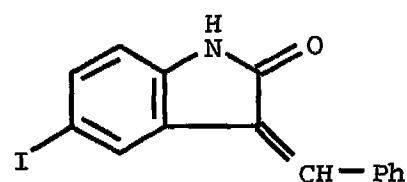
REGISTRY (Copyright (C) 2009 ACS)

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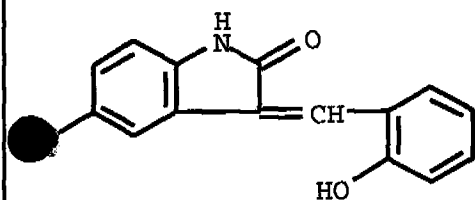
REGISTRY (Copyright (C) 2009 ACS)

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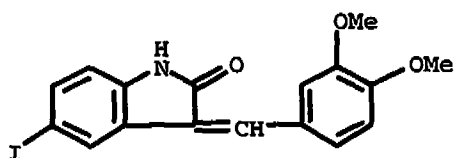
REGISTRY (Copyright (C) 2009 ACS)

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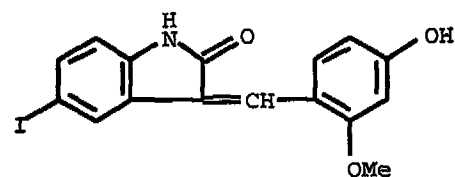
REGISTRY (Copyright (C) 2009 ACS)

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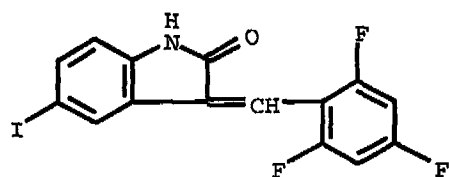
REGISTRY (Copyright (C) 2009 ACS)

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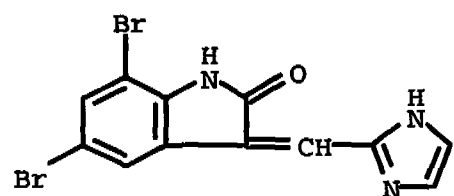
REGISTRY (Copyright (C) 2009 ACS)

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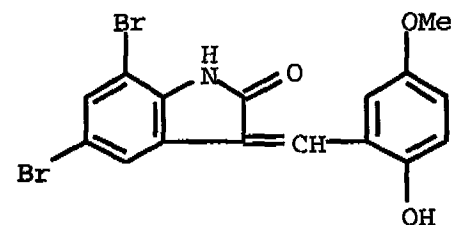
REGISTRY (Copyright (C) 2009 ACS)

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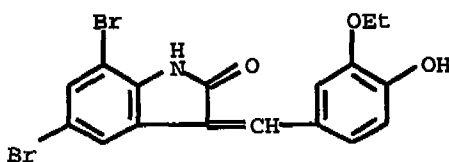
REGISTRY (Copyright (C) 2009 ACS)

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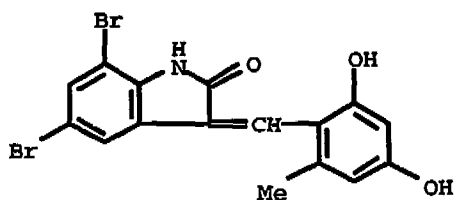
REGISTRY (Copyright (C) 2009 ACS)

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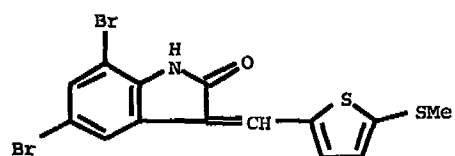
REGISTRY (Copyright (C) 2009 ACS)

Answer 1079:



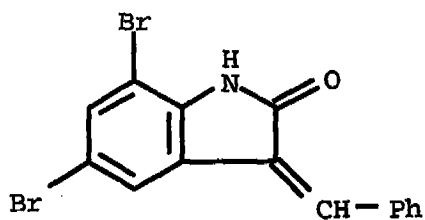
REGISTRY (Copyright (C) 2009 ACS)

Answer 1080:



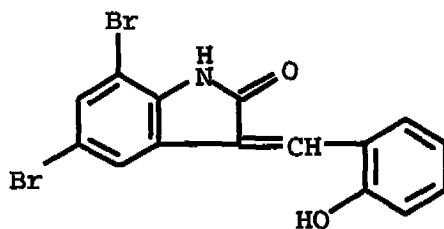
REGISTRY (Copyright (C) 2009 ACS)

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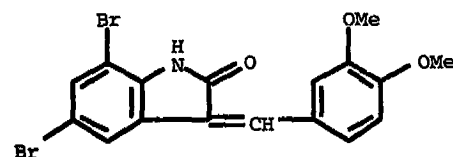
REGISTRY (Copyright (C) 2009 ACS)

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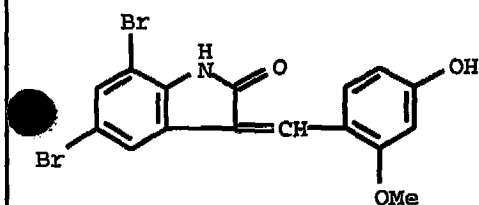
REGISTRY (Copyright (C) 2009 ACS)

Answer 1083:



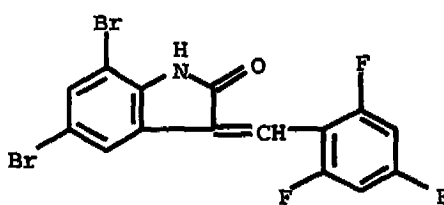
REGISTRY (Copyright (C) 2009 ACS)

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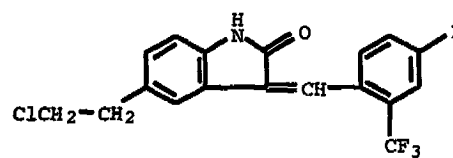
REGISTRY (Copyright (C) 2009 ACS)

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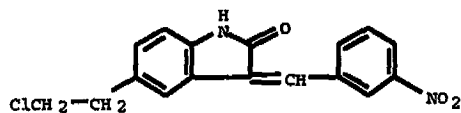
REGISTRY (Copyright (C) 2009 ACS)

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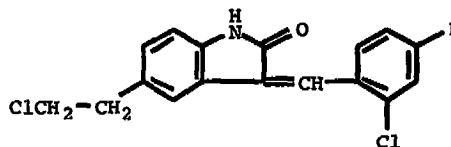
REGISTRY (Copyright (C) 2009 ACS)

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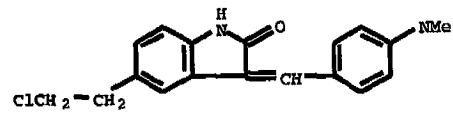
REGISTRY (Copyright (C) 2009 ACS)

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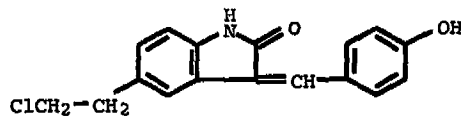
REGISTRY (Copyright (C) 2009 ACS)

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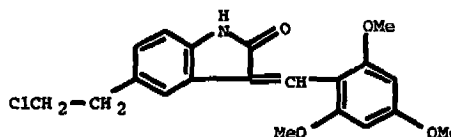
REGISTRY (Copyright (C) 2009 ACS)

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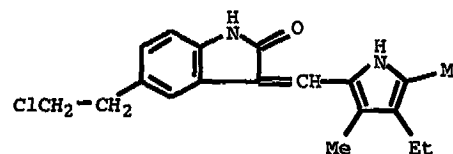
REGISTRY (Copyright (C) 2009 ACS)

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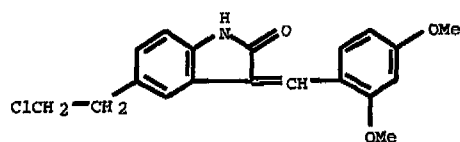
REGISTRY (Copyright (C) 2009 ACS)

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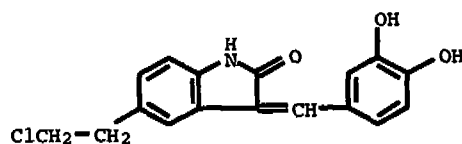
REGISTRY (Copyright (C) 2009 ACS)

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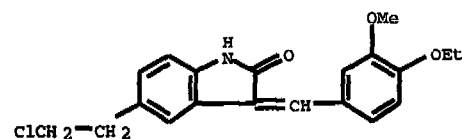
REGISTRY (Copyright (C) 2009 ACS)

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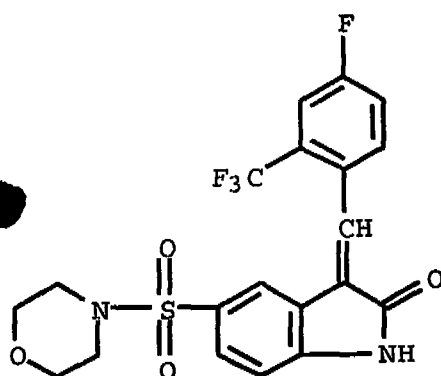
REGISTRY (Copyright (C) 2009 ACS)

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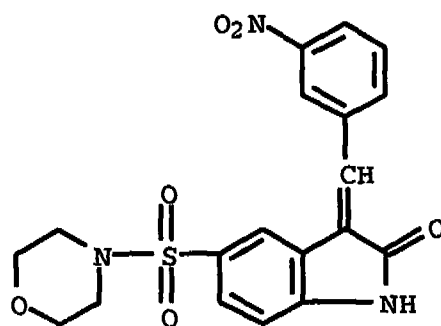
REGISTRY (Copyright (C) 2009 ACS)

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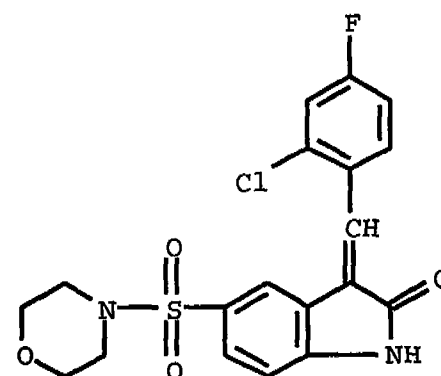
REGISTRY (Copyright (C) 2009 ACS)

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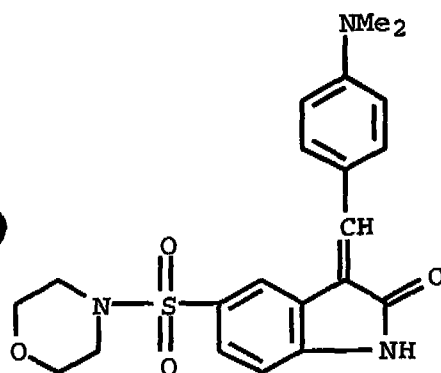
REGISTRY (Copyright (C) 2009 ACS)

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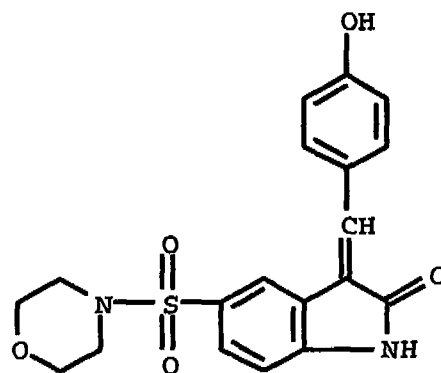
REGISTRY (Copyright (C) 2009 ACS)

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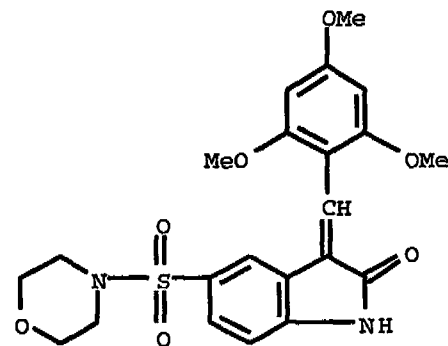
REGISTRY (Copyright (C) 2009 ACS)

Answer 1100:



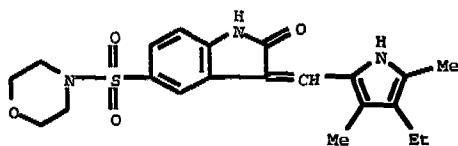
REGISTRY (Copyright (C) 2009 ACS)

Answer 1101:



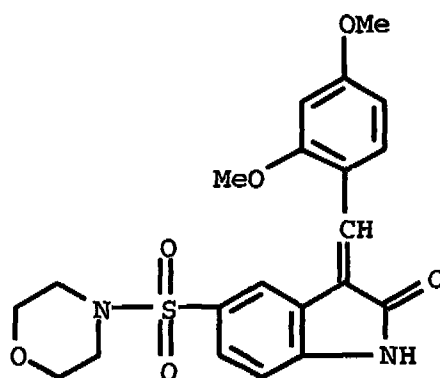
REGISTRY (Copyright (C) 2009 ACS)

Answer 1102:



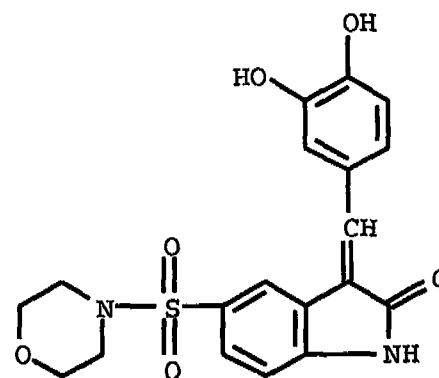
REGISTRY (Copyright (C) 2009 ACS)

Answer 1103:



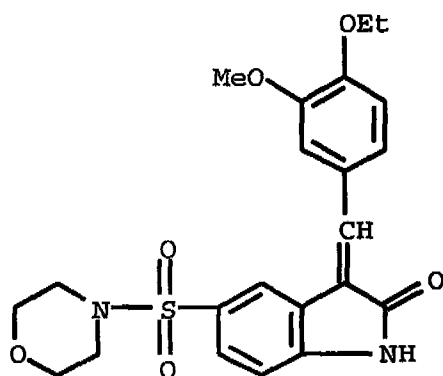
REGISTRY (Copyright (C) 2009 ACS)

Answer 1104:



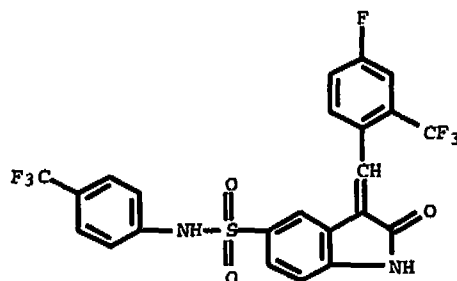
REGISTRY (Copyright (C) 2009 ACS)

Answer 1105:



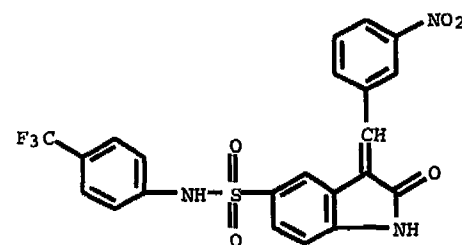
REGISTRY (Copyright (C) 2009 ACS)

Answer 1106:



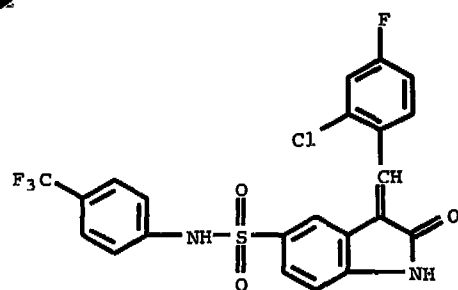
REGISTRY (Copyright (C) 2009 ACS)

Answer 1107:



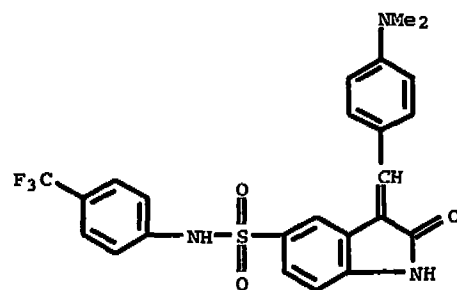
REGISTRY (Copyright (C) 2009 ACS)

Answer 1108:



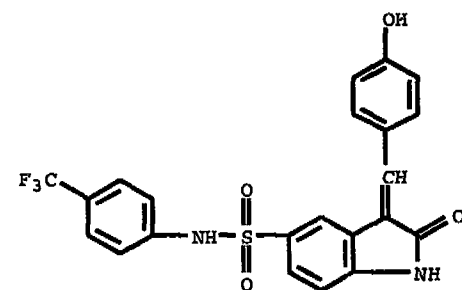
REGISTRY (Copyright (C) 2009 ACS)

Answer 1109:



REGISTRY (Copyright (C) 2009 ACS)

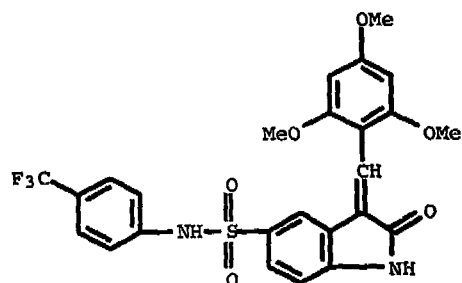
Answer 1110:



REGISTRY (Copyright (C) 2009 ACS)

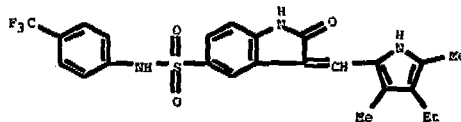
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1242

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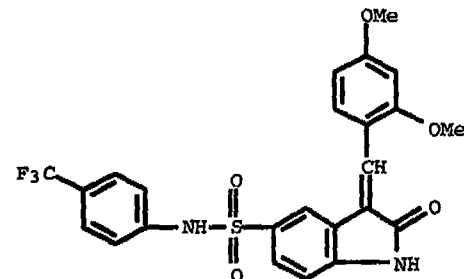
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Answer 1112:



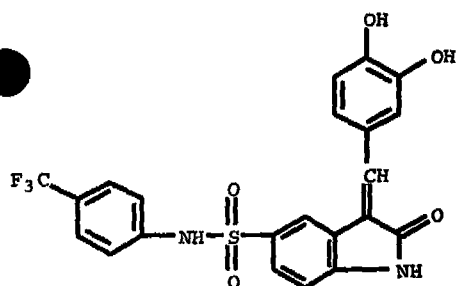
REGISTRY (Copyright (C) 2009 ACS)

Answer 1113:



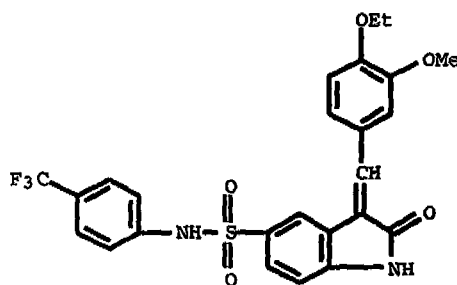
REGISTRY (Copyright (C) 2009 ACS)

Answer 1114:



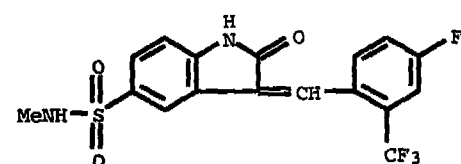
REGISTRY (Copyright (C) 2009 ACS)

Answer 1115:



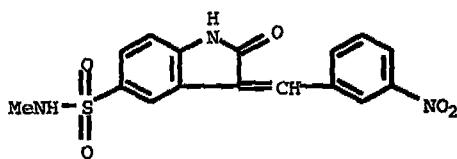
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Answer 1116:



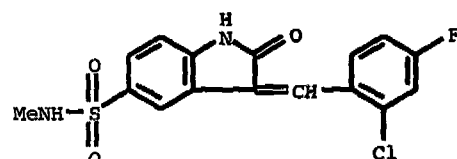
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Answer 1117:



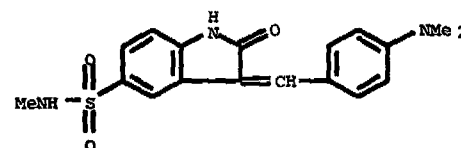
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Answer 1118:



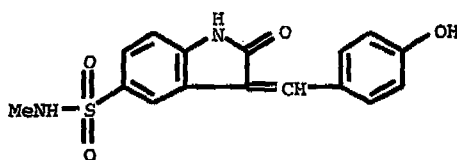
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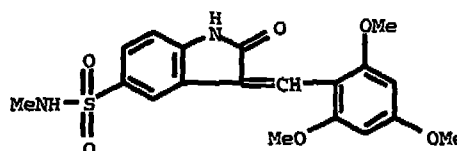
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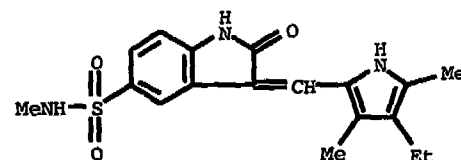
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Answer 1121:



REGISTRY (Copyright (C) 2009 ACS)

Answer 1122:



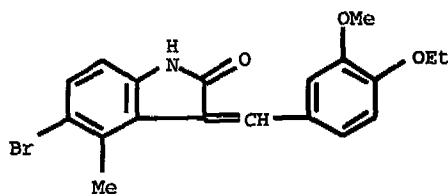
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1243

Answer 1123: REGISTRY (Copyright (C) 2009 ACS)	Answer 1124: REGISTRY (Copyright (C) 2009 ACS)	Answer 1125: REGISTRY (Copyright (C) 2009 ACS)
Answer 1126: REGISTRY (Copyright (C) 2009 ACS)	Answer 1127: REGISTRY (Copyright (C) 2009 ACS)	Answer 1128: REGISTRY (Copyright (C) 2009 ACS)
Answer 1129: REGISTRY (Copyright (C) 2009 ACS)	Answer 1130: REGISTRY (Copyright (C) 2009 ACS)	Answer 1131: REGISTRY (Copyright (C) 2009 ACS)
Answer 1132: REGISTRY (Copyright (C) 2009 ACS)	Answer 1133: REGISTRY (Copyright (C) 2009 ACS)	Answer 1134: REGISTRY (Copyright (C) 2009 ACS)

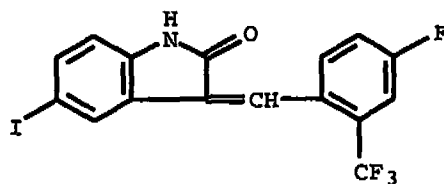
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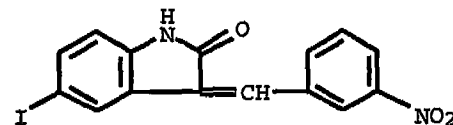
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Answer 1136:



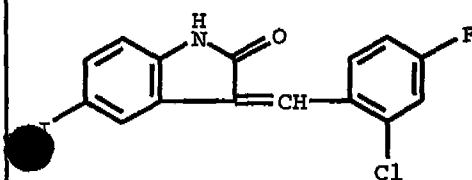
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Answer 1137:



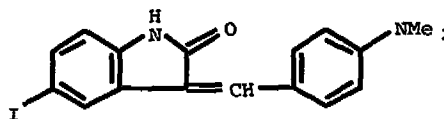
REGISTRY (Copyright (C) 2009 ACS)

Answer 1138:



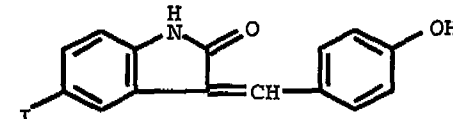
REGISTRY (Copyright (C) 2009 ACS)

Answer 1139:



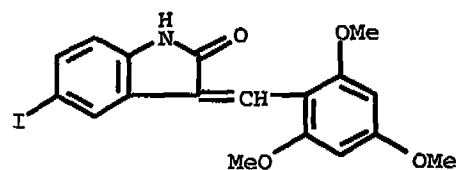
REGISTRY (Copyright (C) 2009 ACS)

Answer 1140:



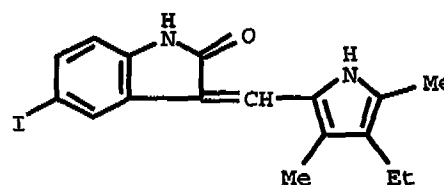
REGISTRY (Copyright (C) 2009 ACS)

Answer 1141:



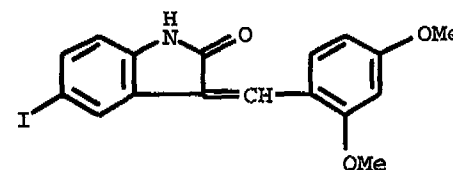
REGISTRY (Copyright (C) 2009 ACS)

Answer 1142:



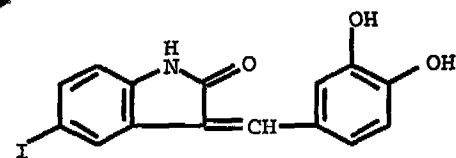
REGISTRY (Copyright (C) 2009 ACS)

Answer 1143:



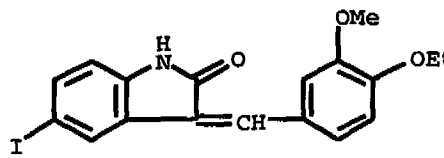
REGISTRY (Copyright (C) 2009 ACS)

Answer 1144:



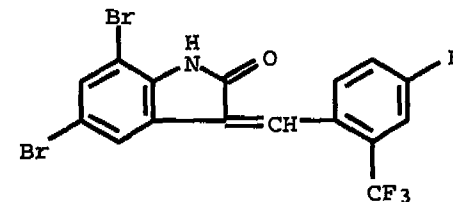
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Answer 1145:



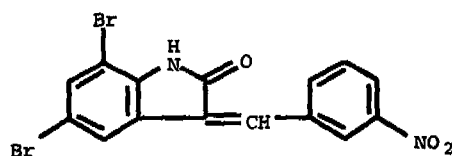
REGISTRY (Copyright (C) 2009 ACS)

Answer 1146:



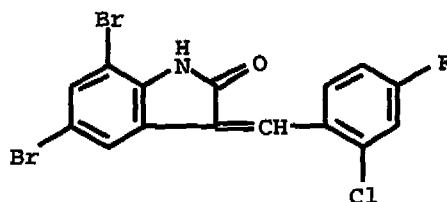
REGISTRY (Copyright (C) 2009 ACS)

Answer 1147:



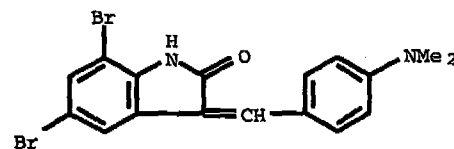
REGISTRY (Copyright (C) 2009 ACS)

Answer 1148:



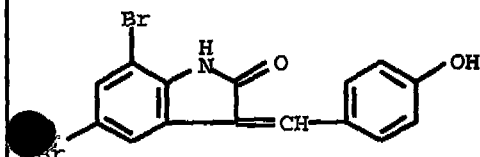
REGISTRY (Copyright (C) 2009 ACS)

Answer 1149:



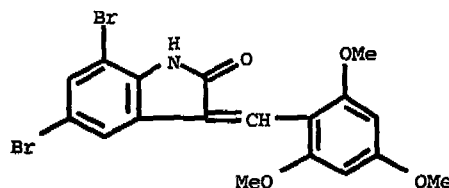
REGISTRY (Copyright (C) 2009 ACS)

Answer 1150:



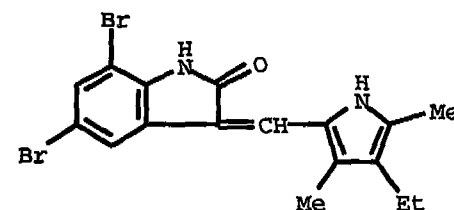
REGISTRY (Copyright (C) 2009 ACS)

Answer 1151:



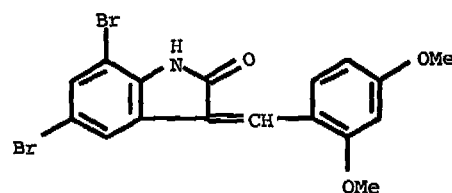
REGISTRY (Copyright (C) 2009 ACS)

Answer 1152:



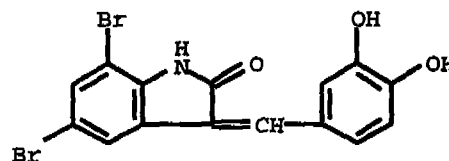
REGISTRY (Copyright (C) 2009 ACS)

Answer 1153:



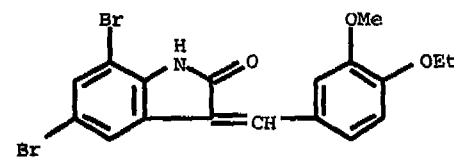
REGISTRY (Copyright (C) 2009 ACS)

Answer 1154:



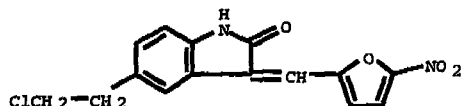
REGISTRY (Copyright (C) 2009 ACS)

Answer 1155:



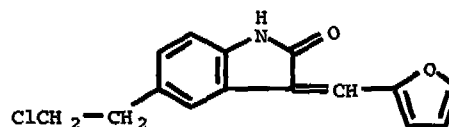
REGISTRY (Copyright (C) 2009 ACS)

Answer 1156:



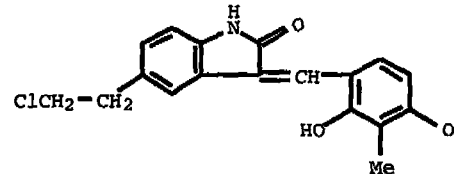
REGISTRY (Copyright (C) 2009 ACS)

Answer 1157:



REGISTRY (Copyright (C) 2009 ACS)

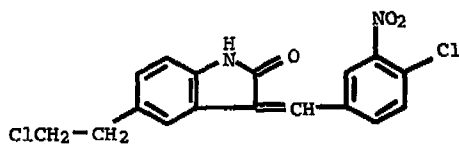
Answer 1158:



REGISTRY (Copyright (C) 2009 ACS)

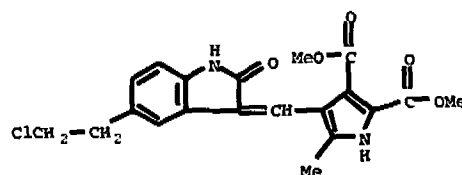
1213
117
1246

Answer 1159:



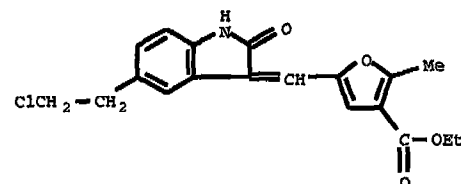
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Answer 1160:



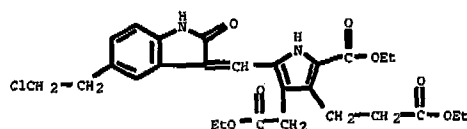
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Answer 1161:



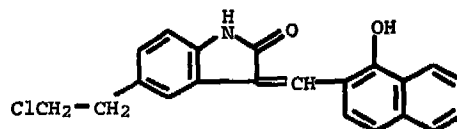
REGISTRY (Copyright (C) 2009 ACS)

Answer 1162:



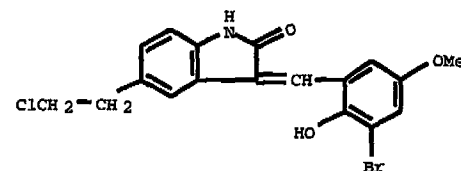
REGISTRY (Copyright (C) 2009 ACS)

Answer 1163:



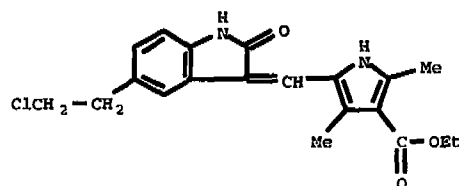
REGISTRY (Copyright (C) 2009 ACS)

Answer 1164:



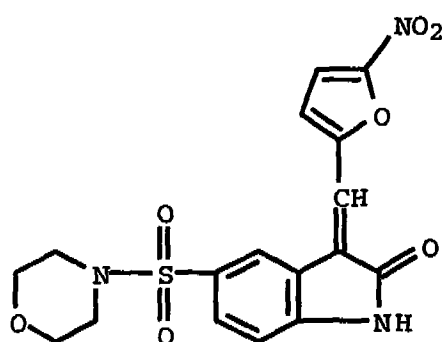
REGISTRY (Copyright (C) 2009 ACS)

Answer 1165:



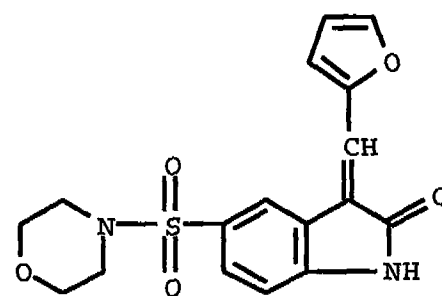
REGISTRY (Copyright (C) 2009 ACS)

Answer 1166:



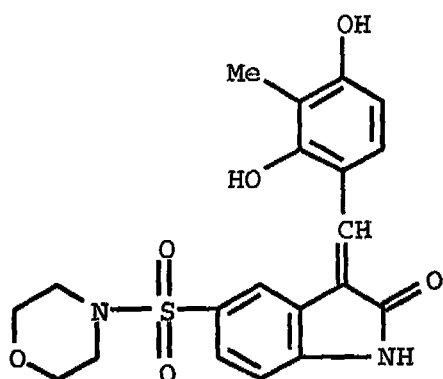
REGISTRY (Copyright (C) 2009 ACS)

Answer 1167:



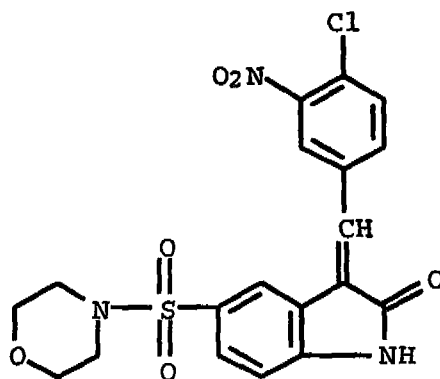
REGISTRY (Copyright (C) 2009 ACS)

Answer 1168:



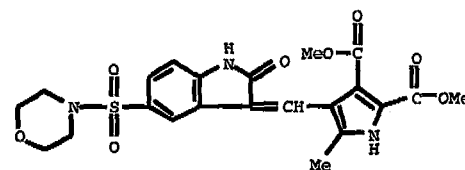
REGISTRY (Copyright (C) 2009 ACS)

Answer 1169:



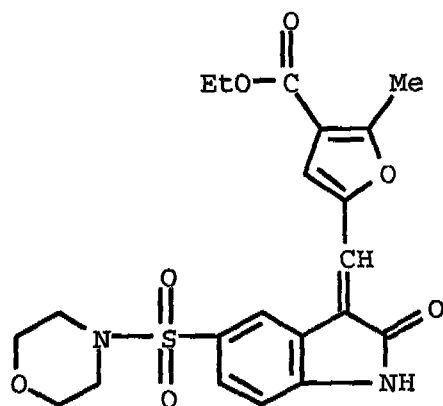
REGISTRY (Copyright (C) 2009 ACS)

Answer 1170:



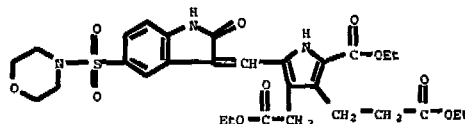
REGISTRY (Copyright (C) 2009 ACS)

Answer 1171:



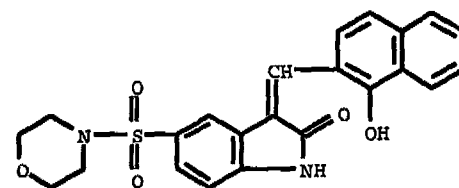
REGISTRY (Copyright (C) 2009 ACS)

Answer 1172:



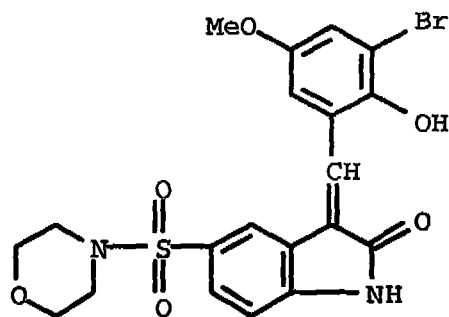
REGISTRY (Copyright (C) 2009 ACS)

Answer 1173:



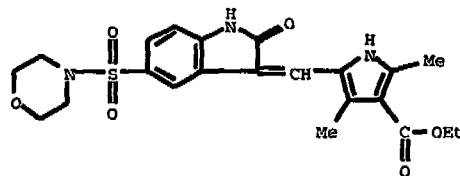
REGISTRY (Copyright (C) 2009 ACS)

Answer 1174:



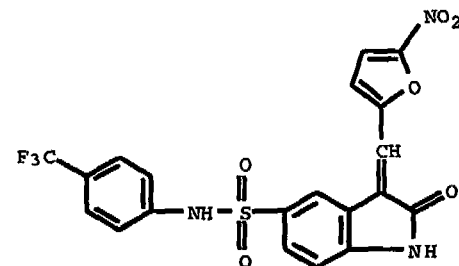
REGISTRY (Copyright (C) 2009 ACS)

Answer 1175:



REGISTRY (Copyright (C) 2009 ACS)

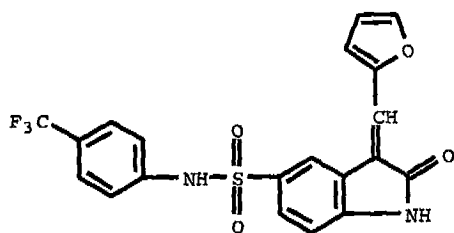
Answer 1176:



REGISTRY (Copyright (C) 2009 ACS)

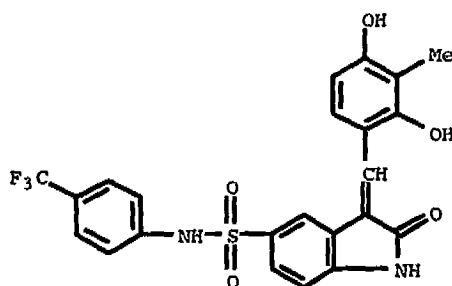
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Answer 1177:



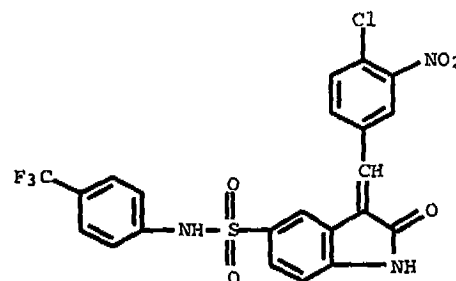
REGISTRY (Copyright (C) 2009 ACS)

Answer 1178:



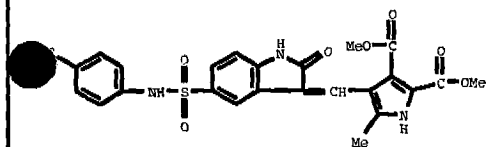
REGISTRY (Copyright (C) 2009 ACS)

Answer 1179:



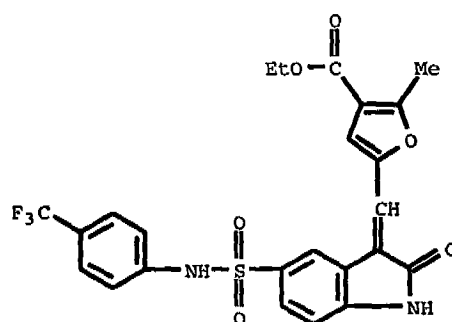
REGISTRY (Copyright (C) 2009 ACS)

Answer 1180:



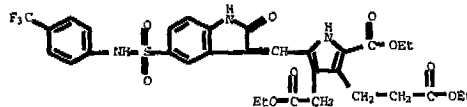
REGISTRY (Copyright (C) 2009 ACS)

Answer 1181:



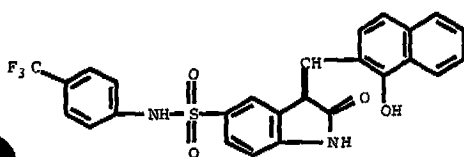
REGISTRY (Copyright (C) 2009 ACS)

Answer 1182:



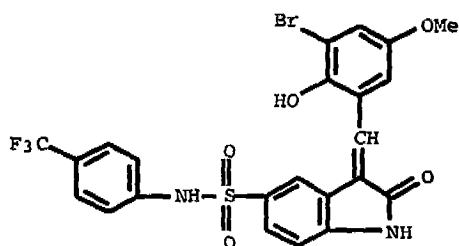
REGISTRY (Copyright (C) 2009 ACS)

Answer 1183:



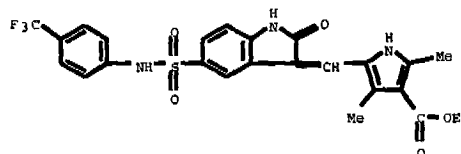
REGISTRY (Copyright (C) 2009 ACS)

Answer 1184:



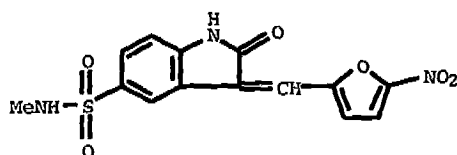
REGISTRY (Copyright (C) 2009 ACS)

Answer 1185:



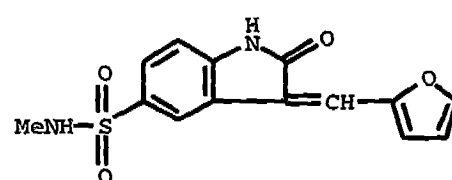
REGISTRY (Copyright (C) 2009 ACS)

Answer 1186:

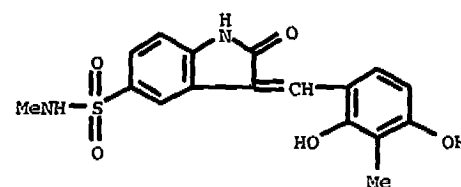


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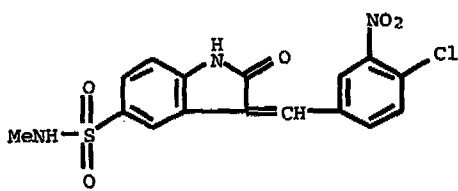
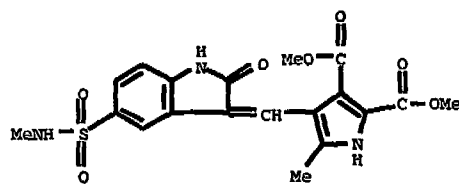
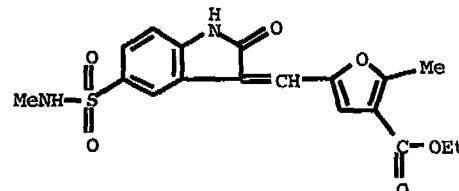
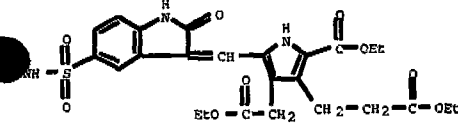
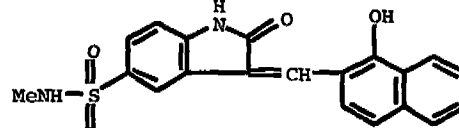
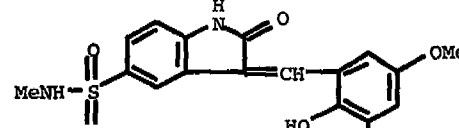
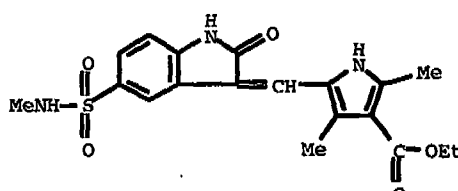
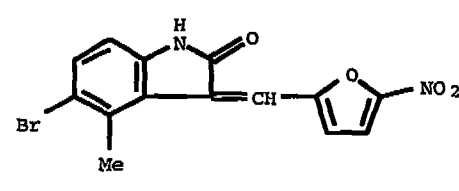
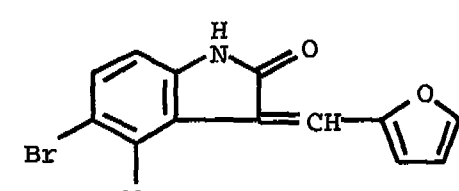
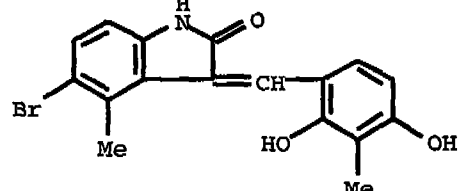
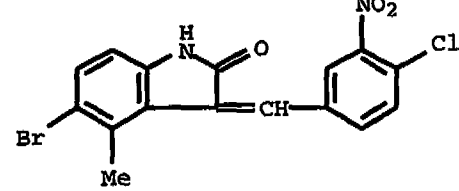
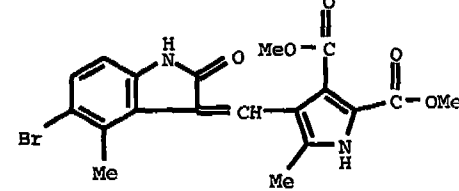
Answer 1187:



Answer 1188:

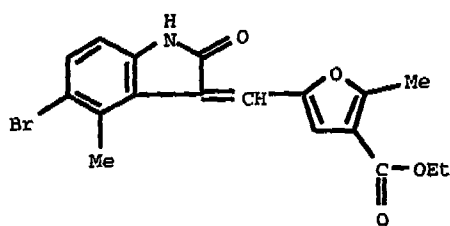


1246
172
1249

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Answer 1189: 	Answer 1190: 	Answer 1191: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 1192: 	Answer 1193: 	Answer 1194: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 1195: 	Answer 1196: 	Answer 1197: 
REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)	REGISTRY (Copyright (C) 2009 ACS)
Answer 1198: 	Answer 1199: 	Answer 1200: 
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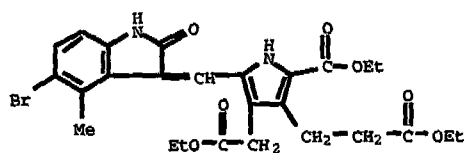
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121
1250

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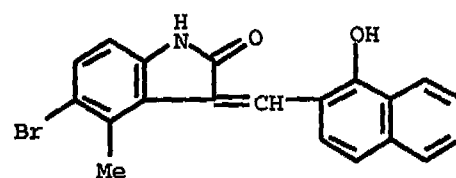
REGISTRY (Copyright (C) 2009 ACS)

Answer 1202:



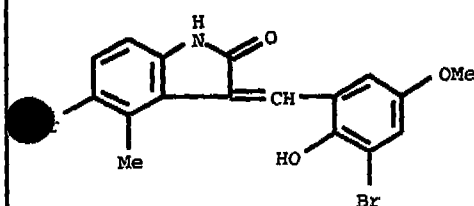
REGISTRY (Copyright (C) 2009 ACS)

Answer 1203:



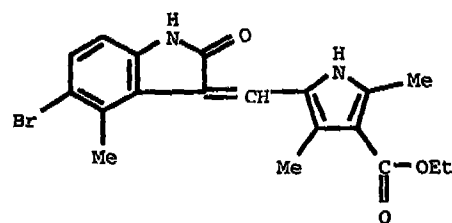
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Answer 1204:



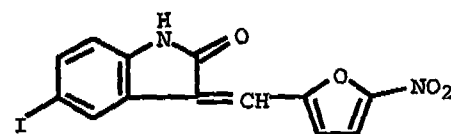
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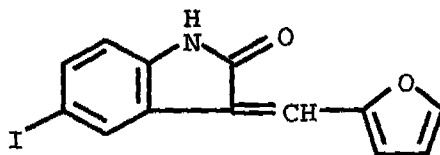
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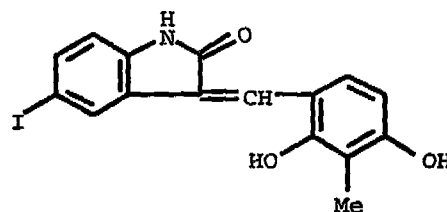
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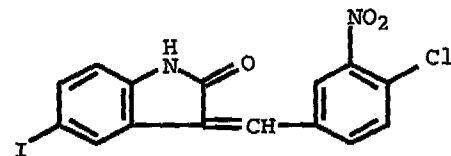
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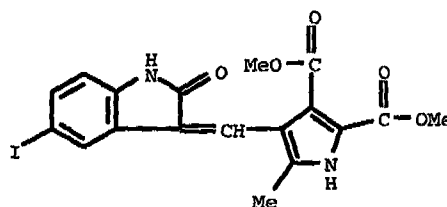
REGISTRY (Copyright (C) 2009 ACS)

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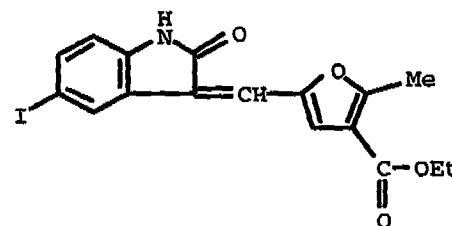
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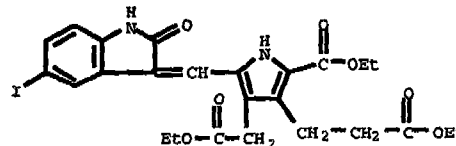
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Answer 1211:



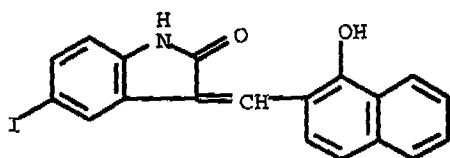
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Answer 1212:



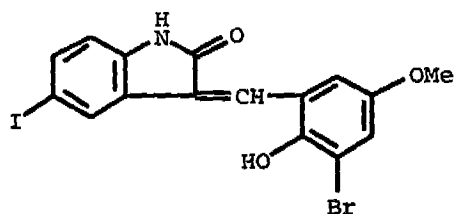
REGISTRY (Copyright (C) 2009 ACS)

Answer 1213:



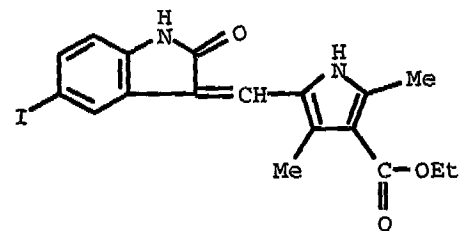
REGISTRY (Copyright (C) 2009 ACS)

Answer 1214:



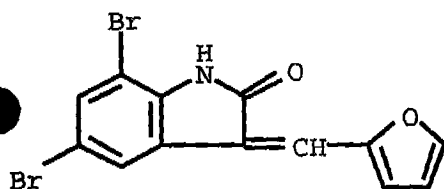
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Answer 1215:



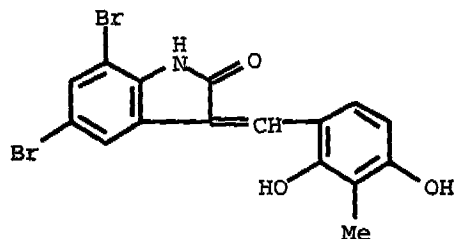
REGISTRY (Copyright (C) 2009 ACS)

Answer 1216:



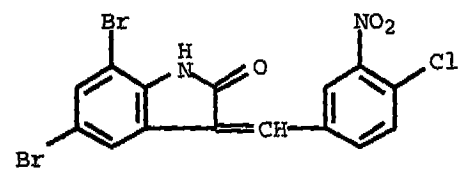
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Answer 1217:



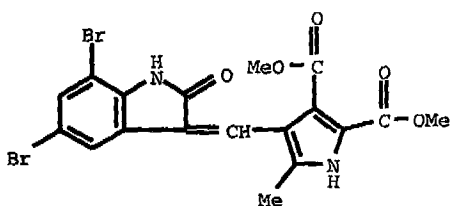
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Answer 1218:



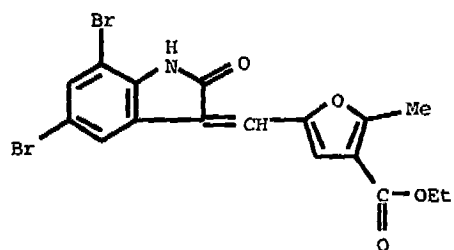
REGISTRY (Copyright (C) 2009 ACS)

Answer 1219:



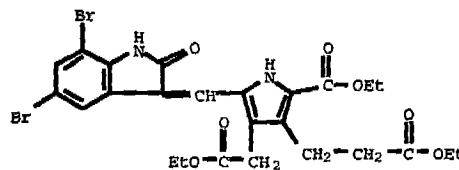
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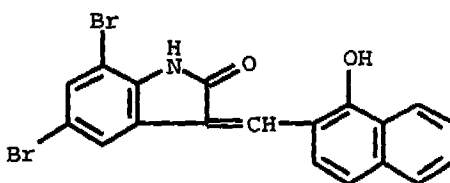
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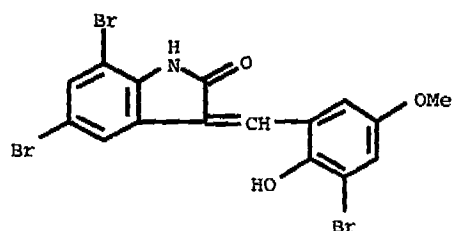
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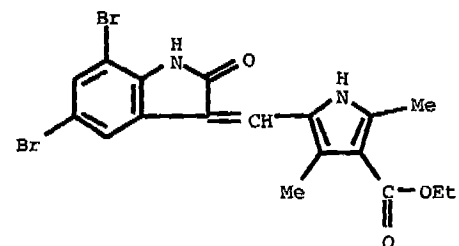
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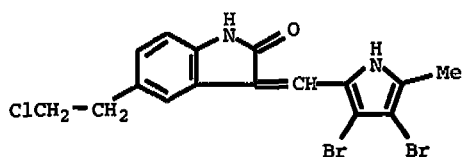
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Answer 1224:



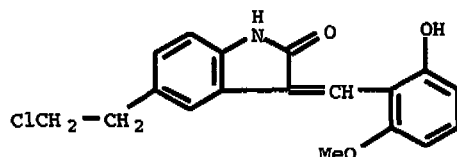
REGISTRY (Copyright (C) 2009 ACS)

Answer 1225:



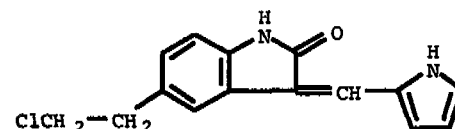
REGISTRY (Copyright (C) 2009 ACS)

Answer 1226:



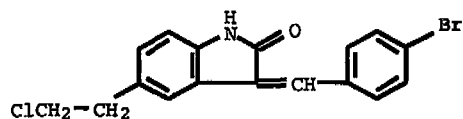
REGISTRY (Copyright (C) 2009 ACS)

Answer 1227:



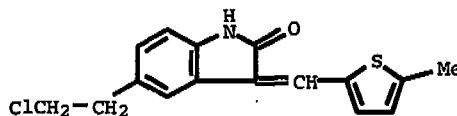
REGISTRY (Copyright (C) 2009 ACS)

Answer 1228:



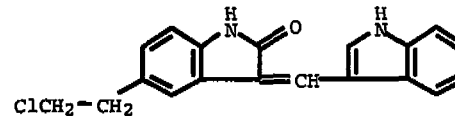
REGISTRY (Copyright (C) 2009 ACS)

Answer 1229:



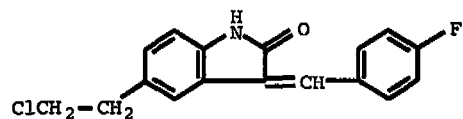
REGISTRY (Copyright (C) 2009 ACS)

Answer 1230:



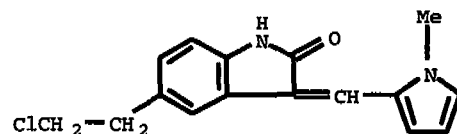
REGISTRY (Copyright (C) 2009 ACS)

Answer 1231:



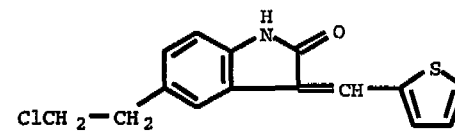
REGISTRY (Copyright (C) 2009 ACS)

Answer 1232:



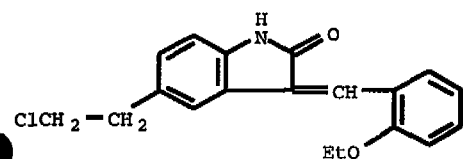
REGISTRY (Copyright (C) 2009 ACS)

Answer 1233:



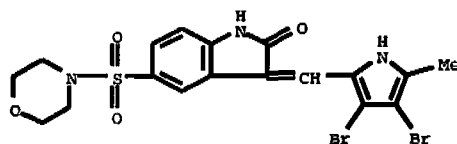
REGISTRY (Copyright (C) 2009 ACS)

Answer 1234:



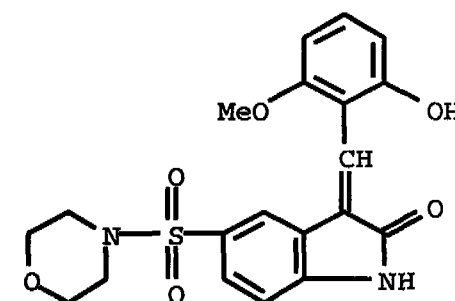
REGISTRY (Copyright (C) 2009 ACS)

Answer 1235:



REGISTRY (Copyright (C) 2009 ACS)

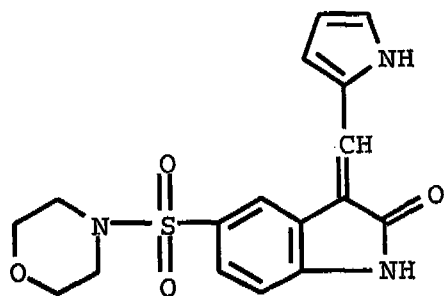
Answer 1236:



REGISTRY (Copyright (C) 2009 ACS)

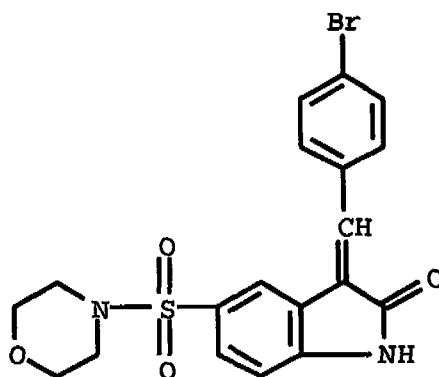
1229
124
1253

Answer 1237:



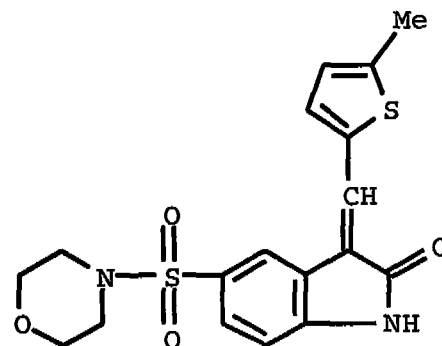
REGISTRY (Copyright (C) 2009 ACS)

Answer 1238:



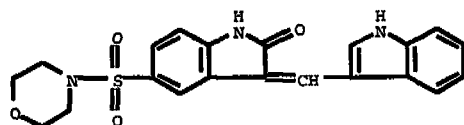
REGISTRY (Copyright (C) 2009 ACS)

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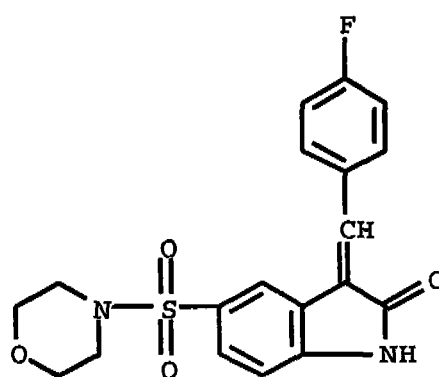
REGISTRY (Copyright (C) 2009 ACS)

Answer 1240:



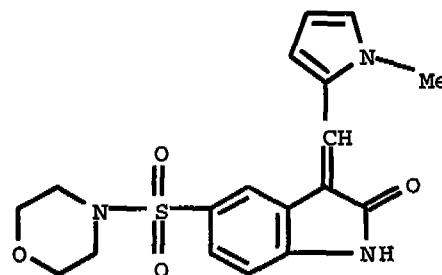
REGISTRY (Copyright (C) 2009 ACS)

Answer 1241:



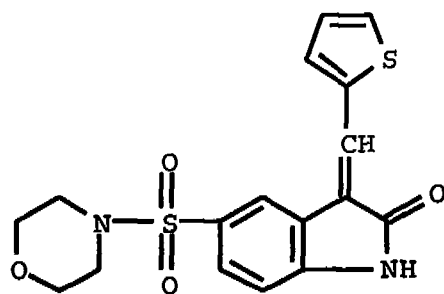
REGISTRY (Copyright (C) 2009 ACS)

Answer 1242:



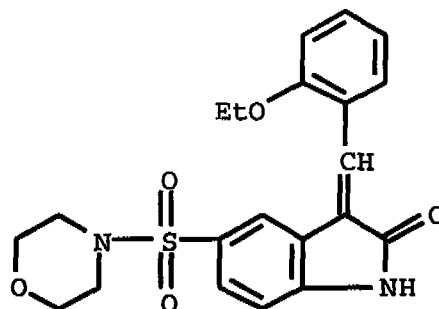
REGISTRY (Copyright (C) 2009 ACS)

Answer 1243:



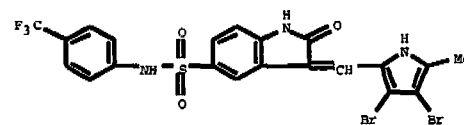
REGISTRY (Copyright (C) 2009 ACS)

Answer 1244:



REGISTRY (Copyright (C) 2009 ACS)

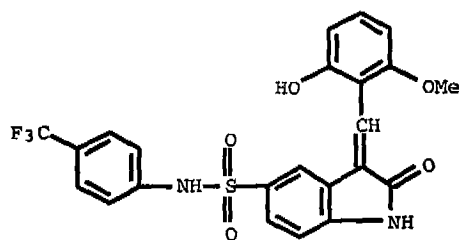
Answer 1245:



REGISTRY (Copyright (C) 2009 ACS)

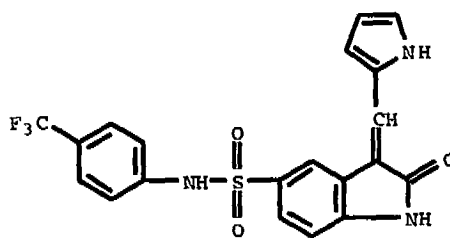
1254

Answer 1246:



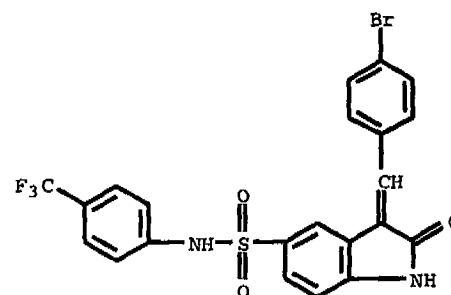
REGISTRY (Copyright (C) 2009 ACS)

Answer 1247:



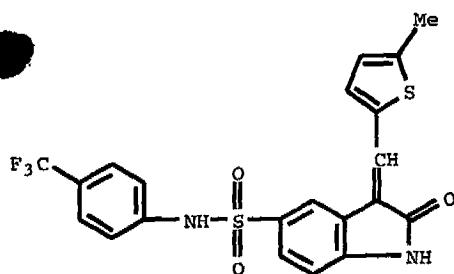
REGISTRY (Copyright (C) 2009 ACS)

Answer 1248:



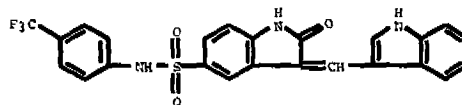
REGISTRY (Copyright (C) 2009 ACS)

Answer 1249:



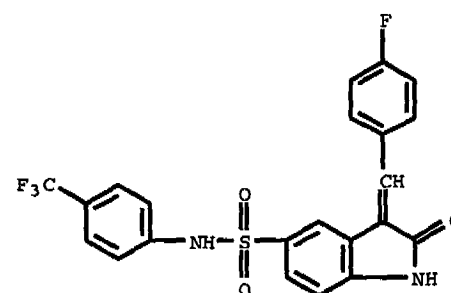
REGISTRY (Copyright (C) 2009 ACS)

Answer 1250:



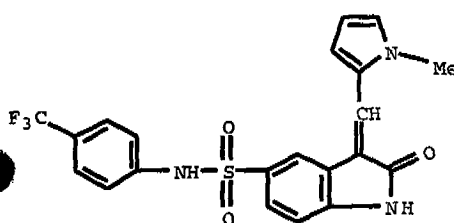
REGISTRY (Copyright (C) 2009 ACS)

Answer 1251:



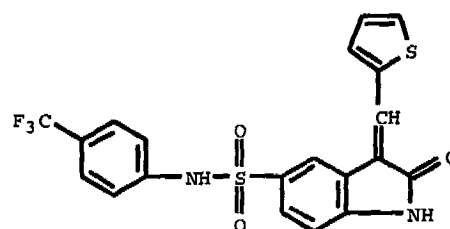
REGISTRY (Copyright (C) 2009 ACS)

Answer 1252:



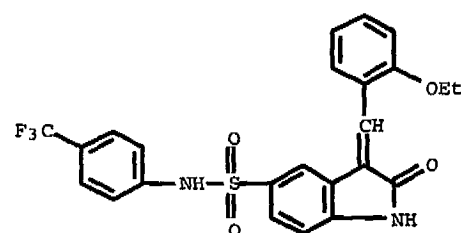
REGISTRY (Copyright (C) 2009 ACS)

Answer 1253:



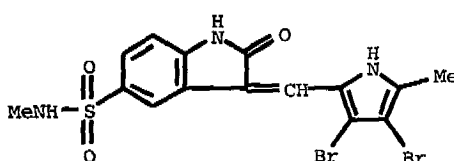
REGISTRY (Copyright (C) 2009 ACS)

Answer 1254:



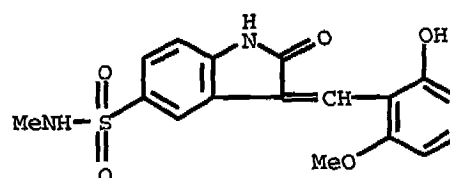
REGISTRY (Copyright (C) 2009 ACS)

Answer 1255:



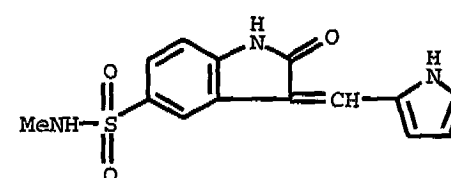
REGISTRY (Copyright (C) 2009 ACS)

Answer 1256:



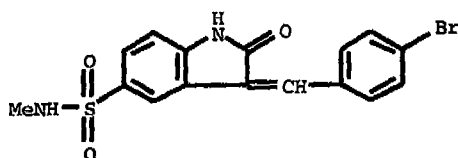
REGISTRY (Copyright (C) 2009 ACS)

Answer 1257:



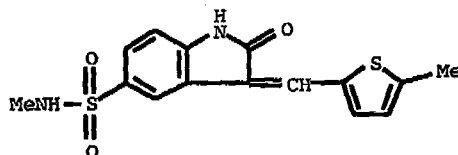
REGISTRY (Copyright (C) 2009 ACS)

Answer 1258:



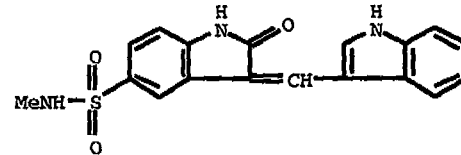
REGISTRY (Copyright (C) 2009 ACS)

Answer 1259:



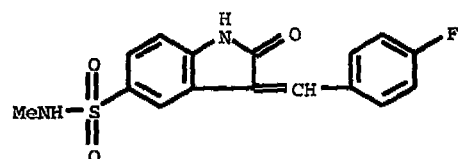
REGISTRY (Copyright (C) 2009 ACS)

Answer 1260:



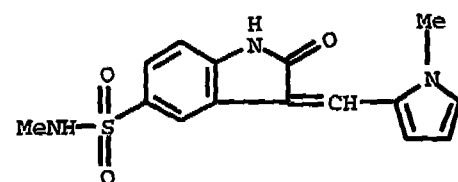
REGISTRY (Copyright (C) 2009 ACS)

Answer 1261:



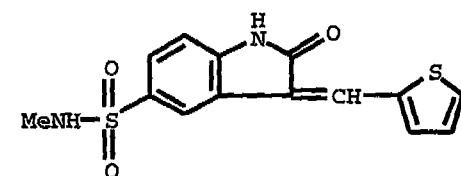
REGISTRY (Copyright (C) 2009 ACS)

Answer 1262:



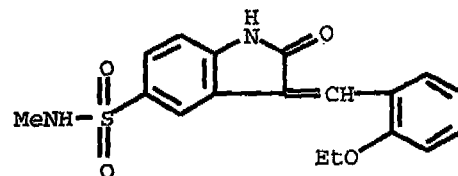
REGISTRY (Copyright (C) 2009 ACS)

Answer 1263:



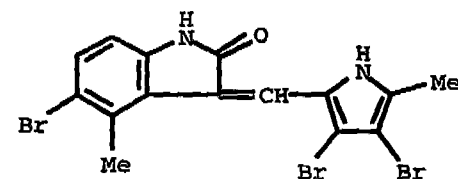
REGISTRY (Copyright (C) 2009 ACS)

Answer 1264:



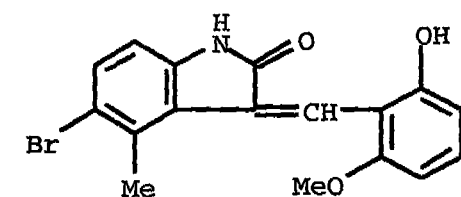
REGISTRY (Copyright (C) 2009 ACS)

Answer 1265:



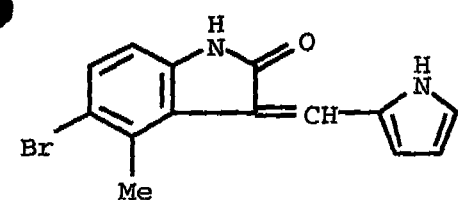
REGISTRY (Copyright (C) 2009 ACS)

Answer 1266:



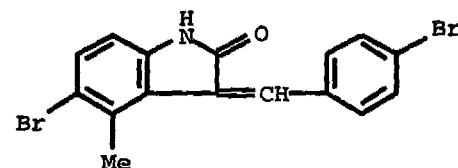
REGISTRY (Copyright (C) 2009 ACS)

Answer 1267:



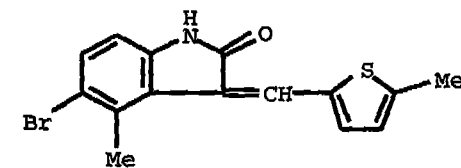
REGISTRY (Copyright (C) 2009 ACS)

Answer 1268:



REGISTRY (Copyright (C) 2009 ACS)

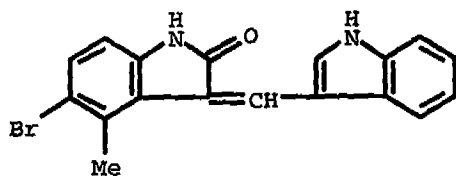
Answer 1269:



REGISTRY (Copyright (C) 2009 ACS)

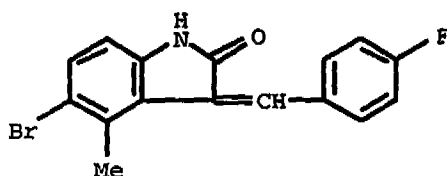
1222
127
1256

Answer 1270:



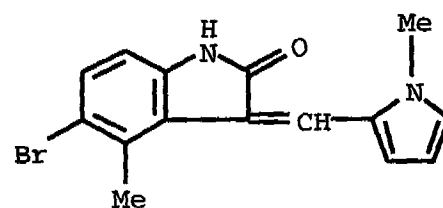
REGISTRY (Copyright (C) 2009 ACS)

Answer 1271:



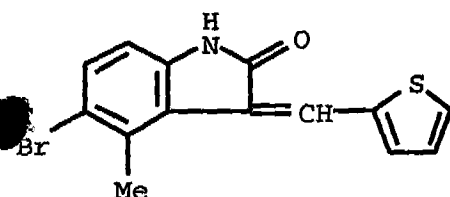
REGISTRY (Copyright (C) 2009 ACS)

Answer 1272:



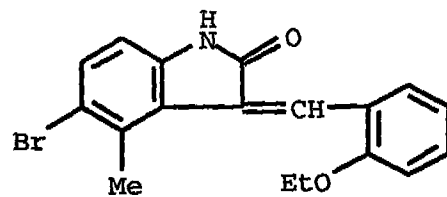
REGISTRY (Copyright (C) 2009 ACS)

Answer 1273:



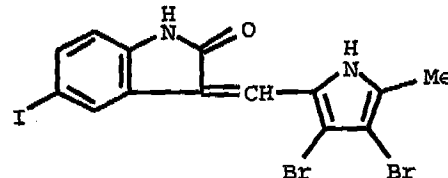
REGISTRY (Copyright (C) 2009 ACS)

Answer 1274:



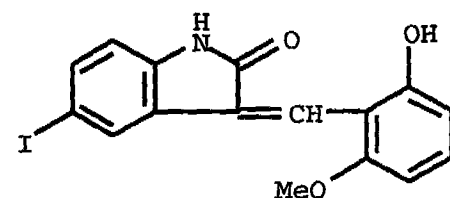
REGISTRY (Copyright (C) 2009 ACS)

Answer 1275:



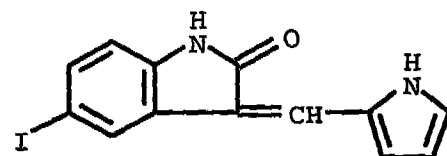
REGISTRY (Copyright (C) 2009 ACS)

Answer 1276:



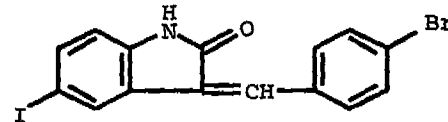
REGISTRY (Copyright (C) 2009 ACS)

Answer 1277:



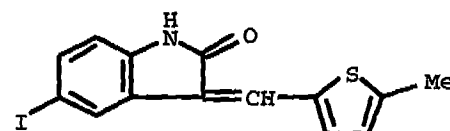
REGISTRY (Copyright (C) 2009 ACS)

Answer 1278:



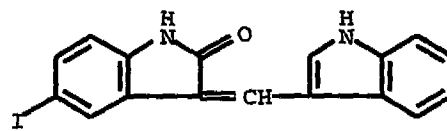
REGISTRY (Copyright (C) 2009 ACS)

Answer 1279:



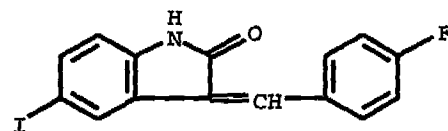
REGISTRY (Copyright (C) 2009 ACS)

Answer 1280:



REGISTRY (Copyright (C) 2009 ACS)

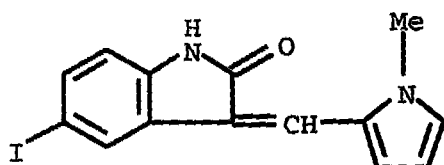
Answer 1281:



REGISTRY (Copyright (C) 2009 ACS)

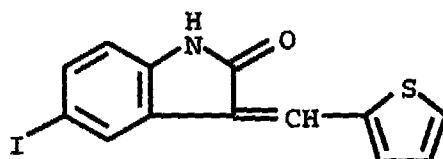
1224
128
1257

Answer 1282:



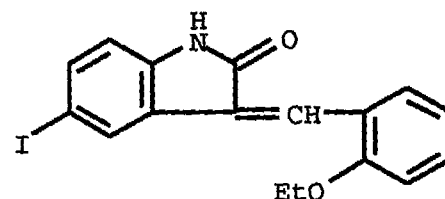
REGISTRY (Copyright (C) 2009 ACS)

Answer 1283:



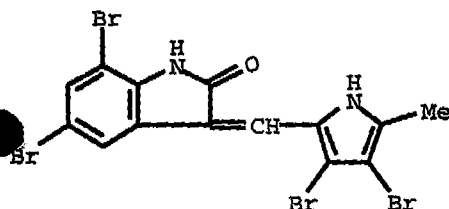
REGISTRY (Copyright (C) 2009 ACS)

Answer 1284:



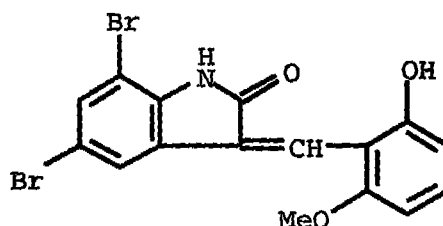
REGISTRY (Copyright (C) 2009 ACS)

Answer 1285:



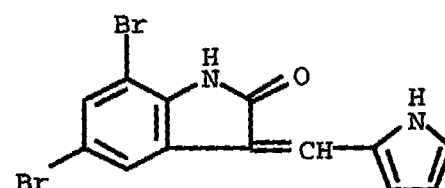
REGISTRY (Copyright (C) 2009 ACS)

Answer 1286:



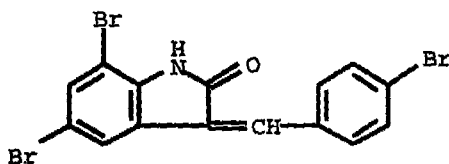
REGISTRY (Copyright (C) 2009 ACS)

Answer 1287:



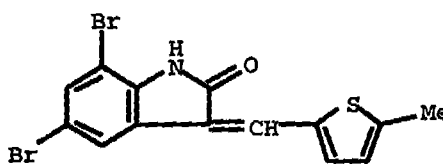
REGISTRY (Copyright (C) 2009 ACS)

Answer 1288:



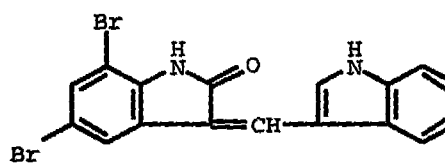
REGISTRY (Copyright (C) 2009 ACS)

Answer 1289:



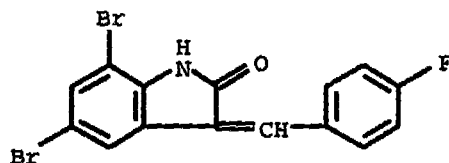
REGISTRY (Copyright (C) 2009 ACS)

Answer 1290:



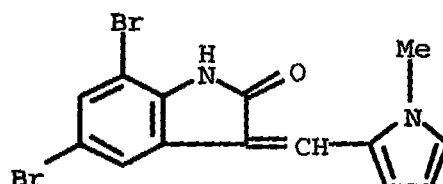
REGISTRY (Copyright (C) 2009 ACS)

Answer 1291:



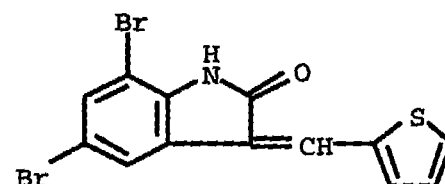
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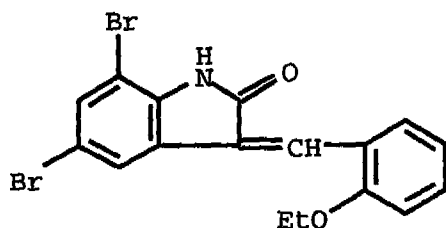
REGISTRY (Copyright (C) 2009 ACS)

Answer 1293:



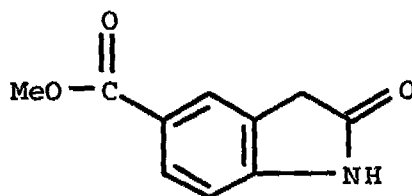
REGISTRY (Copyright (C) 2009 ACS)

Answer 1294:



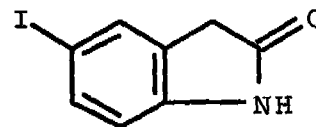
REGISTRY (Copyright (C) 2009 ACS)

Answer 1295:



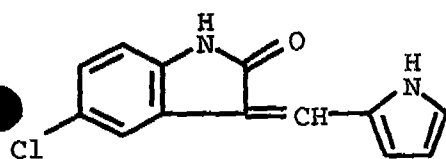
REGISTRY (Copyright (C) 2009 ACS)

Answer 1296:



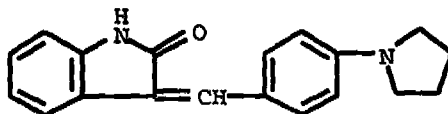
REGISTRY (Copyright (C) 2009 ACS)

Answer 1297:



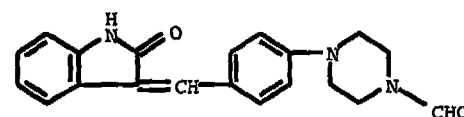
REGISTRY (Copyright (C) 2009 ACS)

Answer 1298:



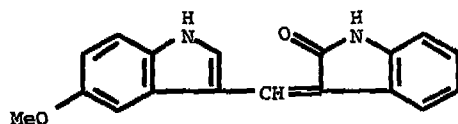
REGISTRY (Copyright (C) 2009 ACS)

Answer 1299:



REGISTRY (Copyright (C) 2009 ACS)

Answer 1300:



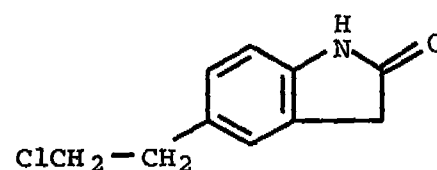
REGISTRY (Copyright (C) 2009 ACS)

Answer 1301:

No Structure
Diagram
Available

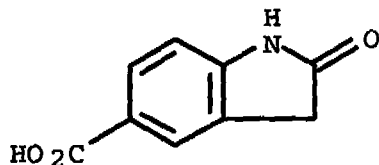
REGISTRY (Copyright (C) 2009 ACS)

Answer 1302:



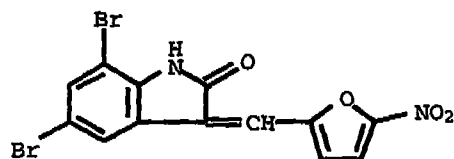
REGISTRY (Copyright (C) 2009 ACS)

Answer 1303:



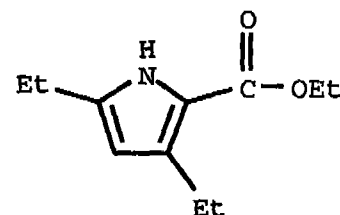
REGISTRY (Copyright (C) 2009 ACS)

Answer 1304:



REGISTRY (Copyright (C) 2009 ACS)

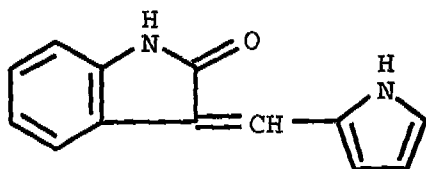
Answer 1305:



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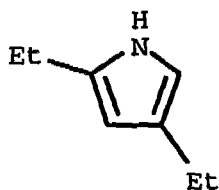
~~301226~~ Page: 1259

Answer 1306:



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Answer 1307:



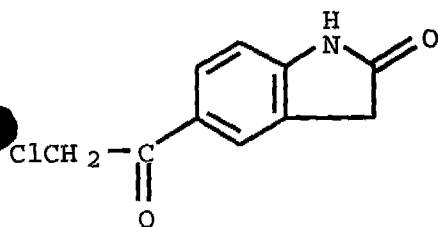
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Answer 1308:

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Diagram
Available**

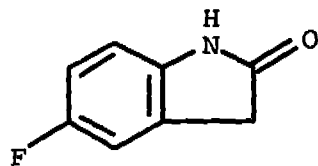
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Answer 1309:



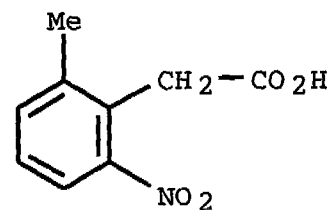
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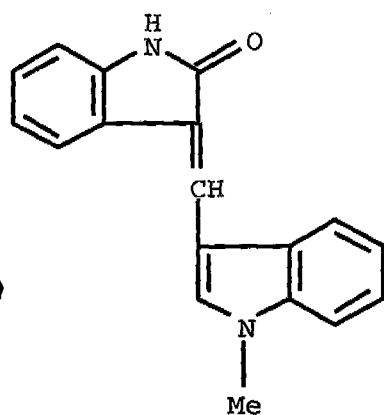
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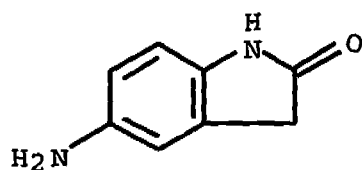
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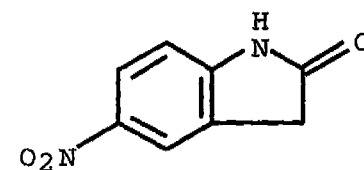
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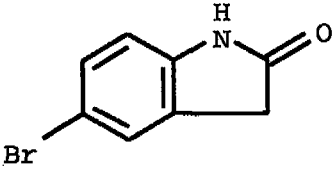
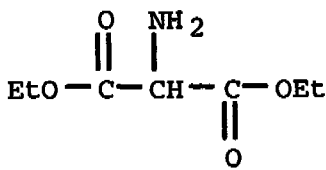
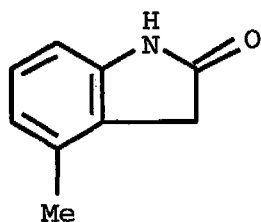
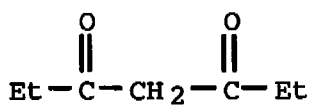
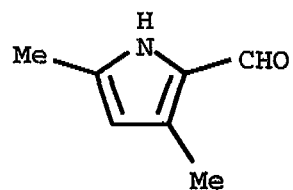
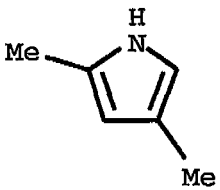
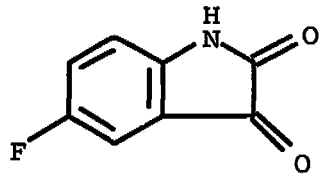
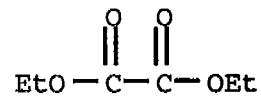
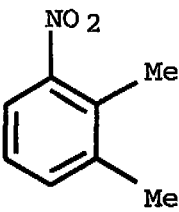
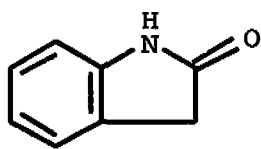


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Answer 1314:



REGISTRY (Copyright (C) 2009 ACS)

Answer 1315:  REGISTRY (Copyright (C) 2009 ACS)	Answer 1316:  • HCl REGISTRY (Copyright (C) 2009 ACS)	Answer 1317:  REGISTRY (Copyright (C) 2009 ACS)
Answer 1318: Structure Diagram Available REGISTRY (Copyright (C) 2009 ACS)	Answer 1319:  REGISTRY (Copyright (C) 2009 ACS)	Answer 1320:  REGISTRY (Copyright (C) 2009 ACS)
Answer 1321:  REGISTRY (Copyright (C) 2009 ACS)	Answer 1322:  REGISTRY (Copyright (C) 2009 ACS)	Answer 1323:  REGISTRY (Copyright (C) 2009 ACS)
Answer 1324:  REGISTRY (Copyright (C) 2009 ACS)	Answer 1325:  REGISTRY (Copyright (C) 2009 ACS)	

1270 1228 1262

Discovery of 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic Acid (2-Diethylaminoethyl)amide, a Novel Tyrosine Kinase Inhibitor Targeting Vascular Endothelial and Platelet-Derived Growth Factor Receptor Tyrosine Kinase

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Received September 24, 2002

Abstract: To improve the antitumor properties and optimize the pharmaceutical properties including solubility and protein binding of indolin-2-ones, a number of different basic and weakly basic analogues were designed and synthesized. 5-[5-Fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide (**12b** or SU11248) has been found to show the best overall profile in terms of potency for the VEGF-R2 and PDGF-R β tyrosine kinase at biochemical and cellular levels, solubility, protein binding, and bioavailability. **12b** is currently in phase I clinical trials for the treatment of cancers.

Introduction. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors have been well validated as targets for the treatment of cancers because of their critical roles in tumor growth and survival via autocrine and paracrine loops.^{1–4} In this regard, both receptor tyrosine kinases (RTKs) have been found to be expressed on the tumor cells and to directly affect tumor cell proliferation (e.g., VEGF receptor in melanoma and PDGF receptor in gliomas).¹ In addition, both RTKs have been found to play prominent roles in tumor angiogenesis by participating in the transmission of proliferation, migration, differentiation, and survival signals between tumor cells and endothelial cells.^{2–4} Thus, simultaneous inhibition of both endothelial growth factor receptor-2 (VEGF-R2) and platelet-derived growth factor receptor- β (PDGF-R β) might be expected to show better antitumor activity than by inhibiting only one of these RTKs. In the past several years, we have taken two indolin-2-ones, **5a** (SU5416) (VEGF-R selective inhibitor⁵) and **5b** (SU6668) (a potent and selective PDGF-R β tyrosine kinase inhibitor) (Table 1), to clinical trials for the treatment of cancers.^{6,7} However, their low solubility and/or high protein binding properties were considered to be potential liabilities. In this study, efforts have been focused on finding an indolin-2-one analogue that shows potent and broad inhibitory activity against both VEGF-R2 and

PDGF-R β and has good pharmaceutical properties with regard to solubility and protein binding. We report here the design, synthesis, and structure–activity relationship that led to the discovery of **12b** (Table 1),⁸ a potent tyrosine kinase inhibitor targeting VEGF-R2 and PDGF-R β .

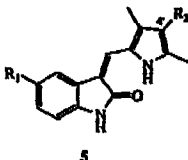
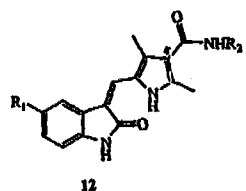
Chemistry. To broaden the kinase selectivity spectrum of indolin-2-one for both VEGF-R2 and PDGF-R β and to optimize the pharmaceutical properties including solubility and protein binding, diversification at the C-4' position on the pyrrole ring of **5a** has been explored. We learned from the past structure–activity relationship studies on indolin-2-ones that modifications at the C-4' position could lead to compounds with different kinase inhibition profiles for VEGF-R2 and PDGF-R β . In this regard, neutral **5a**¹² has been found to be a potent and selective inhibitor for VEGF-R2 while acidic **5b**⁶ was found to be such for PDGF-R β . On the other hand, the cocrystal structure of **5b** in the catalytic domain of the FGF-R1 kinase,⁶ a kinase that has high amino acid sequence homology for the ATP-binding pocket to VEGF-R2, revealed that the substitution at the C-4' position on the pyrrole ring is positioned close to the opening of the binding pocket and could be exposed to solvent. Thus, substitution at this position might serve as a handle for improving pharmaceutical properties of the indolin-2-ones. In this study, various basic side chains have been introduced at the C-4' position of **5a** to broaden the kinase inhibition spectrum and to improve pharmaceutical properties (particularly solubility) of indolin-2-ones. First, we introduced the aminoalkyl functionality in **5a** (**5c** in Scheme 1). Starting from **1**,⁷ **5c** was synthesized by amidation with *N*-methylpiperazine, reduction of the amide functionality, formylation, and condensation with the indolin-2-one. Further diversification at the C-4' position on the pyrrole ring of **5a** was also explored by introducing soluble aminoalkylamide functionalities as depicted in Scheme 2 (**12a–j**). The key intermediate **10** was prepared from commercially available 3-oxobutyric acid *tert*-butyl ester (**6**) by condensing with sodium nitrite in acetic acid, reductive cyclization with 3-oxobutyrate ethyl ester, hydrolytic decarboxylation and formylation, and base hydrolysis of the ethyl ester of **9**. Condensation of **10** with different indolin-2-ones afforded **11**, which upon amidation with different amines was converted to **12a–j**. Most of the amines used are commercially available except the amines (Scheme 3) for synthesizing **12g** and **12j**.

Results and Discussion. The biological activities of the synthesized compounds were first evaluated in biochemical assays measuring tyrosine phosphorylation of VEGF-R2, PDGF-R β , fibroblast growth factor receptor-1 (FGF-R1), and epidermal growth factor receptor (EGF-R) as described previously.^{6,7} Potent and selective inhibitors against VEGF-R2 and PDGF-R β were subsequently tested for their kinase inhibitory activity in 3T3 cells, inhibitory activity against ligand-induced cellular proliferation (as measured by BrdU incorporation in 3T3 cells), cellular cytotoxicity, and solubility at pH 2 and 6 (Table 1).

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Table 1. In Vitro Kinase Inhibitory Activities and Solubility

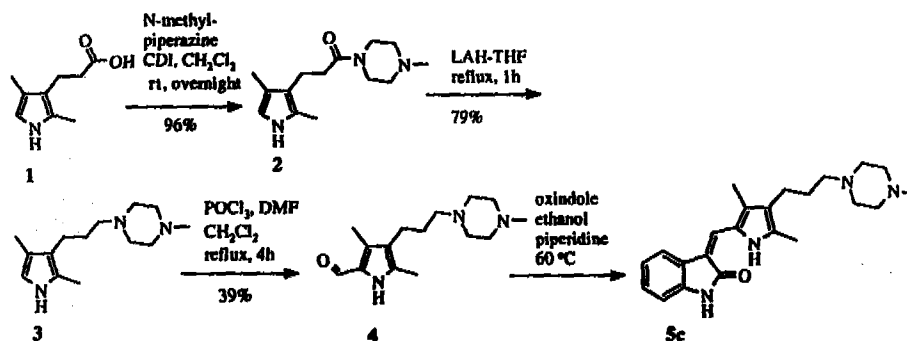
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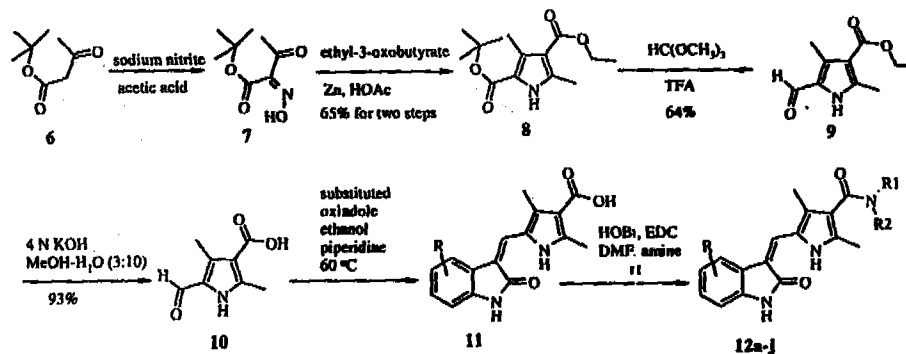
compd	R1	R2	biochemical activity against kinases IC ₅₀ , ^a μM				cellular kinase activity in 3T3 cell IC ₅₀ , ^a μM		PDGF-induced BrdU incorporation IC ₅₀ , ^a μM	cytotoxicity LD ₅₀ , ^a μM	solubility, ^b μg/mL	
			VEGF-R2	PDGF-Rβ	FGF-R1	EGF-R	VEGF	PDGF			pH 2	pH 6
5a	H	H	1.23	22.9	>100	>100	1.04	20.3	4.05	>50	<1	<1
5b	H	(CH ₂) ₂ COOH	2.4	0.060	3.00	>20	1–2	0.1–1.0	16	>50	<5	18
5c	H	(CH ₂) ₃ N(CH ₂ CH ₂) ₂ NCH ₃	0.3	0.060	4.20	>20	<i>d</i>	<i>d</i>	0.20	>50	<i>d</i>	<i>d</i>
12a	H	(CH ₂) ₂ N(C ₂ H ₅) ₂	0.050	0.017	0.88	>20	0.05–0.5	0.1–1.0	<0.07	>50	3022	511
12b	F	(CH ₂) ₂ N(C ₂ H ₅) ₂	0.080	0.0020	2.90	>20	0.005–0.05	0.01	0.008	48.9	2582	364
12c	Cl	(CH ₂) ₂ N(C ₂ H ₅) ₂	0.027	0.0030	0.17	>20	0.005–0.05	0.01–0.1	<0.07	15.6	3259	186
12d	Br	(CH ₂) ₂ N(C ₂ H ₅) ₂	0.032	0.0050	0.73	10	0.005–0.05	0.01–0.1	<0.07	16.3	1299	101
12e	F	(CH ₂) ₂ N(CH ₃) ₂	0.080	0.0005	1.60	>20	0.005–0.05	0.01–0.1	0.015	48.5	3012	75
12f	F	(CH ₂) ₂ -pyrrolidin-1-yl	0.060	0.0010	3.90	>20	0.005–0.05	0.01–0.1	<0.07	49	3319	9
12g	F	CH ₂ CH(CH ₂ CH ₂) ₂ N–CH ₃	0.025	0.0030	0.20	<i>d</i>	0.04	0.02	0.030	38	>250	>250
12h	F	(CH ₂) ₂ -morpholin-4-yl	0.090	0.0020	2.60	>20	0.05	<i>d</i>	0.037	>50	2945	0.3
12i	F	CH ₂ -pyridin-4-yl	<0.16	0.0010	3.10	>20	0.005–0.05	0.01–0.1	0.080	>50	486	<LD ^c
12j	F	(CH ₂) ₂ -triazol-1-yl	0.085	0.010	17.1	>20	0.05–0.5	0.1–1.0	0.19	>50	2	6

^a IC₅₀ and LD₅₀ values were determined by at least two separate tests and reported as mean values. ^b Solubility of the compounds was determined in 20 mM buffered solutions (pH 2, KCl/HCl; pH 6, phosphate) after shaking for 24 h at 22 °C. Data presented are from a single determination or an average of two determinations. ^c LD = limit for detection. ^d Not tested.

Scheme 1. Synthesis of 5c



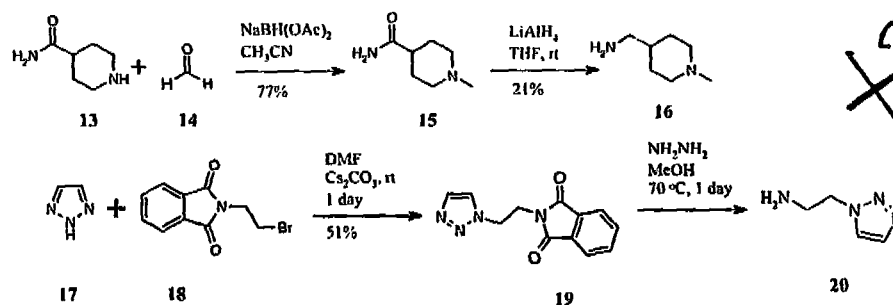
Scheme 2. Synthesis of 4'-Carboxamide Analogues of 5a



It is interesting to note that converting the carboxylate group of 5b into an amino group (5c) enhances the inhibitory activity for VEGF-R2 (8-fold) while retaining the potency against PDGF-Rβ kinase (Table 1). The difference in potency observed between inhibition of PDGF-induced BrdU incorporation and inhibition of cellular kinase activity for 5b might be related to its poor solubility (Table 1). The SAR implied that a basic analogue of 5a has shown broader kinase inhibitory

activity against both VEGF-R2 and PDGF-Rβ than neutral (i.e. 5a-VEGF-R-selective inhibitor⁹) and an acid analogue (i.e., 5b-PDGF-Rβ-selective inhibitor), confirming that the kinase selectivity could be affected by a substituent at the C-4' position.

Being encouraged by the above results, we extensively explored other possibilities for diversification at the C-4' position and found that the readily accessible 12 showed the most promise. 12a–d exhibited even more potent

Scheme 3. Synthesis of Amines for **12g** and **12j**Table 2. Effects of Protein Binding on Cellular Potency^a

compd	PDGF-induced BrdU Incorporation in 3T3 cells, IC ₅₀ (μM)				
	at 0% BSA	at 0.1% BSA	at 0.5% BSA	at 1% BSA	at 5% BSA
5b	0.3	9.5	16	36	49
12b	<0.007	<0.007	<0.007	0.008	0.083

^a BSA = bovine serum albumin.

inhibitory activity against VEGF-R2 and PDGF-R β in biochemical and cellular assays (Table 1). Specifically, compared to **5b**, **12b** is about 30-fold more potent against VEGF-R2 and PDGF-R β in biochemical assays, over 10-fold more potent in cellular kinase assays, and significantly more soluble under neutral (20-fold) and acidic (>500-fold) conditions. Among **12a–d**, **12a** is markedly less potent in inhibiting PDGF-R β phosphorylation in biochemical and cellular assays. Different halogen substitutions had little effect on the biochemical activities against VEGF-R2 and PDGF-R β but affected the cytotoxicity profiles. For example, the bulkier Cl and Br substitutions (**12c** and **12d**) show some extent of cytotoxicity when compared to the F analogue (**12b**) (Table 1). Therefore, diversification on **12b** has been further explored.

Surprisingly, replacing the diethylamine in **12b** by either dimethylamine (**12e**) or pyrrolidine (**12f**) markedly reduced solubility at pH 6 (from 364 to 75 or 9 μg/mL, respectively), while the *in vitro* activities were similar (Table 1). The more basic *N*-methylpiperidine group (which significantly increased solubility of the quinazoline-based VEGF-R2/EGF-R inhibitor (4-bromo-2-fluorophenyl)[6-methoxy-7-(1-methylpiperidin-4-yl-methoxy)quinazolin-4-yl]amine or ZD6474)¹⁰ also improved the solubility of **12g**. However, this substitution increased cytotoxicity and decreased metabolic stability because of *N*-demethylation (data not shown). The much less basic morpholino analogue **12h** is also much less soluble at pH 6, though it has good solubility at pH 2. The basic heteroaromatic analogues **12i** and **12j** have generally poor solubility and sometimes reduced cellular potency (**12j** in Table 1).

To assess the protein binding properties for **5b** and **12b**, cellular IC₅₀ values were measured in the presence or absence of serums. Compared to **5b**, the cellular potency of **12b** against PDGF-induced proliferation is less likely to be affected by the presence of serum protein (Table 2). In this regard, **12b** was still a very potent inhibitor of PDGF-R β while **5b** became inactive in the presence of a high level of serum.

In conclusion, **12b** possesses the best overall profile in terms of inhibitory potency against the VEGF-R2 and

PDGF-R β targets in biochemical and cellular assays, solubility under both neutral and acidic conditions, and protein binding properties. Additional kinase selectivity study revealed that **12b** is also a good inhibitor of KIT and FLT-3.¹³ Furthermore, **12b** also had very good oral bioavailability, was highly efficacious in a number of preclinical tumor models, and was well tolerated at efficacious doses.⁸ It is currently in clinical phase I trials for the treatment of cancers.

Supporting Information Available: Synthesis of **12b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- Cherrington, J. M.; Strawn, L. M.; Shawver, L. K. New paradigms for the treatment of cancer; the role of anti-angiogenesis agents. In *Advances in Cancer Research*, 1st ed.; Klein, G., Van de Woude, G. F., Eds.; Academic Press: San Diego, CA, 2000; Vol. 70, pp 1–38.
- Gale, N. W.; Yancopoulos, G. D. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. *Genes Dev.* **1999**, *13*, 1055–1066.
- Ferrara, N. VEGF: an update on biological and therapeutic aspects. *Curr. Opin. Biotechnol.* **2000**, *11*, 617–624.
- Rosenkranz, S.; Kazlauskas, A. Evidence for distinct signaling properties and biological responses induced by the PDGF receptor alpha and beta subtypes. *Growth Factors* **1999**, *16*, 201–216.
- Sun, L.; Tran, N.; Tang, T.; App, H.; Hirth, P.; McMahon, G.; Tang, C. Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity towards particular receptor tyrosine kinases. *J. Med. Chem.* **1998**, *41* (14), 2588–2603.
- Laird, A. D.; Vajkoczy, P.; Shawver, L. K.; Thurnher, A.; Liang, C.; Mohammadi, M.; Schlessinger, J.; Ullrich, A.; Hubbard, S. R.; Blake, R. A.; Fong, A. T.; Strawn, L. M.; Sun, L.; Tang, C.; Hawtin, R.; Tang, F.; Shenoy, N.; Hirth, K. P.; McMahon, G.; Cherrington, J. M. SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res.* **2000**, *60*, 4152–4160.
- Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L. K.; McMahon, G.; Tang, C. Design, synthesis, and evaluations of substituted 3-[(3- or 4-carboxyethyl)pyrrol-2-ylmethylidene]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF receptor tyrosine kinases. *J. Med. Chem.* **1999**, *42*, 5120–5130.
- Mendel, D. B.; Laird, A. D.; Xin, X.; Louie, S. G.; Christensen, J. G.; Li, G.; Schreck, R. E.; Abrams, T. J.; Ngai, T. J.; Lee, L. B.; Murray, L. J.; Carver, J.; Chan, E.; Moss, K. G.; Haznedar, J. O.; Sukbunthorn, J.; Blake, R. A.; Sun, L.; Tang, C.; Miller, T.; Shirazian, S.; McMahon, G.; Cherrington, J. M. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting VEGF and PDGF receptors: Determination of a pharmacokinetic/pharmacodynamic relationship. *Clin. Cancer Res.* **2003**, *9*, 327–337.
- Fong, A. T. T.; Shawver, L. K.; Sun, L.; Tang, C.; App, H.; Powell, T. J.; Kim, Y. H.; Schreck, R.; Wang, X. Y.; Risau, W.; Ullrich, A.; Hirth, K. P.; McMahon, G. SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res.* **1999**, *59*, 99–106.

- (10) Hennequin, L. F.; Stokes, E. S. E.; Thomas, A. P.; Johnstone, C.; Ple, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. Novel 4-anilinoquinazolines with C-7 basic side chains: Design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *J. Med. Chem.* **2002**, *45*, 1300-1312.
- (11) Liao, A. T.; Chien, M. B.; Shenoy, N.; Mendel, D. B.; McMahon, G.; Cherrington, J. M.; London, C. A. Inhibition of constitutively active form of mutant kit by multitargeted indolinone tyrosine kinase inhibitors. *Blood* **2002**, *100*, 585-593.
- (12) Fong, T. A. T.; Shawver, L. K.; Sun, L.; Tang, C.; App, H.; Powell, T. J.; Kim, Y. H.; Schreck, R.; Wang, X.; Risau, W.; Ullrich, A.; Hirth, K. P.; McMahon, G. SU5416 is a potent and selective

inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res.* **1999**, *59* (1), 99-106.

- (13) Mendel, D. B.; Laird, A. D.; Xin, X.; Abrams, T. J.; Li, G.; Schreck, R. E.; Carver, J.; Louie, S. G.; Ngai, T. J.; Lee, L. B.; Nannini, M. A.; Murray, L. J.; Tang, C.; McMahon, G.; Cherrington, J. M. In vivo antitumor and mechanism of action studies of SU11248, a potent and selective inhibitor of the VEGF and PDGF receptors. Presented at the American Association for Cancer Research, 2002; Poster 5349.

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D₂.

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1266

unsubstituted lower alkyl and carbonyl substituted with hydrogen.

Examples of specific compounds of this invention are shown in Tables 1, 2, 3 and 4, below. The compounds in
5 Tables 1, 2, 3, and 4 are shown by way of example and are not to be construed as limiting the scope of this invention in any manner whatsoever.

Table 1

10	indolinone	3-[3-(2-carboxyethyl-4-methylpyrrol-2-methylidenyl)-2-	1
	linone	3- ~~2-methyl-3,4-dimethylpyrrol-5-methylidenyl~~ -2-indo-	2
		denyl]-2-indolinone	
		3-[4-(2-methoxycarbonyl-ethyl-3-methylpyrrol-2-methylidenyl)-2-indolinone	3
15	2-indolinone	3-(2,4-dimethyl-3-ethoxycarbonylpyrrol-5-methylidenyl)-	4
		2-indolinone	
		3-[2-ethoxycarbonyl-3-(2-ethoxycarbonyl-ethyl)-4-ethoxycarbonylmethylpyrrol-5-methylidenyl]-2-indolinone	5
20	indolinone	3-(2-carboxy-4-ethyl-3-methylpyrrol-5-methylidenyl)-2-	6
		indolinone	
		3-[2-chloro-4-methoxycarbonyl-3-methoxycarbonylmethylpyrrol-5-methylidenyl]-2-indolinone	7
		3-(4-acetyl-2-ethoxycarbonyl-3-methylpyrrol-5-methylidenyl)-2-indolinone	
25	indolinone	3-(4-ethoxycarbonyl-3-methylpyrrol-2-methylidenyl)-2-	
		indolinone	
		3-[4-(2-methoxycarbonyl-ethyl)-3-methylpyrrol-2-methylidenyl]-5,6-dimethoxy-2-indolinone	
		3-(2,4-dimethyl-3-ethoxycarbonylpyrrol-5-methylidenyl)-	
30	5-(4-methoxycarbonylbenzamido)-2-indolinone		

1233
1267

TABLE 3

MASTER BARCODE	NAME
10717/H02	3-(2-ethoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10717/H03	3-[(thien-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10717/H04	3-[(1-methylpyrrol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10717/H05	3-(4-fluorobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10717/H06	3-[(indol-3-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10717/H07	3-[(2-methylthien-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10717/H08	3-(4-bromobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10717/H09	3-[(pyrrol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10717/H10	3-(2-hydroxy-6-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10717/H11	3-[(3,4-dibromo-2-methylpyrrol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H02	3-[(2,4-dimethyl-3-ethoxycarbonylpyrrol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H03	3-(3-bromo-2-hydroxy-5-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10718/H04	3-[(1-hydroxynaph-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H05	3-[(2-ethoxycarbonyl-3-(2-ethoxycarbonyl)ethyl-4-(ethoxycarbonylmethyl)pyrrol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H06	3-[(2-methyl-3-ethoxycarbonylfuran-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H07	3-[(2,3-dimethoxycarbonyl-5-methylpyrrol-4-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H08	3-(4-chloro-3-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10718/H09	3-(2,4-dihydroxy-3-methylbenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10718/H10	3-[(furan-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10718/H11	3-[(2-nitrofuran-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10719/H02	3-(4-ethoxy-3-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H03	3-(3,4-dihydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H04	3-(2,4-dimethoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H05	3-[(2,4-dimethyl-3-ethylpyrrol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10719/H06	3-(2,4,6-trimethoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H07	3-(4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H08	3-(4-dimethylaminobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H09	3-(2-chloro-4-fluorobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H10	3-(3-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10719/H11	3-[4-fluoro-2-(trifluoromethyl)benzylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10720/H02	3-(2,4,6-trifluorobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H03	3-(4-hydroxy-2-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H04	3-(3,4-dimethoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H05	3-(2-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H06	3-benzylidenyl-5,7-dibromo-4-methyl-2-indolinone
10720/H07	3-[(2-methylmercaptothien-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10720/H08	3-(2,4-dihydroxy-6-methylbenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H09	3-(3-ethoxy-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H10	3-(2-hydroxy-5-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10720/H11	3-[(imidazol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H02	3-[(1-methylbenzimidazol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone

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10721/H03	3-[(4-chloro-1-methylpyrazol-3-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H04	3-[(2,3-dimethylthien-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H05	3-[(4,5,6,7-tetrahydroindol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H06	3-(3-chloromethyl-2-hydroxy-5-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10721/H07	3-[(2-chlorothien-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H08	3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10721/H09	3-(3-t-butyl-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10721/H10	3-(3-bromo-5-t-butyl-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10721/H11	3-(3,5-di-t-butyl-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H02	3-(3-t-butyl-4-hydroxy-5-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H03	3-(2,4,6-trihydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H04	3-[(2-nitrothien-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10722/H05	3-(4-carboxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H06	3-(2,4-difluorobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H07	3-(3,5-dimethyl-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H08	3-(3-t-butyl-5-chloro-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H09	3-[(2-nitrothien-4-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10722/H10	3-(4-di-n-butylaminobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10722/H11	3-[4-(trifluoromethyl)benzylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10723/H02	3-(2,3,4-trihydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H03	3-(2-hydroxy-3-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H04	3-(3-bromo-4,5-dihydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H05	3-(3,4-diacetoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H06	3-(4-hydroxy-3-methylbenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H07	3-(2-bromobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H08	3-(2,4-dihydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H09	3-(2-hydroxy-4-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H10	3-(3-bromobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10723/H11	3-(3,5-di-t-butyl-2-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10724/H02	3-[(1-dimethylaminonaph-4-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10724/H03	3-(4-hydroxy-3-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10724/H04	3-(3-hydroxy-4-nitrobenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10724/H05	3-[(6-hydroxy-2,3,8,7-tetrahydro-1H,5H-benzo[<i>h</i>]quinolizin-9-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10724/H06	3-(3,5-diisopropyl-4-hydroxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10724/H07	3-[(benzo[<i>b</i>]furan-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10724/H08	3-(2-hydroxy-4,6-dimethoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10724/H09	3-[1-(4-chlorophenyl)pyrrol-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10724/H10	3-[(2-ethylfuran-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10724/H11	3-[(3,4-dimethylthieno[2,3- <i>b</i>]thien-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10725/H02	3-[(3-bromothien-2-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10725/H03	3-(2-bromo-6-hydroxy-5-methoxybenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10725/H04	3-[(2-methylfuran-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10725/H05	3-[(3-methylpyrazol-5-yl)methylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10725/H06	3-(2-hydroxy-6-methoxy-4-methylbenzylidenyl)-5,7-dibromo-4-methyl-2-indolinone
10725/H07	3-[4-(4-formylpiperazin-1-yl)benzylidenyl]-5,7-dibromo-4-methyl-2-indolinone
10725/H08	3-[4-(morpholin-1-yl)benzylidenyl]-5,7-dibromo-4-methyl-2-indolinone

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Table 13

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MASTER BARCODE	PLATE ROW	PLATE COLUMN	NAME
10717	A	2	3-(2-ethoxybenzylidenyl)-5,7-dibromo-2-indolinone
10717	A	3	3-[(thien-2-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	A	4	3-[(1-methylpyrrol-2-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	A	5	3-(4-fluorobenzylidenyl)-5,7-dibromo-2-indolinone
10717	A	6	3-[(indol-3-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	A	7	3-[(2-methylthien-5-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	A	8	3-(4-bromobenzylidenyl)-5,7-dibromo-2-indolinone
10717	A	9	3-[(pyrrol-2-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	A	10	3-(2-hydroxy-6-methoxybenzylidenyl)-5,7-dibromo-2-indolinone
10717	A	11	3-[(3,4-dibromo-2-methylpyrrol-5-yl)methylidenyl]-5,7-dibromo-2-indolinone
10717	B	2	3-(2-ethoxybenzylidenyl)-5-iodo-2-indolinone
10717	B	3	3-[(thien-2-yl)methylidenyl]-5-iodo-2-indolinone
10717	B	4	3-[(1-methylpyrrol-2-yl)methylidenyl]-5-iodo-2-indolinone
10717	B	5	3-(4-fluorobenzylidenyl)-5-iodo-2-indolinone
10717	B	6	3-[(indol-3-yl)methylidenyl]-5-iodo-2-indolinone

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Table 13
(continued)

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10717	B	7	3-[(2-methylthien-5-yl)methylidenyl]-5-iodo-2-indolinone
10717	B	8	3-(4-bromobenzylidenyl)-5-iodo-2-indolinone
10717	B	9	3-[(pyrrol-2-yl)methylidenyl]-5-iodo-2-indolinone
10717	B	10	3-(2-hydroxy-6-methoxybenzylidenyl)-5-iodo-2-indolinone
10717	B	11	3-[(3,4-dibromo-2-methylpyrrol-5-yl)methylidenyl]-5-iodo-2-indolinone
10717	C	2	3-(2-ethoxybenzylidenyl)-5-bromo-4-methyl-2-indolinone
10717	C	3	3-[(thien-2-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	C	4	3-[(1-methylpyrrol-2-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	C	5	3-(4-fluorobenzylidenyl)-5-bromo-4-methyl-2-indolinone
10717	C	6	3-[(indol-3-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	C	7	3-[(2-methylthien-5-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	C	8	3-(4-bromobenzylidenyl)-5-bromo-4-methyl-2-indolinone
10717	C	9	3-[(pyrrol-2-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	C	10	3-(2-hydroxy-6-methoxybenzylidenyl)-5-bromo-4-methyl-2-indolinone
10717	C	11	3-[(3,4-dibromo-2-methylpyrrol-5-yl)methylidenyl]-5-bromo-4-methyl-2-indolinone
10717	D	2	3-(2-ethoxybenzylidenyl)-5-methylaminosulfonyl-2-indolinone

Table 14

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Barcode/ Plate Row- Plate Column	Fik Kinase	Biochem EGFR	PDGF Kinase	Met Kinase
	%Inhibition	%Inhibition	%Inhibition	% Inhibition
10717/A02	0.1	24.3		46.1
10717/A03	2.9	1.0		54.6
10717/A04	-4.5	29.0		37.4
10717/A05	-2.6	16.6		35.6
10717/A06	-10.8	-7.8		31.7
10717/A07	-6.4	20.2		29.2
10717/A08	-5.2	39.1		21.7
10717/A09	-3.9	37.7		9.4
10717/A10	-3.3	8.1		71.6
10717/A11	-5.8	59.9		64.8
10717/B02	5.0	31.7		87.5
10717/B03	-8.8	7.3		90.5
10717/B04	-18.3	10.3		70.0
10717/B05	1.0	31.7		87.4
10717/B06	5.4	-30.8		89.5
10717/B07	-18.3	58.3		90.0
10717/B08	-0.9	60.5		88.8
10717/B09	-40.7	78.3		88.8
10717/B10	-2.3	18.1		56.1
10717/B11	11.4	82.7		91.0
10717/C02	4.1	-0.4		29.7
10717/C03	-7.7	18.0		25.3
10717/C04	-0.8	14.4		25.0
10717/C05	-2.3	13.1		44.6
10717/C06	7.6	-49.7		44.1
10717/C07	1.6	28.7		16.5
10717/C08	7.0	24.3		27.3
10717/C09	77.1	8.1		47.7
10717/C10	-8.0	17.5		22.8
10717/C11	4.6	67.3		71.8
10717/D02	5.1	10.1		28.6
10717/D03	1.1	-1.4		11.1
10717/D04	-2.1	4.9		21.0
10717/D05	-3.8	-2.8		23.8
10717/D06	1.0	-23.4		33.8
10717/D07	-8.4	-4.5		16.8
10717/D08	6.8	-7.8		16.0
10717/D09	-55.0	6.8		29.1
10717/D10	-6.0	3.5		15.6
10717/D11	11.6	59.1		55.3
10717/E02	17.9	17.2		24.0
10717/E03	19.0	11.7		52.2
10717/E04	6.1	-28.3		29.4
10717/E05	13.2	22.4		39.1
10717/E06	7.5	-28.1		24.8
10717/E07	15.3	-7.8		41.0
10717/E08	13.2	28.2		51.7
10717/E09	-1.1	-5.8		19.2
10717/E10	4.7	-6.1		35.9
10717/E11	8.9	44.9		75.1

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Epidermal Growth Factor Stimulates Vascular Endothelial Growth Factor Production by Human Malignant Glioma Cells: A Model of Glioblastoma Multiforme Pathophysiology

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Submitted November 9, 1992; Accepted December 2, 1992

Hypervascularity, focal necrosis, persistent cerebral edema, and rapid cellular proliferation are key histopathologic features of *glioblastoma multiforme* (GBM), the most common and malignant of human brain tumors. By immunoperoxidase and immunofluorescence, we definitively have demonstrated the presence of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFr) in five out of five human glioma cell lines (U-251MG, U-105MG, D-65MG, D-54MG, and CH-235MG) and in eight human GBM tumor surgical specimens. In vitro experiments with glioma cell lines revealed a consistent and reliable relation between EGFr activation and VEGF production; namely, EGF (1–20 ng/ml) stimulation of glioma cells resulted in a 25–125% increase in secretion of bioactive VEGF. Conditioned media (CM) prepared from EGF-stimulated glioma cell lines produced significant increases in cytosolic free intracellular concentrations of Ca^{2+} ($[\text{Ca}^{2+}]_i$) in human umbilical vein endothelial cells (HUVECs). Neither EGF alone or CM from glioma cultures prepared in the absence of EGF induced $[\text{Ca}^{2+}]_i$ increases in HUVECs. Preincubation of glioma CM with A4.6.1, a monoclonal antibody to VEGF, completely abolished VEGF-mediated $[\text{Ca}^{2+}]_i$ transients in HUVECs. Likewise, induction by glioma-derived CM of von Willebrand factor release from HUVECs was completely blocked by A4.6.1 pretreatment. These observations provide a key link in understanding the basic cellular pathophysiology of GBM tumor angiogenesis, increased vascular permeability, and cellular proliferation. Specifically, EGF activation of EGFr expressed on glioma cells leads to enhanced secretion of VEGF by glioma cells. VEGF released by glioma cells in situ most likely accounts for pathognomonic histopathologic and clinical features of GBM tumors in patients, including striking tumor angiogenesis, increased cerebral edema and hypercoagulability manifesting as focal tumor necrosis, deep vein thrombosis, or pulmonary embolism.

INTRODUCTION

It is an intriguing fact that *glioblastoma multiforme* (GBM), the most anaplastic of malignant brain tumors, is also the most common (>65%) of primary intracranial malignant tumors in adults. In almost every biochemical, immunological, morphological, and biological category examined, GBM tumors exhibit striking heterogeneity

(Bigner *et al.*, 1981; Wikstrand *et al.*, 1985). The degree of heterogeneity associated with these tumors has made it difficult to unravel the molecular and cellular mechanisms underlying GBM pathogenesis.

Histopathologically, GBM are characterized by striking pleomorphism in cellular and nuclear detail with pathognomonic features of focal necrosis and neovascularization. It is the appearance of necrotic foci that

shifts the diagnosis from anaplastic astrocytoma (grade III) to GBM (grade IV) (Fulling and Nelson, 1984; Burger and Kleihues, 1989), which has a documented poorer prognosis (Burger and Green, 1987). Other characteristic histopathological features of this uniformly fatal brain tumor relevant to this report include marked endothelial cell proliferation and increased vascular permeability. Biochemical analyses of tissue culture media conditioned either by GBM tumor explants or U-251MG, an established glioma cell line, indicated the presence of vascular permeability factor (VPF)-like activity (Crisuolo *et al.*, 1989).

VPF, also known as vascular endothelial cell growth factor (VEGF), is a potent and specific endothelial cell mitogen *in vitro* (Leung *et al.*, 1989) and induces angiogenesis *in vivo* (Conn *et al.*, 1990). VEGF is 1000-fold more potent than histamine in inducing capillary permeability (Connolly, 1991). Furthermore, VEGF increases von Willebrand Factor (vWF) release (Brock *et al.*, 1991) and thromboplastin activity (Clauss *et al.*, 1990) in human endothelial cells, two physiological changes that could enhance coagulation. Taken together, these important biological activities indicate that VEGF may play major roles in the angiogenesis and increased edema characteristic of malignant gliomas and may contribute to hypercoagulability in these patients (Bostrom *et al.*, 1986).

Recent studies indicate that almost all malignant glioma cells and cell lines express the receptor for epidermal growth factor (EGFr), and the majority of GBM tumors show amplification or rearrangement of the EGFr gene (Sang *et al.*, 1989; Blumenstock *et al.*, 1991; Maruno *et al.*, 1991). Furthermore, Maruno *et al.* (1991) have shown an increased proliferative index for EGFr-positive malignant glioma cells as compared with glioma cells that do not express this receptor. The implication is that activation of EGFr may play a role in the persistent proliferation of GBM. EGF is a low molecular weight (6 kDa) growth factor that is a normal constituent of human plasma (Tuomela, 1990) and is mitogenic for cells expressing EGFr. Several groups also have demonstrated that some gliomas show increased mRNA levels and express transforming growth factor- α (TGF- α), which can activate EGFr *in vitro* (Maxwell *et al.*, 1991) in an equimolar fashion equivalent to EGF. Therefore, the ligand(s) responsible for activating EGFr, i.e., EGF and TGF- α , are either constitutively present in plasma and plasma ultrafiltrates or can be produced by glioma cells themselves.

Until now, there has been no demonstrated relation between EGFr expression in gliomas and associated vascularity of these tumors. In this study we demonstrate the simultaneous expression of VEGF and EGFr in five out of five brain tumor cell lines examined. In eight postoperative surgical specimens, both VEGF and EGFr were determined by immunohistochemical means to be variably expressed. More importantly, we dem-

onstrate for the first time that activation of EGFr by EGF leads to increased secretion of bioactive VEGF by malignant glioma cells. This physiological relation provides a useful paradigm to explain the paradoxical existence of intense neovascularity and necrotic foci in GBM tumors.

MATERIALS AND METHODS

Biochemical and Biological Reagents

Dulbecco's modified Eagle's medium mixed 1:1 with Ham's F-12 (DMEM/F12; Sigma Chemical, St. Louis, MO) and MCDB 105 (Sigma) supplemented to 2 mM with L-glutamine were used for culture of all glioma cells. Medium 199 (M199; GIBCO, Grand Island, NY) was used as the culture medium for human umbilical vein endothelial cells (HUVEC). Fetal bovine serum (FBS) and calf serum (Hyclone, Logan, UT) were heat inactivated (56°C, 45 min). EGF (human, receptor grade; Collaborative Research, Bedford, MA), dexamethasone (Sigma), β -phorbol (Sigma), and phorbol myristate acetate (PMA; Sigma) were all diluted at specified concentrations in Dulbecco's phosphate-buffered saline (PBS, pH 7.2). Transforming growth factor- β 1 (TGF- β 1) (R&D Systems, Minneapolis, MN) was prepared as 1 μ g/ml stock solution by dilution in endpilot (25% isopropyl alcohol + 0.1% trifluoroacetic acid). Further dilution was carried out in PBS containing 3 mM CHAPS (Boehringer Mannheim, Mannheim, Germany). Recombinant VEGF (rhVEGF; 165 amino acid isoform) was purified at Genentech (South San Francisco, CA). Purified guinea pig VPF was used as a positive control for vWF assay and was obtained as a generous gift from Dr. Donald Senger (Beth Israel Hospital, Boston, MA). Fura-2/AM was purchased from Molecular Probes (Junction City, OR). Culture media and sera were determined to have <100 pg/ml of endotoxin by the Limulus amoebocyte lysate assay.

Cell Lines

GBM cell lines U-251MG, U105MG, D-65MG, and D-54MG were obtained as a gift from Dr. Darell D. Bigner (Duke University, Durham, NC) and have been described in detail (Ponten and MacIntyre, 1968; Ponten and Westermarck, 1978; Bigner *et al.*, 1981). CH-235MG was cultured from a portion of a GBM tumor removed in 1979 from a 60-year-old female and recently has been characterized (Bethea *et al.*, 1992a). Karyotypic analyses of these cell lines have revealed that each is karyotypically unique with complex numerical and structural deviations. Karyotypes for U-251MG, U105MG, D-65MG, and D-54MG were similar to those previously reported (Bigner and Mark, 1984; Bigner *et al.*, 1985). Moreover, restriction fragment length polymorphism mapping profiles produced by restricting cell line DNA with *Hae* III and probing Southern blots with YNH24 (locus D2S44, 0.96 polymorphic discrimination) and pH30 (locus D4S139, 0.99 polymorphic discrimination) were nonidentical, corroborating that these tumor cell lines originated from different patients. All of the glioma cell lines have been passaged >40 times, and, for experiments, the cell lines were maintained in 150-cm² plastic tissue culture flasks (Falcon Plastics, Lincoln Park, NJ) in complete culture media (DMEM/F12 + 2 mM L-glutamine + 8% FBS) at 37°C and 7.5% CO₂. Antibiotics were not used routinely, and all cell lines were found by routine testing to be negative for mycoplasma by Hoechst 33258 stain (Chen, 1977). Cells were grown to postconfluence with medium changes every third day and were harvested using brief incubation (10 min, room temperature [RT]) in 7 ml 0.05% Trypsin/0.53 mM EDTA solution [GIBCO]. FBS (2 ml) was added to the flask to inactivate the trypsin, and adherent cells were released by trituration, pelleted by centrifugation (200 $\times$ g, 8 min, 22°C), and resuspended in 10 ml of DMEM/F12. Viable cell counts were determined by trypan blue exclusion.

Human Endothelial Cell Cultures

Primary cultures of human endothelial cells (HUVECs) were established from pooled umbilical cords and were propagated (passages

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Article

Design, Synthesis, and Evaluations of Substituted 3-[(3- or 4-Carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as Inhibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases

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J. Med. Chem., 1999, 42 (25), pp 5120-5130

DOI: 10.1021/jm9904295

Publication Date (Web): November 19, 1999

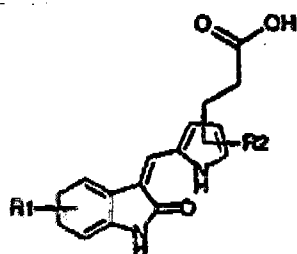
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Abstract

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Receptor tyrosine kinases (RTKs) have been implicated as therapeutic targets for the treatment of human diseases including cancers, inflammatory diseases, cardiovascular diseases including arterial restenosis, and fibrotic diseases of the lung, liver, and kidney. Three classes of 3-substituted indolin-2-ones containing propionic acid functionality attached to the pyrrole ring at the C-3 position of the core have been identified as catalytic inhibitors of the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) RTKs. Some of the compounds were found to inhibit the tyrosine kinase activity associated with isolated vascular endothelial growth factor receptor 2 (VEGF-

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Sun, Li

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History

Published In Issue
December 16, 1999

R2) [fetal liver tyrosine kinase 1 (Flk-1)/kinase insert domain-containing receptor (KDR)], fibroblast growth factor receptor (FGF-R), and platelet-derived growth factor receptor (PDGF-R) tyrosine kinase with IC_{50} values at nanomolar level. Thus, compound 1 showed inhibition against VEGF-R2 (Flk-1/KDR) and FGF-R1 tyrosine kinase activity with IC_{50} values of 20 and 30 nM, respectively, while compound 16f inhibited the PDGF-R tyrosine kinase activity with IC_{50} value of 10 nM. Structural models and structure-activity relationship analysis of these compounds for the target receptors are discussed. The cellular activities of these compounds were profiled using cellular proliferation assays as measured by bromodeoxyuridine (BrdU) incorporation. Specific and potent inhibition of cell growth was observed for some of these compounds. These data provide evidence that these compounds can be used to inhibit the function of these target receptors.

Received August 23, 199

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Journal of Medicinal Chemistry

Article

Synthesis and Biological Evaluations of 3-Substituted Indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particular Receptor Tyrosine Kinases

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J. Med. Chem., 1998, 41 (14), pp 2588-2603

DOI: 10.1021/jm980123i

Publication Date (Web): June 9, 1998

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Abstract

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Abstract

3-Substituted indolin-2-ones have been designed and synthesized as a novel class of tyrosine kinase inhibitors which exhibit selectivity toward different receptor tyrosine kinases (RTKs). These compounds have been evaluated for their relative inhibitory properties against a panel of RTKs in intact cells. By modifying the 3-substituted indolin-2-ones, we have identified compounds which showed selective inhibition of the ligand-dependent autophosphorylation of various RTKs at submicromolar levels in cells. Structure-activity analysis for these compounds and their relative potency and selectivity to inhibit particular RTKs has determined that (1) 3-[(five-membered heteroaryl ring)methylidenyl]indolin-2-ones are highly specific against the VEGF (*Flk-1*) RTK activity, (2) 3-(substituted benzylidenyl)indolin-2-ones containing bulky group(s) in the phenyl ring at the C-3 position of indolin-2-ones showed high selectivity toward the EGF and Her-2 RTKs, and (3) the compound containing an extended side chain at the C-3 position of the indolin-2-one (16) exhibited high potency and selectivity when tested against the PDGF and VEGF (*Flk-1*) RTKs. Recent published crystallographic data for two of these 3-substituted indolin-2-ones provides a rationale to suggest that these compounds may bind in the ATP binding pocket of RTKs. The structure-activity analysis supports the use of subsets of

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History

Published In Issue
July 02, 1998

Received February 24, 1998

These compounds as specific chemical leads for the development of RTK-specific drugs with broad application for the treatment of human diseases.

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Small Molecule Inhibitors of the Platelet-Derived Growth Factor Receptor, the Fibroblast Growth Factor Receptor, and Src Family Tyrosine Kinases

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ABSTRACT. The inhibition of tyrosine kinases involved in growth factor signal transduction pathways represents an attractive strategy for controlling aberrant cellular growth. Over the last 4-5 years, there have been numerous reports on the discovery of small molecule inhibitors for potential therapeutic applications to a number of proliferative diseases, principally cancer and restenosis, where the over-expression of certain tyrosine kinases has been demonstrated. These include, amongst others, the platelet-derived growth factor receptor, the fibroblast growth factor receptor, and the nonreceptor c-Src tyrosine kinase. This review compiles published reports and patent filings from 1995 to mid-1997 that include data directly related to inhibition of the platelet-derived growth factor receptor, fibroblast growth factor receptor, and Src family tyrosine kinases. Potential clinical applications for selected classes of tyrosine kinase inhibitors reviewed herein will likely depend on the demonstration of meaningful activity in a variety of therapeutic targets in animal models. PHARMACOL. THER. 76(1-3):55-71, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Small molecule inhibitors, platelet-derived growth factor receptor (PDGFr) tyrosine kinase, fibroblast growth factor receptor (FGFr) tyrosine kinase, Src family tyrosine kinases, phosphorylation.

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ABBREVIATIONS. aFGF, acidic FGF; bFGF, basic FGF; CHO, Chinese hamster ovary; COX, cyclooxygenase; EGF, epidermal growth factor; EGFr, EGF receptor; FGF, fibroblast growth factor; FGFr, FGF receptor; Hck, haematopoietic cell kinase; IL, interleukin; Ins, insulin; LPS, lipopolysaccharide; MAP, mitogen-activated protein; PDGF, platelet-derived growth factor; PDGFr, PDGF receptor; PI, phosphatidylinositol; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; Sam, Src associated in mitosis; SAR, structure-activity relationship.

1. INTRODUCTION

The response of cells to secreted growth factors and other mitogens is mediated by complex intracellular networks that regulate metabolism, differentiation, and proliferation. These networks are made up of specific receptor and nonreceptor proteins, including protein tyrosine kinases (Saltiel, 1995).

Occasionally, these regulatory networks cease to function properly, due to overactivation or mutation of these kinases, resulting in the over-expression of growth factors or their cognate receptors. This state of dysregulation of signaling pathways has been shown to lead to various diseases including proliferative disorders, such as cancer (Kelloff *et al.*, 1996), atherosclerosis (Reidy and Bendeck, 1997), immunodeficiency (Ochs, 1996), and restenosis (Bornfeldt, 1996). Thus, the inhibition of tyrosine kinases involved in

[‡]Corresponding author.

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growth factor signal transduction pathways represents an attractive strategy for controlling aberrant cellular growth.

The proliferation of vascular smooth muscle cells of the arterial wall has been associated with the formation and progression of lesions in restenosis following angioplasty (Jackson and Schwartz, 1992). Numerous *in vivo* studies reveal that this is mediated in part by the platelet-derived growth factor (PDGF) (Lindner *et al.*, 1995) and the fibroblast growth factor (FGF) (Lindner and Reidy, 1993). Additionally, c-Src, a nonreceptor membrane-associated tyrosine kinase that binds via its Src homology-2 domain and becomes phosphorylated by the PDGF receptor (PDGFr) (Alonso *et al.*, 1995), is thought to be important for the mitogenic effects of PDGF. It has been suggested that restenosis may in part result from a cascade of early events that trigger the early expression of cytokines and growth factors, which, in turn, could stimulate multiple cell types via paracrine and autocrine mechanisms to regulate their own expression. Thus, redundant signaling pathways could be involved that could circumvent any therapeutic agent aimed at blocking a specific growth factor or cytokine. Within this context, we have embarked on a program for the potential treatment of restenosis by attempting to uncover broadly acting, nonselective small molecule inhibitors of the PDGFr, FGFr, and c-Src tyrosine kinases that might serve to overcome these redundancies in growth signaling and prevent the accelerated proliferation and migration of cells that contribute to the formation of the restenotic lesion.

A second component of our program has been to initiate studies toward evaluating these same inhibitors in a number of tumor models that over-express these three tyrosine kinases. The PDGFr has been implicated in the mitogenesis and progression of a variety of tumor cell lines and types (Ross *et al.*, 1986; Heldin and Westermark, 1991; Claesson-Welsh, 1994). Ligand binding to this receptor has been shown to cause receptor dimerization, activation of the receptor tyrosine kinase, and the generation of subsequent phosphorylation-dependent mitogenic and proliferative signals. Inhibition of the kinase activity, therefore, may lead to suppression of signals that support proliferation in transformed cells. c-Src kinase and other members of this family participate in a number of signal transduction pathways that are important in proliferation and activation of cellular processes (Bolen *et al.*, 1997). Although evidence for the participation of c-Src alone in transforming events leading to uncontrolled proliferation in tumors is not strong, increased expression or activity of c-Src (or another member of this family) has been associated with transformed phenotypes in connection with other transforming genes (Talamonti *et al.*, 1993; Twamley-Stein *et al.*, 1993). Since c-Src is likely involved in cellular signaling and attenuation, or amplification, of mitogenic signals initiated by other branches of signal transduction pathways, the inhibition of c-Src and other Src kinase family members may provide opportunities for therapeutic intervention in cancer, as well as a variety of other proliferative diseases. Finally, since angiogenesis is an obligatory step in the growth and survival of

solid tumors, this process has been targeted for therapeutic intervention. One approach taken has been the inhibition or neutralization of angiogenic factors elaborated by many tumors. One such factor excreted by some tumor types is FGF. Consequently, inhibition of FGFr kinase may be an effective mechanism to control inappropriate vascularization associated with tumor growth (Castl *et al.*, 1997).

A third component of our program has been to find selective inhibitors of these kinases for use as tools in an attempt to dissect various components of these multiple pathways. There is a burgeoning impetus toward this approach as a result of the recent disclosures of the crystal structures of the FGFr tyrosine kinase in complex with two inhibitors (Mohammadi *et al.*, 1997), and the structures of the Src family tyrosine kinases c-Src (Xu *et al.*, 1997), and the haematopoietic cell kinase (Hck) (Sicheri *et al.*, 1997).

This review broadly covers the literature on studies of small molecule inhibitors of the PDGFr, FGFr, and Src family tyrosine kinases from 1995 through mid-1997. Included are several new studies with older, well-known inhibitors, along with studies of a number of novel small molecule templates. The patent literature during this period is also extensively covered. This review expands significantly on recently published articles that cover small molecule inhibitors of a range of tyrosine kinase classes (Fry, 1994; Fry and Bridges, 1995; Shugar, 1995; Spada and Myers, 1995; Kelloff *et al.*, 1996; Patrick and Heimbrook, 1996), and more specialized coverage such as inhibitors in restenosis (Golomb and Fishbein, 1997), inhibitors of the PDGFr (Bilder and Rojas, 1996), and inhibitors of Src kinase (Levitzi, 1996). A much larger body of literature during this same period covers multiple effects of ligand binding and its antagonism and downstream signal transduction effects. However, these studies were deemed to be outside of the scope of this review. Sample reports of these studies include antagonism with peptides (Edison *et al.*, 1995; Nishi *et al.*, 1996; Lou *et al.*, 1996, 1997), antisense oligonucleotides (Liu *et al.*, 1995; Wang *et al.*, 1996), and immunoconjugates (Chen *et al.*, 1996; Mattar *et al.*, 1996; Uckun, 1996).

2. INHIBITORS OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR TYROSINE KINASE

2.1. Natural Products

Natural product inhibitors of PDGFr tyrosine kinase are shown in Fig. 1. The natural product glucopiericidin A isolated from a *Streptomyces* species has been shown to be a somewhat selective inhibitor of the PDGFr autophosphorylation relative to epidermal growth factor receptor (EGFr) kinase and to affect the PDGF-stimulated turnover of phosphatidylinositol (PI) pools in NIH 3T3 mouse fibroblasts transfected with phospholipase C (PLC)- γ 1 (Ahn *et al.*, 1995). In this system, PI turnover can be mediated through the activation of PDGFr by its ligand. The IC_{50} for PI turnover of this compound in cells was approximately 5 μ M. PLC- γ 1 activity itself was not directly inhibited by this

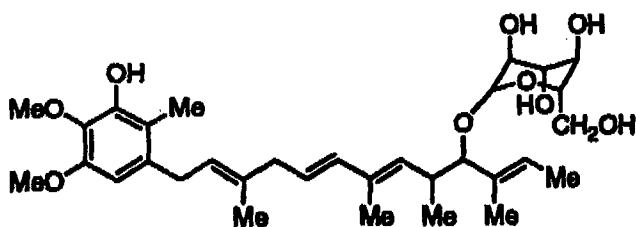
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compound. Inhibition of PDGF-stimulated whole cell phosphotyrosine levels in the PLC- γ 1 transfected NIH 3T3 cells was also apparent after a 5-min pretreatment with 5 μ M compound prior to addition of PDGF and lysis. In contrast, no effect on cellular phosphotyrosine was apparent in A431 carcinoma cells stimulated with EGF after treatment with glucopiericidin A, leading to the conclusion that this compound is PDGF-selective.

Herbimycin A, another *Streptomyces*-derived natural product previously described in the literature as a tyrosine kinase inhibitor, has been examined recently for its effects on vascular smooth muscle proliferation and PDGFr phosphorylation (Shimokado *et al.*, 1995). PDGF-stimulated [^{3} H]thymidine incorporation into rat aorta smooth muscle cells was inhibited with an IC_{50} of 170 nM after a 10-min incubation with compound. However, when examined for inhibition of PDGFr autophosphorylation, herbimycin A was ineffective at concentrations up to 1.7 μ M (highest concentration tested). In addition, PDGF-stimulated expression of c-Fos, a downstream effector of mitogenic signal transduction, was also not inhibited. These data show that herbimycin A is not an inhibitor of PDGF-stimulated tyrosine phosphorylation in cells, and that its effects on DNA synthesis likely arise from other mechanisms.

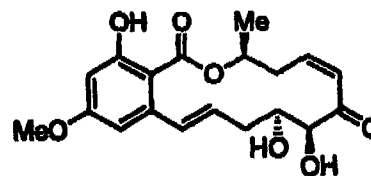
A third natural product with PDGFr kinase inhibitory

activity has been disclosed in a patent application from the group at Cor Therapeutics (South San Francisco, CA, USA) (Giese and Lokker, 1996). This macrocyclic lactone, designated as C292, was derived from fermentation of a *Curvularia* species. The human PDGF- β receptor transfected into a HR5 Chinese hamster ovary (CHO) cell line was used to assess inhibition of PDGFr autophosphorylation. Using immunopurified receptor from cells treated with compound for 15 min prior to stimulation and lysis, an IC_{50} of 6.2 nM was determined for C292. The nonreceptor tyrosine kinase c-Src was inhibited with an IC_{50} of 1 μ M in a purified enzyme assay. EGF α or FGFr autophosphorylation was not affected by concentrations of C292 as high as 30 μ M. Protein kinases A (PKA) and C (PKC) were also not inhibited in isolated enzyme assays by this compound up to 40 μ M. A thymidine incorporation assay was used to determine if C292 was an inhibitor of PDGF-stimulated mitogenesis in a human primary fibroblast cell line (HS68). A 30-min pretreatment of cells with C292, followed by PDGF-BB and 18 hr of incubation with [^{3} H]-labeled thymidine in the presence of the inhibitor, resulted in an IC_{50} of 16 nM. The effect of the inhibitor on proliferation of cells over 96 hr was also investigated. In the same HS68 cells, as well as in 3 other human cell lines (CCD18, HS27, WS1), the IC_{50} was >30 μ M. These data suggest that this com-



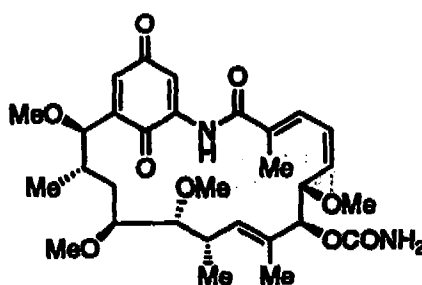
Glucopiericidin A

(Ahn *et al.*, 1995)



C292

(Giese and Lokker, 1996)



Herbimycin A

(Shimokado *et al.*, 1995)

FIGURE 1. Natural product inhibitors of PDGFr tyrosine kinase.

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pound should be able to inhibit PDGFr phosphorylation events at concentrations well below those that might cause general cytotoxic effects. This would make C292 a candidate for therapeutic use in a situation dependent upon PDGF stimulation, such as restenosis, as opposed to a purely antiproliferative utility, as might be envisioned for an anti-tumor agent.

2.2. Synthetic Agents

Synthetic inhibitors of PDGFr tyrosine kinase are shown in Fig. 2. Small molecule synthetic inhibitors of PDGFr kinase have been the focus of effort from a number of groups. Direct comparisons among different compounds are made difficult by the diversity of enzyme assays and cell lines used in the various reports. Most compounds have been characterized at the least by using PDGFr kinase partially purified from a variety of cell lines. Additionally, many reports (but not all) have included some indication of cellular activity of the inhibitors. The potency of the inhibitors against PDGFr kinase autophosphorylation in partially purified enzyme ranges from an IC_{50} of <20 nM (Myers *et al.*, 1995) to 65 nM (Hirth *et al.*, 1995).

2.2.1. Quinoxalines and quinolines. A series of pyrazolo-[3,4-g]quinoxalines has been described in a patent application from Rhone-Poulenc Rorer (Collegeville, PA, USA) to be potent inhibitors of the PDGFr tyrosine kinase (Myers *et al.*, 1995). One of the exemplified compounds (Compound 1, Fig. 2) was reported to have an IC_{50} of <20 nM in an assay with partially purified PDGFr from NIH 3T3 cells. No additional information regarding selectivity or mode of inhibition was given.

Another compound described by a group at Sugen (Redwood City, CA, USA) (Hirth *et al.*, 1995) is a quinoxaline labeled as G25. It showed an IC_{50} of $\sim 1 \mu M$ for inhibition of PDGFr autophosphorylation and an IC_{50} of $\sim 50 \mu M$ for inhibition of proliferation of C6 rat glioma cells in culture. The IC_{50} for inhibition of kinase activity from EGFr, Her-2, or Flk-1 was $>50 \mu M$, suggesting that this compound may have some selectivity toward PDGFr kinase inhibition.

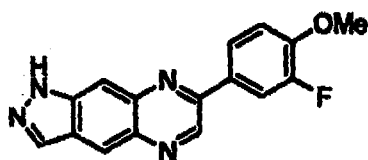
Another closely related quinoxaline (Compound 2, Fig. 2) of a large series structurally related to G25 has been briefly characterized for its PDGFr inhibition in whole Swiss 3T3 cells treated for 15 min with inhibitor prior to PDGF stimulation and lysis of the cells (Gazit *et al.*, 1996). Compound 2 had an IC_{50} of 400 nM. These workers also reported that the quinoline congener, Compound 3, displayed an IC_{50} of $1.5 \mu M$ against PDGFr from membrane fragments (as opposed to the whole cell assay of Compound 3), and it showed ~ 30 -fold greater potency toward inhibition of PDGFr than EGFr kinase.

Still another related quinoxaline compound, AG1296, has been thoroughly characterized for its inhibitory activity toward the partially purified PDGF- β receptor from canine kidney epithelial cells (Kovalenko *et al.*, 1997). This compound was shown not to inhibit the binding of PDGF to its receptor or the dimerization of PDGFr monomers. A ki-

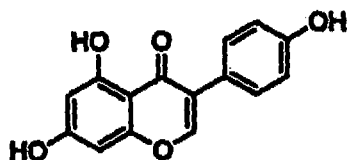
netic analysis of inhibition of PDGFr kinase of purified enzyme suggested that in unactivated kinase (no PDGF stimulation), AG1296 inhibits by an ATP-competitive mechanism. Activated kinase appeared to be inhibited via a mixed competitive mode with respect to ATP. In both cases, the mechanism was mixed-competitive with respect to an exogenous peptide substrate. PI-3 kinase activity was inhibited in an immunoprecipitate assay from cells treated with AG1296, but this downstream effector of PDGFr kinase appeared to be inhibited by virtue of PDGFr inhibition, based on the relative concentrations of AG1296 required for direct inhibition of recombinant purified PI-3 kinase activity in a parallel assay.

2.2.2. Tyrphostins. Recent kinase inhibitory activity in two human cell lines for two compounds, RG 50875 and RG 13022 drawn from a large tyrphostin class, has been published (Weernink *et al.*, 1996). The human glioblastoma A172 and the human glioma Hs683 cell lines were used to evaluate growth and mitogenic effects of these agents. PDGF-stimulated receptor phosphorylation was inhibited by the reference compound genistein at $185 \mu M$ in both cell lines. Genistein also completely inhibited growth factor- or serum-stimulated thymidine incorporation in these cell lines. Due to the apparent nonspecific kinase inhibition and toxicity of genistein in both cell lines, the authors examined the inhibition by the two tyrphostins of thymidine incorporation stimulated by PDGF, EGF, or serum. The glioma line Hs683 was preincubated with inhibitor for 1 hr prior to stimulation with mitogen and the subsequent DNA synthesis assessed. RG 13022 inhibited the process with an IC_{50} of $\sim 20 \mu M$ for both PDGF and EGF; the serum effect was less potently inhibited. RG 50875 inhibited PDGF- or EGF-stimulated DNA synthesis only at $IC_{50} > 50 \mu M$. Because of the inhibition of DNA synthesis in Hs683, cell growth in serum was evaluated as well. RG 13022 inhibited growth with an IC_{50} of $17 \mu M$, and RG 50875 with an IC_{50} of $35 \mu M$. Since RG 50875 exerts its growth-delaying effects at concentrations lower than that seen for inhibition of PDGF-stimulated thymidine incorporation, the question arises as to whether the primary mechanism of action of this compound is via PDGFr inhibition. RG 13022 inhibited cellular growth at a concentration similar to that seen for inhibition of mitogenesis. These data are consistent for this compound's mode of action being the inhibition of PDGFr in this cell line, although data for the direct inhibition of the receptor kinase activity were not presented. RG 13022 was evaluated by another group for *in vivo* efficacy in the human head and neck carcinoma cell line HN5 (McLeod *et al.*, 1996). Examination of the pharmacokinetics showed a rapid elimination of the compound from plasma; no antitumor efficacy was found upon chronic administration. Whether or not RG 13022 directly inhibits PDGFr kinase, whole animal pharmacokinetic results appear to lead to the conclusion that the compound does not remain in plasma with a sufficient half-life or concentration necessary for *in vivo* activity.

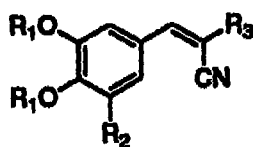
An indolic-type tyrphostin (Compound 4, Fig. 2) has been characterized for its cellular inhibition of PDGF in the



Compound 1
(Myers *et al.*, 1995)



Genistein
(Weemink *et al.*, 1996)

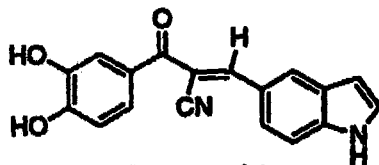


$R_1 = \text{H}, R_2 = \text{OH}, R_3 = \text{CN};$
RG 50875

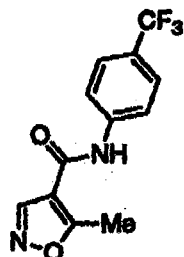
(Weemink *et al.*, 1996)

$R_1 = \text{Me}, R_2 = \text{H}, R_3 = 3\text{-pyridyl};$
RG 13022

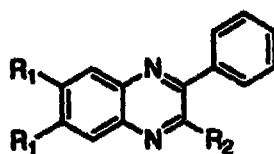
(McLeod *et al.*, 1996)
(Weemink *et al.*, 1996)



Compound 4
(Gazit *et al.*, 1996)



A10 (Leflunomide)
(Hirth *et al.*, 1995)
(Xu *et al.*, 1995)



$R_1 = \text{Me}, R_2 = \text{CO}_2\text{H};$ G25

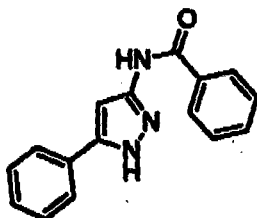
(Hirth *et al.*, 1995)

$R_1 = \text{Me}, R_2 = \text{H};$ Compound 2

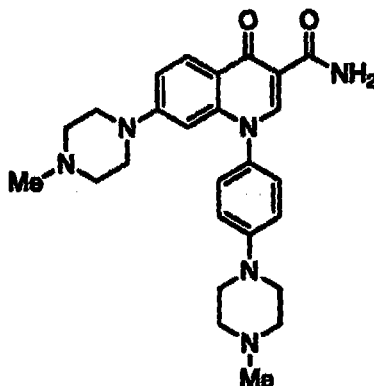
(Gazit *et al.*, 1996)

$R_1 = \text{OMe}, R_2 = \text{H};$ AG1296

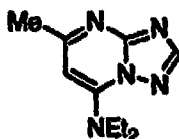
(Kovalenko *et al.*, 1997)



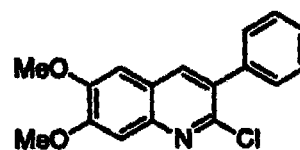
Compound 5
(Giese *et al.*, 1996)



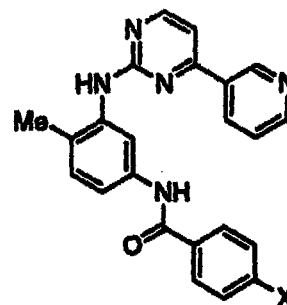
WIN 61651
(Faltynek *et al.*, 1995b)



Trapidil
(Bando *et al.*, 1995)



Compound 3
(Gazit *et al.*, 1996)

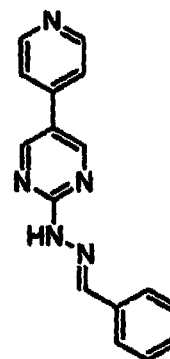


$X = \text{H};$ CGP 53716

(Buchdunger *et al.*, 1995)
(Zhen *et al.*, 1997)

$X = \text{Me};$ CGP 57148

(Buchdunger *et al.*, 1996)
(Zimmerman *et al.*, 1996, 1997)



WIN 41662
(Sawutz *et al.*, 1996)

FIGURE 2. Synthetic inhibitors of PDGFr tyrosine kinase.

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1283

same fashion described in Section 2.2.1 for Compound 3 (Gazit *et al.*, 1996). Compound 4 displayed an IC_{50} of 1 μ M and was ~30-fold more potent as an inhibitor of PDGFr than EGFr kinase.

2.2.3. 2-Anilinoimidazoles. Of all the reported bona fide PDGFr tyrosine kinase inhibitors, perhaps the best characterized for antiproliferative activity is the series of 2-anilinoimidazoles from a group at Ciba-Geigy (Basel, Switzerland). In one study with CGP 53716 (Buchdunger *et al.*, 1995), this compound was shown to inhibit PDGFr kinase autophosphorylation of the isolated enzyme with an IC_{50} of ~100 nM, and did so selectively relative to a large number of other tyrosine and serine/threonine kinases. Of the 21 other kinases evaluated, only v-Abl, c-Fgr, and the cell cycle kinase CDC2/cyclin B were inhibited at concentrations below 30 μ M. The compound also inhibited cellular PDGFr autophosphorylation with an IC_{50} of 30–100 nM. Reduction of PDGF-stimulated c-Fos message and proliferation in BALB/c 3T3 cells with an IC_{50} of 100–300 nM and 600 nM, respectively, suggested that CGP 53716 is a PDGFr kinase inhibitor with activity in some cell lines. It was a much less potent inhibitor of proliferation in an H-Ras transformed bladder carcinoma (T24) with an IC_{50} of 19.1 μ M. The inhibitor also was reported to possess *in vivo* activity against Sis-transformed BALB/c 3T3 cells in xenograft testing. In another study with CGP 53716 (Zhen *et al.*, 1997), preincubation of MC3T3-E1 osteoblast-like cells with 5 μ M of inhibitor for 90 min completely blunted the change in protein tyrosine phosphorylation and the binding of p85 to tyrosine-phosphorylated protein induced by PDGF. Associated with these effects of CGP 53716 on activation of signaling molecules, inorganic phosphate transport-stimulation induced by PDGF was dose-dependent and maximally inhibited by 5 μ M of this agent. These results strongly suggest that tyrosine phosphorylation induced by the PDGFr, including its autophosphorylation, is the initial event responsible for inorganic phosphate transport stimulation induced by PDGF.

A closely related compound in this series, CGP 57148, was also extensively studied relative to its inhibition of the v-Abl kinase (Buchdunger *et al.*, 1996; Zimmermann *et al.*, 1996, 1997). CGP 57148 inhibited PDGFr kinase with potencies similar to those of CGP 53716. In addition, most of the other cellular parameters examined did not differ appreciably from CGP 53716. Since the activities of the two compounds are very similar, functional selectivity between the two, relative to PDGFr kinase vs. v-Abl kinase, is difficult to characterize. One possible approach to address these issues may be to examine the PDGFr status and its phosphorylation state in cells that are sensitive to these compounds (for example, inhibition of proliferation) compared with cell lines that are not as sensitively inhibited. If the (as yet unproved) assumption is correct that a cell that expresses PDGFr is dependent upon it for proliferation or survival, inhibition of proliferation in such a cell line by one of these inhibitors should correlate with PDGFr inhibition.

2.2.4. Other agents. A synthetic small molecule inhibitor of PDGFr has been reported in a patent application (Giese *et al.*, 1996). A substituted pyrazole (Compound 5, Fig. 2) has been described as having an IC_{50} of 220 nM in an assay of PDGFr- β autophosphorylation (PDGF-BB-stimulated) in a transfected CHO cell line. PDGFr- α autophosphorylation stimulated by PDGF-AA was inhibited by Compound 5 with an IC_{50} of 170 nM. In other cell culture lines overexpressing EGFr or FGFr-1, receptor autophosphorylation was not markedly inhibited at inhibitor concentrations of up to 30 μ M. The IC_{50} against isolated c-Src inhibition was 8 μ M. Therefore, this compound is about 3- to >200-fold selective for PDGFr kinase inhibition vs. other tyrosine kinases when assessed by the described assays. One consideration with these numbers that make meaningful comparisons difficult is that the assays vary considerably from one another within this report (isolated enzyme catalytic inhibition data in some instances to immunoprecipitated receptor phosphotyrosine determinations by ELISA in other cases). PKA and PKC (serine/threonine kinases) were not inhibited by Compound 5 at concentrations as high as 40 μ M in isolated enzyme assays. To assess the effect of Compound 5 on mitogenesis, the mouse haematopoietic cell line 32D- α R was used in a PDGF-stimulated labeled thymidine incorporation assay. The IC_{50} in this assay was 700 nM. In other human cell lines (CCD18, HS27, WS1, HS68), the IC_{50} for proliferation in the presence of serum was >30 μ M; in the HR5- β R CHO cell line, the IC_{50} was 28 μ M. Direct comparisons of inhibition between serum- or PDGF-stimulated growth were not made. Neither were such comparisons made for inhibition of serum- or PDGF-stimulated mitogenesis. Therefore, the 40-fold apparent therapeutic index of mitogenesis vs. proliferation is difficult to interpret.

Although directed toward discovery of T-cell-selective tyrosine kinase inhibitors, a program at Sterling-Winthrop (Collegeville, PA, USA) led to the discovery of the quinoline carboxamide WIN 61651, which inhibited PDGFr autophosphorylation with an IC_{50} of 10 μ M (Faltynek *et al.*, 1995b). It also inhibited p56^{lck}, a Src-family kinase important in T-cell signaling, with an IC_{50} of 18 μ M. In the Lck kinase assay, WIN 61651 was characterized as an ATP-competitive inhibitor and was noncompetitive with respect to peptide substrate. Selectivity studies *in vitro* against other kinases showed no meaningful inhibition against PKC or PKA, nor against EGFr, insulin (Ins) receptor, or ErbB-2 kinases at concentrations exceeding 500 μ M when measuring autophosphorylation. WIN 61651 did inhibit tyrosine phosphorylation in a T-cell line assessed by whole cell lysate Western blotting of phosphotyrosine beginning at ~50 μ M of inhibitor and at higher concentrations. Interleukin (IL)-2 production was inhibited by the compound in the 10–30 μ M range in activated CD4⁺ lymphocytes. Since most of the cellular effects of WIN 61651 were described in T-cell systems, the relevance of the *in vitro* PDGFr inhibition data with respect to the mechanism of action of WIN 61651, as it has been characterized, is not clear. From comparisons

with other tyrosine kinase inhibitors, WIN 61651 is neither a potent nor a selective compound.

In a series of biarylhydrazone tyrosine kinase inhibitors, also from the Sterling-Winthrop group, WIN 41662 has been the best characterized of this class (Sawutz *et al.*, 1996). In contrast to WIN 61651, WIN 41662 is a potent and much more selective inhibitor of PDGFr kinase. The partially purified enzyme autophosphorylation IC_{50} was 60 nM and the cellular PDGFr autophosphorylation IC_{50} value was estimated to be 30–100 nM. Since the IC_{50} value increased linearly with the ATP concentration in the enzyme assay, the authors concluded that the inhibition is competitive with ATP, giving a K_i of 15 nM (± 5 nM; $n = 4$). WIN 41662 was examined for inhibitory activity toward other kinases in *in vitro* assays and was found to be inactive. Of the kinases examined (p56^{lck}, EGFr, ErbB-2, PKA, and PKC), only p56^{lck} was modestly inhibited ($IC_{50} = 30 \mu M$). In two other PDGF-stimulated cellular processes, WIN 41662 was also found to be active. Mobilization of intracellular Ca^{2+} is an early step in mitogen-stimulated signaling. In a human vascular smooth muscle cell line, a 5-min pretreatment of cells with inhibitor prior to PDGF stimulation resulted in inhibition of Ca^{2+} release with an IC_{50} of 430 nM. PDGF stimulation of thymidine incorporation in another human vascular smooth muscle line was inhibited with an IC_{50} of 2.0 μM after a 2-hr preincubation step with WIN 41662, followed by a 24-hr period of compound plus PDGF, and an additional 24 hr of label incorporation. This result appears not to be a nonspecific toxicity effect, since only at a much greater concentration of compound was a general cytotoxic effect noted. Although WIN 41662 was a reasonably potent inhibitor of thymidine incorporation stimulated by PDGF, no data were presented demonstrating an effect against either serum-stimulated mitogenesis or against PDGF-stimulated growth (as opposed to only thymidine incorporation), so the effect of the compound in more rigorous cell growth conditions, where multiple signals for mitogenesis and growth are likely to be present, is unknown. Based on a limited set of data for structural analogues of WIN 41662, the authors speculate that the activity of this particular compound may lie in part in the orientation of the phenyl substituent relative to the pyridyl-pyrimidine nucleus.

The effects of trapidil, a triazolo[1,5-a]pyrimidine, on the *in vitro* human glioma cell line T98G stimulated with PDGF or EGF have been described (Bando *et al.*, 1995). Two parameters were characterized: effect on mitogen-stimulated Ca^{2+} flux and effect on whole cell lysate phosphotyrosine levels. Cells pretreated for 5 min with 48 μM trapidil and then stimulated with PDGF were examined for increased Ca^{2+} flux by means of intracellular fluorescence of a Ca^{2+} -sensitive dye. Trapidil completely inhibited this flux, as well as that resulting from stimulation of cells with EGF. The effect of the compound on PDGFr autophosphorylation was examined in whole cell lysates of the T98G cell lines. After a 16-hr preincubation, 240 μM trapidil completely blocked the PDGF-stimulated phosphorylation of a 185 kDa protein band in an antiphosphotyrosine Western

blot that the investigators believed to be the PDGFr. The same concentration of trapidil only partially inhibited the phosphotyrosine level of a 170 kDa band (likely the EGFr) in cells stimulated by EGF. Based on this differential effect on receptor phosphorylation, some selectivity of trapidil for PDGF inhibition was proposed. In this study, no cellular growth or mitogenic effects were described for trapidil, nor was any description of a direct inhibition of the tyrosine kinase activity of purified PDGFr provided. Therefore, the investigators' speculation regarding direct effects of trapidil on PDGF-stimulated proliferation can be considered only provisional.

The inhibition of PDGFr kinase by leflunomide (designated as A10) has been described by a group from Sugen (Hirth *et al.*, 1995). PDGFr autophosphorylation was inhibited with an IC_{50} of 65 mM in U1242 cells assessed by an ELISA immunoprecipitation assay. In these same cells, proliferation (growth on plastic) was inhibited with an IC_{50} of 700 mM. As stated elsewhere (see Section 4.2.3), since the primary leflunomide metabolite inhibits the biosynthesis of pyrimidines with an IC_{50} of 220 nM, the tyrosine kinase inhibitory activity of this compound and its metabolites does not likely contribute to its cellular or *in vivo* mechanism of action.

Further interest in the overall class of PDGFr tyrosine kinase inhibitors reviewed herein will likely depend on the demonstration of meaningful activity in a variety of therapeutic targets in animal models. To date, only the 2-anilino-pyrimidines have been reported to have *in vivo* activity linked to inhibition of the PDGFr kinase.

3. INHIBITORS OF FIBROBLAST GROWTH FACTOR RECEPTOR TYROSINE KINASE

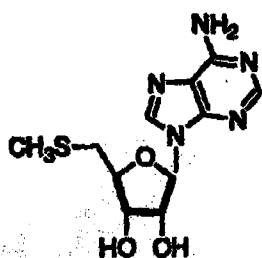
3.1. Natural Products

Inhibitors of FGFr tyrosine kinase are shown in Fig. 3. Abundant FGFr-1 expression and low levels of FGFr-2 are detected in human malignant glioma. Additionally, malignant gliomas express an alternatively spliced form of FGFr-1 (FGFr-1- β) containing two immunoglobulin-like disulfide groups. A study was carried out to examine the effects of the methyltransferase inhibitor 5'-methylthioadenosine on cell growth and FGFr tyrosine kinase activity in human glioma cells (Miyaji *et al.*, 1995). Against these lines, this agent inhibited in a specific and dose-dependent fashion both bFGF-stimulated cell growth and protein tyrosine phosphorylation by blocking both receptor autophosphorylation and substrate phosphorylation. Complete inhibition of cell growth was observed only at 2 mM and of tyrosine phosphorylation at 3 mM. The inhibition was both rapid and readily reversible. 5'-Methylthioadenosine had no effect on either EGF- or PDGF-stimulated protein tyrosine phosphorylation in glioma cells. These investigators concluded that because 5'-methylthioadenosine had no effect on the number and dissociation constants of high- and low-affinity bFGF receptors, the inhibition of bFGF-stimulated bFGFr tyrosine kinase activity was likely not due to a reduction in both bFGF ligand and receptor binding capacity.

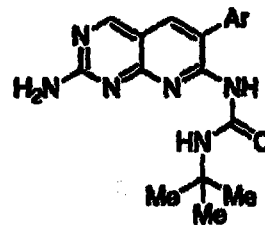
3.2. Synthetic Agents

3.2.1. Pyrido[2,3-d]pyrimidines. In a program initially designed to uncover small molecule inhibitors of the PDGFr- β tyrosine kinase, the Parke-Davis group (Ann Arbor, MI,

USA) uncovered a novel class of inhibitors, the 6-arylpyrido[2,3-d]pyrimidines, against this and other tyrosine kinases through screening of their compound library (Blankley et al., 1996a,b). In detailed studies on the initial lead



5'-Methylthioadenosine
(Miyaji et al., 1995)

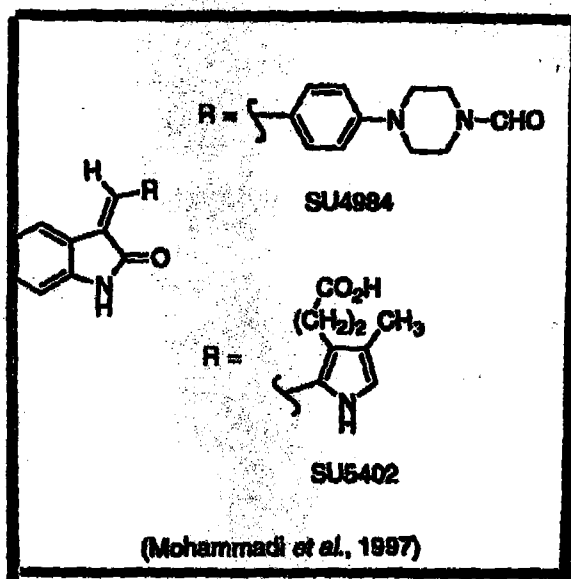


Ar = 2,6-dichlorophenyl; PD 089828

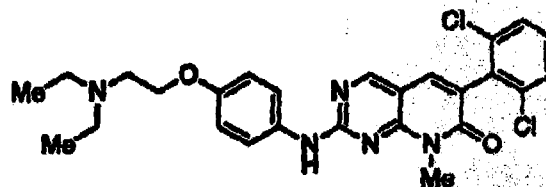
(Dahring et al., 1997)

Ar = 3,5-dimethoxyphenyl;
Compound 6

(Hamby et al., 1997)

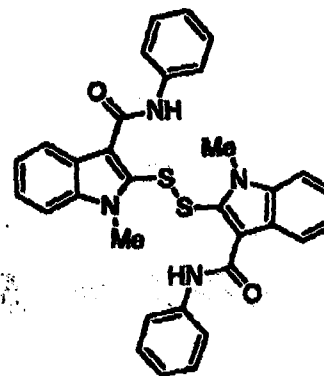


(Mohammadi et al., 1997)



PD 166285

(Panek et al., 1997)



PD 145709

(Fry and Nelson, 1995)

FIGURE3. Inhibitors of FGFr tyrosine kinase.

(Dahring *et al.*, 1997), PD 089828 was found to inhibit human full-length FGFr (FGFr-1), PDGFr- β , EGFr, and c-Src nonreceptor tyrosine kinases with IC_{50} values of 0.15, 1.76, 5.47, and 0.18 μ M, respectively. Against FGFr-1, PD 089828 was characterized as a reversible ATP-competitive inhibitor. PD 089828 inhibited PDGF- and EGF-stimulated receptor autophosphorylation in vascular smooth muscle cells, and basic FGF (bFGF)-mediated tyrosine phosphorylation in A121 cells with IC_{50} values similar to those observed against the isolated kinases. Additionally, in functional assays, this agent inhibited PDGF-stimulated DNA synthesis, PDGF-directed migration, and serum-stimulated growth of vascular smooth muscle cells, all to the same extent as PDGFr autophosphorylation (IC_{50} = 0.8, 4.5, and 1.8 μ M, respectively). Similarly, increases in DNA synthesis stimulated by bFGF-induced mitogenesis were inhibited by PD 089828 with an IC_{50} of 0.48 μ M. Because of its broad activity as a protein kinase inhibitor, the effects of PD 089828 on growth factor-induced functional responses may be due to inhibition of other protein kinases associated with growth factor receptor signaling, including mitogen-activated protein (MAP) kinase.

In a second study on this novel class of compounds (Hamby *et al.*, 1997), structure-activity relationship (SAR) studies were conducted around the C-2 amino and C-6 aryl positions in an attempt to uncover analogues that would display improved potency, solubility, and bioavailability relative to the initial lead. From this study, the 3', 5'-dimethoxyphenyl compound (Compound 6, Fig. 3) was found to be a selective inhibitor of the FGFr tyrosine kinase relative to a panel of five tyrosine kinases screened. The IC_{50} for FGFr tyrosine kinase inhibition was 0.08 μ M for Compound 6 and >50 μ M for each of PDGF, FGF, EGF, Ins receptor, and c-Src tyrosine kinases. Based on this selectivity pattern, it was not surprising that Compound 6 did not inhibit PDGF-stimulated cellular autophosphorylation (IC_{50} >50 μ M) in rat aortic vascular smooth muscle cells.

A third study on this class of compounds profiled PD 166285, a more potent and soluble analogue of an early screening lead, in which the urea function of PD 089828 was replaced by a carbonyl (Panek *et al.*, 1997). PD 166285 was found to inhibit c-Src, FGFr-1, EGFr, and PDGFr- β tyrosine kinases in an ATP-competitive fashion with IC_{50} values of 8.4 nM, 39.3 nM, 87.5 nM, and 98.3 nM, respectively. PD 166285 also demonstrated inhibitory activity against MAP kinase (IC_{50} = 5 μ M) and PKC (IC_{50} = 22.7 μ M). In addition, PD 166285 inhibited PDGF- and EGF-stimulated receptor autophosphorylation in vascular smooth muscle cells and in A431 cells, respectively, and bFGF-mediated tyrosine phosphorylation in Sf9 cells with IC_{50} values of 6.5 nM, 1.9 μ M and 97.3 nM, respectively. The inhibition of PDGFr autophosphorylation in vascular smooth muscle cells was long-lasting and persisted for 4 days after a single 1-hr exposure followed by extensive washing. The PDGF-induced tyrosine phosphorylation of the 44 and 42 kDa MAP kinase isoforms was also blocked as a result of the inhibition of PDGF-stimulated receptor autophosphoryla-

tion by PD 166285 in vascular smooth muscle cells. Inhibitory effects of PD 166285 were demonstrated in a number of functional assays of cell attachment, migration, and proliferation.

The results from the above three studies highlight the biological characteristics of many members of the 6-arylpyrido[2,3-d]pyrimidines as broadly active protein tyrosine kinase inhibitors capable of potently suppressing a number of kinase-mediated cellular functions, including cell attachment, movement, and replication. The therapeutic utility of these inhibitors as antiproliferative and antimigratory agents could extend to such diseases as cancer, atherosclerosis, and restenosis, where redundancies in protein kinase signaling pathways are known to exist. Initial SAR studies also demonstrate that it is possible to design selectivity within this series, especially toward inhibition of the FGFr tyrosine kinase.

3.2.2. 3-Alkylidene oxindoles. Recently, two members of a new class of tyrosine kinase inhibitors based on an oxindole core template have been described (Mohammadi *et al.*, 1997). Both SU4984 and SU5402 inhibited the purified kinase activity of FGFr-1 with an IC_{50} of 10–20 μ M in the presence of 1 mM ATP. In NIH 3T3 cells stimulated with acidic FGF (aFGF), autophosphorylation of FGFr-1 was inhibited by the two compounds with an IC_{50} of 20–40 μ M for SU4984 and 10–20 μ M for SU5402. These two compounds also inhibited, with similar IC_{50} values, aFGF-induced tyrosine phosphorylation of a 90 kDa phosphoprotein (pp90) and the MAP kinases, ERK1 and ERK2, two intracellular events that are dependent on the kinase activity of FGFr-1. Additionally, both compounds also inhibited [³H]thymidine incorporation in response to aFGF stimulation. The effects of the inhibitors on downstream signaling events were also examined. In NIH 3T3 cells, NIH IR cells, and HER 14 cells, the phosphorylation of downstream proteins in response to stimulation by either PDGF, Ins, or EGF, respectively, was inhibited by SU4984 and SU5402 in direct correlation with inhibition of receptor autophosphorylation. The crystal structures of the tyrosine kinase domain of FGFr-1 in complex with each inhibitor were determined. For each complex, the oxindole template occupies the site in which the adenine of ATP binds, whereas the moiety extending out from the alkylidene function contacts residues in the hinge region between the two kinase lobes. The more specific inhibitor of FGFr1, SU5402, induces a conformational change in the nucleotide-binding loop.

3.2.3. Other agents. The effect of PD 145709, one member of the thioindole class of tyrosine kinase inhibitors (see also Section 4.2.4), on bFGF-mediated tyrosine phosphorylation in Swiss 3T3 murine fibroblasts and in a human breast carcinoma line has been evaluated (Fry and Nelson, 1995). Against Swiss 3T3 cells, half-maximal suppression was attained at 4.5 μ M of inhibitor. Little or no inhibition of EGF- or PDGF-mediated tyrosine phosphorylation was observed at concentrations as high as 50 μ M. The inhibi-

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tion of bFGF-mediated phosphorylation occurred rapidly, and once established, was irreversible and remained so for at least 2 hr after PD 145709 had been removed from the extracellular medium. PD 145709 also inhibited bFGF-mediated phosphorylation, as well as FGFR autophosphorylation, in a human breast carcinoma, MDA-MB-134, which over-expresses the FGFR-1. PD 145709 caused an increase of c-Jun mRNA in response to EGF, PDGF, bFGF, and serum, and was a potent inhibitor of protein synthesis in fibroblasts with an IC_{50} of 0.5 μ M. The data are compatible with a mechanism by which PD 145709 interferes with signals or protein factors that are involved in the initiation of protein synthesis.

4. INHIBITORS OF SRC FAMILY TYROSINE KINASES

4.1. Natural Products

Natural product inhibitors of SRC family tyrosine kinases are shown in Fig. 4. Radicicol, a fungal antibiotic previously shown to be a potent inhibitor of $p60^{src}$ tyrosine kinase (Kown *et al.*, 1992), is reported to potently suppress tyrosine phosphorylation of other Src family tyrosine kinases in lipopolysaccharide (LPS)-stimulated macrophages (Chamugam *et al.*, 1995). Pretreatment of macrophages with radicicol resulted in the suppression of levels of tyrosine-phosphorylated proteins in a time- and dose-dependent fashion. Antiphosphotyrosine immunoblot analysis of LPS-stimulated macrophages showed that the major tyrosine phosphorylated proteins were in the 55–90 kDa ranges. When antibodies in antiphosphotyrosine immunoblot membranes were stripped off and the membranes reprobed with anti-Src family kinase antibodies, $p60^{src}$, $p58/64^{lck}$, and $p59^{c-fgr}$ were detected in the cell lysates. Doublet bands for $p53/56^{bm}$, detected by immunoblot with anti- $p53/56^{bm}$ antibodies, were superimposed with the two major tyrosine-phosphorylated bands in an antiphosphotyrosine immunoblot of cell lysates. Antiphosphotyrosine immunoblot of immunoprecipitates of cell lysates indicated that $p53/56^{bm}$ and $p59^{c-fgr}$ were the major tyrosine-phosphorylated Src family tyrosine kinases. The mechanism by which radicicol inhibits protein tyrosine kinases is not known, but it is thought that an irreversible binding to a sulfhydryl group(s) of the enzyme occurs, much in the same fashion as for herbimycin A. Radicicol also inhibited the expression of cyclooxygenase (COX)-2 without affecting COX-1 expression in LPS-stimulated macrophages with an IC_{50} value of 27 nM. These results suggest that the activation of tyrosine-specific protein kinases is the proximal obligatory step in the LPS-induced signal transduction pathway, leading to the induction of COX-2 expression in macrophages. Radicicol represents one of the few agents other than glucocorticoids that can selectively suppress the expression of COX-2 without affecting COX-1.

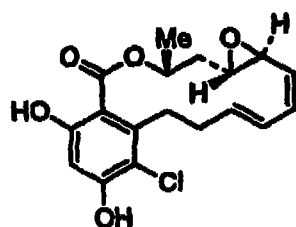
In a second study, the effects of radicicol upon both the phosphorylation of Src associated in mitosis (Sam)68, a protein that associates with and is tyrosine phosphorylated by

Src in a mitosis-specific manner, and upon mitotic progression in Src-transformed mouse fibroblasts, have been examined (Pillay *et al.*, 1996). Radicicol reversibly inhibited the mitosis-specific tyrosine phosphorylation of Sam68 in a Src-transformed mouse fibroblast NIH 3T3 line, which expresses an activated Src mutant (src^{Y527F}), as determined by antiphosphotyrosine immunoblotting. Radicicol inhibited the tyrosine phosphorylation of both free and Src-associated Sam68, suggesting the presence of two intracellular pools of tyrosine-phosphorylated Sam68 in mitotic cells. In addition, radicicol treatment had no effect on the ability of cells to enter mitosis, indicating that tyrosine phosphorylation of Sam68 is probably not important for this event. However, radicicol reversibly retarded the exit of cells from mitosis, as determined by flow cytometric analyses. Radicicol-mediated inhibition of Sam68 tyrosine phosphorylation, and its concurrent ability to block mitotic exit, suggests the possibility of a significant role for Src kinase and the unique mitotic substrate, Sam68, in cell cycle regulation.

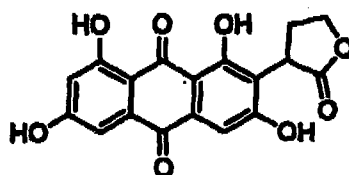
The evaluation of anthraquinone natural products as tyrosine kinase inhibitors continues to be reported in the literature. In one study (Fredenhagen *et al.*, 1995), the protein tyrosine kinase activities for a range of inhibitors related to emodin were examined more closely. Of the 12 compounds evaluated, paecilquinone A demonstrated the best potency against c-Src with an IC_{50} of 2 μ M. It also demonstrated modest potency against v-Abl tyrosine kinase with an IC_{50} of 0.59 μ M. Most of the compounds of this series showed a low degree of selectivity among the kinases investigated and were unable to inhibit autophosphorylation of the EGFR tyrosine kinase and v-Abl substrates within intact cells.

In another study (Faltynek *et al.*, 1995a), damnacanthal, a plant-derived anthraquinone, was found to be a potent and selective inhibitor of $p56^{lck}$ tyrosine kinase activity. It exhibited an IC_{50} of 17 nM for the inhibition of $p56^{lck}$ autophosphorylation and 620 nM for phosphorylation of an exogenous peptide by $p56^{lck}$. Damnacanthal displayed >100-fold selectivity for $p56^{lck}$ over the serine/threonine kinases, PKA, and PKC, and >40-fold selectivity over four receptor tyrosine kinases (EGF, PDGF, ErbB2, Ins). It also demonstrated modest (7- to 20-fold) selectivity for $p56^{lck}$ over the homologous enzymes $p60^{src}$ and $p59^{fms}$. Mechanistic studies demonstrated that damnacanthal was competitive with the peptide binding site, but mixed noncompetitive with the ATP site. Damnacanthal appeared to bind nonspecifically to membrane lipids and was not active in whole cell tyrosine kinase assays.

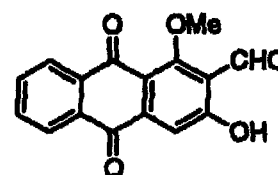
In continuing studies with geldanamycin (Yamaki *et al.*, 1995), this ansamycin antibiotic was shown to selectively inhibit recombinant c-Src tyrosine kinase (IC_{50} = 0.62 μ M) without significantly inhibiting PKA. This agent was also shown to preferentially inhibit the G1/S transition and caused G2/M arrest in human leukemia HL-60 cells. This perturbation of cell cycling was accompanied by marked suppression of c-myc expression and an enhancement in pRb expression. These workers speculated that the inhibition of c-Src signals the coordinative down-regulation of the



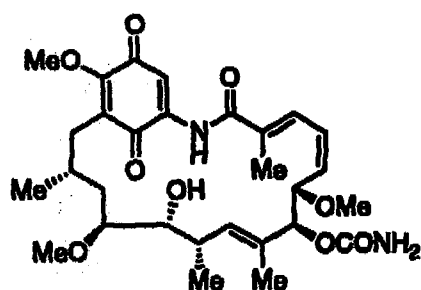
Radicicol
(Chanmugam *et al.*, 1995)



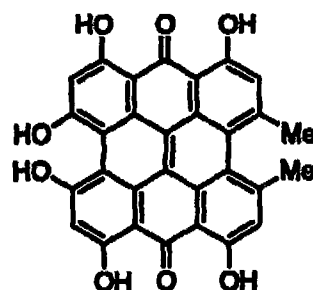
Paecilquinone A
(Fredenhagen *et al.*, 1995)



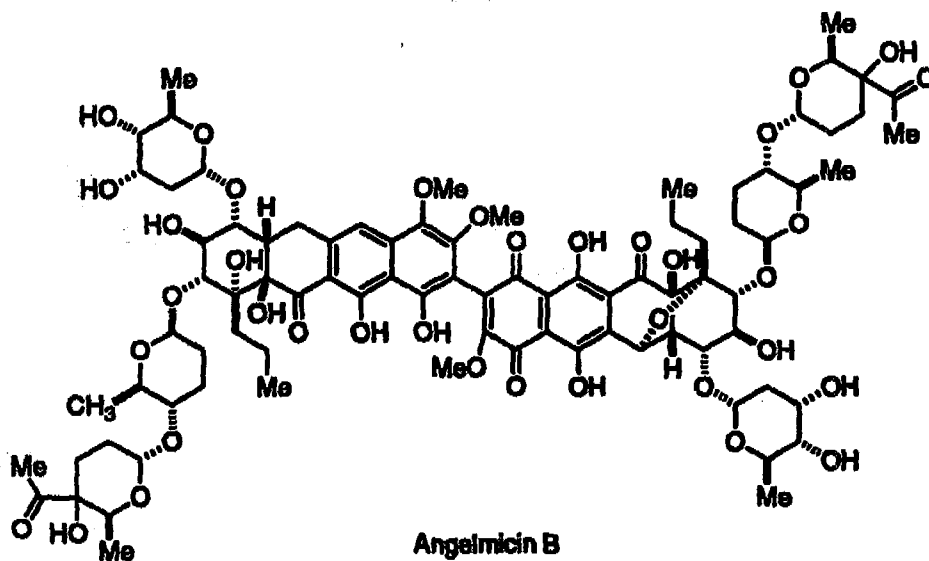
Damnacanthal
(Faltynek *et al.*, 1995a)



Geldanamycin
(Yamakiet *et al.*, 1995)



Hypericin
(Kil *et al.*, 1996)



Angelimycin B
(Yokoyama *et al.*, 1996)

FIGURE 4. Natural product inhibitors of Src family tyrosine kinases.

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early responsive gene *c-myc* and up-regulation of pRb, although no such direct relationship was demonstrated.

In a study with the naphthodianthrone hypericin (Kil *et al.*, 1996), it was found to inhibit the T-cell Src family kinase p56^{lck} in a dose-dependent fashion with an IC₅₀ of 5 μ M. This natural product inhibited EGFr-catalyzed tyrosine phosphorylation of the exogenous peptide substrate RR-SRC with an IC₅₀ of 3 μ M, and also suppressed EGFr-induced autophosphorylation, either *in vitro* or in A431 serum-starved cells, with each process inhibited at an IC₅₀ of 2 μ M.

The structure of angelmicin B, previously isolated from a rare actinomycete, *Microbispora*, and shown to inhibit p60^{v-src} (Uehara *et al.*, 1993), has been fully elucidated (Hori *et al.*, 1996). A study was carried out with this compound and related analogues (A1, A2, C, and D) for their effects on the differentiation of HL-60 cells relative to their inhibition of Src tyrosine kinase (Yokoyama *et al.*, 1996). All analogues had almost equivalent effects on the differentiation of HL-60 cells, although angelmicins C and D inhibited Src tyrosine kinase activity less than the other analogues. Based on its molecular weight, angelmicin B is a relatively weak inhibitor of p60^{v-src} with an IC₅₀ of $\sim$ 30 μ M. The effective concentration of angelmicin B for Src kinase inactivation was about 100-fold higher than that required for growth inhibition and differentiation induction, suggesting that these effects are not directly associated with suppression of Src tyrosine kinase.

4.2. Synthetic Agents

4.2.1. Ring-fused pyrimidines. Synthetic inhibitors of SRC family tyrosine kinases are shown in Fig. 5. A number of reports have appeared in the literature describing exceptionally potent quinazoline-based EGFr inhibitors. However, selective inhibition against a Src family kinase has not been reported for this class of compounds. The Rhone-Poulenc Rorer group have described preliminary results of an SAR study of quinazoline-based tyrosine kinase inhibition against the EGFr and of p56^{lck} tyrosine kinases (Myers *et al.*, 1997). The most potent inhibitor of p56^{lck} was identified as RPR-108518A with an IC₅₀ of 0.50 μ M. This compound displayed identical potent inhibition of EGFr autophosphorylation. Preliminary results using RPR-108518A in intact cell studies revealed that inhibition of p56^{lck} tyrosine kinase activity diminished the levels of substrate tyrosine phosphorylation upon stimulation. IL-2 secretion was also inhibited in Jurkat cells with an IC₅₀ that corresponded to the inhibition of *in situ* p56^{lck} autophosphorylation.

As part of a screening effort directed toward identification of tyrosine-specific kinase activity associated with p60^{c-src}, a member of the 5,10-dihydropyrimido[4,5-*b*]quinolin-4(1H)-one chemical class was discovered to potently inhibit this enzyme. From follow-up SAR studies (Dow, 1996; Dow *et al.*, 1995), Compound 7 was found to possess optimal potency toward c-Src with an IC₅₀ of 0.50 μ M. The compound showed remarkable selectivity (6000-fold) for inhibition of c-Src over PKA and 150-fold selectivity toward EGFr tyrosine kinase. Mode of inhibition studies revealed

that this agent inhibits in a pure-mixed-noncompetitive mode with respect to ATP.

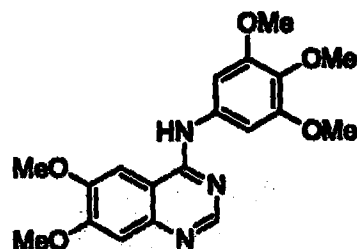
In conjunction with a program to find agents that selectively inhibit certain Src kinase family members, workers at Pfizer (Groton, CT, USA) synthesized a series of 4-aminopyrazolo[3,4-*d*]pyrimidines designed for inhibition of p56^{lck} and p60^{c-src} tyrosine kinases (Dow *et al.*, 1997). One member of this series, PP1, was evaluated in depth (Hanke *et al.*, 1996). Against a panel of kinases, PP1 showed selective inhibition toward p56^{lck} (IC₅₀ = 5 nM) and p59^{hmr} (IC₅₀ = 6 nM). In further selectivity tests against other Src family tyrosine kinases, PP1 also inhibited Src (IC₅₀ = 170 nM) and Hck (IC₅₀ = 20 nM). In contrast, it was 50-fold less active in the inhibition of A-431 EGFr autophosphorylation (IC₅₀ = 250 nM), and it was essentially inactive against ZAP-70 (IC₅₀ = >100 μ M), JAK-2 (IC₅₀ = >50 μ M), and PKA (data not given). In an assay to test its ability to inhibit anti-CD3-stimulated tyrosine phosphorylation in purified human T-cells, PP1 suppressed whole cell phosphorylation following treatment with anti-CD3 with an IC₅₀ of $\sim$ 0.5 μ M. It was less effective at inhibiting the non-T-cell receptor-dependent phorbol 12-myristate 13-acetate/IL-2-induced T-cell proliferation. Additionally, PP1 selectively inhibited the induction of the IL-2 gene, but not the granulocyte-macrophage colony-stimulating factor or IL-2 receptor genes. Preliminary studies with PP1 showed complex kinetics for inhibition of Lck. However, at certain concentrations of ATP, the compound appeared to be competitive with ATP.

c-Src tyrosine kinase has been shown to be essential in the process of osteoclastic bone resorption in mutant mice that do not express c-Src (Soriano *et al.*, 1991). A series of compounds closely related to PP1, the 4-aminopyrazolo[2,3-*d*]pyrimidines, were synthesized by the Ciba-Geigy group (Missbach, 1996; Missbach *et al.*, 1996) in a program to design c-Src inhibitors with greater specificity than herbimycin A for suppression of bone resorption by isolated rat osteoclasts on bovine bone slices. Within this series, one compound, CGP 62464, selectively inhibited c-Src tyrosine kinase (IC₅₀ = 50 nM) over other protein kinases examined, including EGFr (IC₅₀ = 3 μ M), PKC- α (IC₅₀ = 42 μ M), and the cell cycle kinase CDC2 (IC₅₀ = 54 μ M). Inhibition of osteoclastic bone resorption occurred with an IC₅₀ of 0.5 μ M, and for the series as a whole, this suppression correlated with c-Src inhibition in an artificial (cell-free) liquid phase assay. However, these data do not rule out that another kinase present in the osteoclast is inhibited by this chemical class, and inhibition of c-Src activity in intact osteoclasts has yet to be demonstrated. Preliminary kinetic data suggested that CGP 62464 is competitive with respect to ATP.

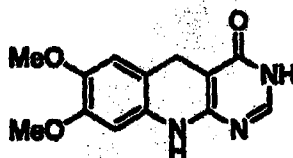
4.2.2. Benzopyrans. Sterling-Winthrop investigators have reported on a mechanism-based approach to the discovery of novel immunosuppressants by screening for selective inhibitors of p56^{lck} tyrosine kinase activity (Miller *et al.*, 1995). Using this approach, three simple nonhydroxylated chromones

were found to inhibit p56^{lck} autophosphorylation with low micromolar potencies and to exhibit some selectivity for p56^{lck} over the serine/threonine (PKC, PKA) and other tyrosine kinases (PDGFr, ErbB2) tested. Against p56^{lck}, KAN 104141 gave an IC₅₀ of 17 μM for inhibition of peptide

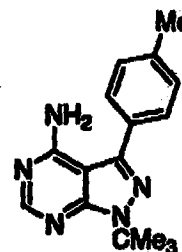
phosphorylation and 3.6 μM for inhibition of receptor autophosphorylation. It was also the most selective of these compounds, exhibiting more than 10-fold greater potency toward p56^{lck} over the serine/threonine or tyrosine kinases examined.



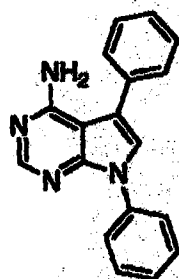
RPR-108518A
(Myers *et al.*, 1997)



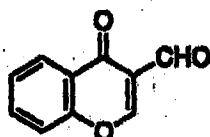
Compound 7
(Dow *et al.*, 1995)



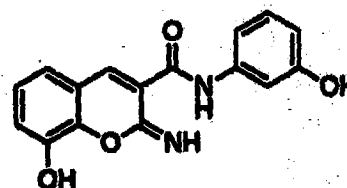
PP1
(Hanke *et al.*, 1996)



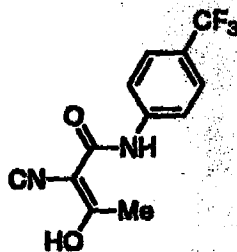
CGP 62464
(Missbach, 1996)



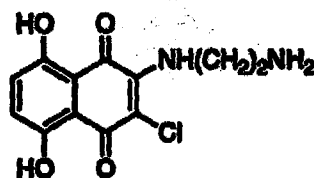
KAN 104141
(Miller *et al.*, 1995)



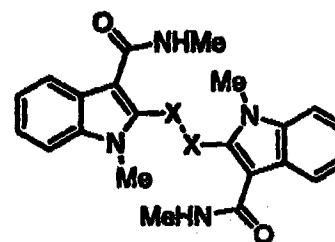
9TA
(Huang *et al.*, 1995)



A77 1726
(Xu *et al.*, 1996)



Compound 8
(Frew *et al.*, 1995)



X = Se; Compound 9
(Showalter *et al.*, 1997)

X = S; Compound 10
(Palmer *et al.*, 1995)

FIGURE 5. Synthetic inhibitors of Src family tyrosine kinases.

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A related class of compounds, the polyhydroxylated 3-(N-phenyl)carbamoyl-2-iminochromenes, have been synthesized (Huang, 1996; Huang *et al.*, 1995). The pattern of hydroxylation on both the iminochrome ring and the N-phenylcarbamoyl side chain appears to play an important role in the determination of potency within this class. Against a number of Src family kinases, compound 9TA showed the best overall potency with an IC_{50} of 0.12 μ M vs. p60^{c-src}, 2.1 μ M vs. p56^{lck}, and >30 μ M vs. p56^{lyn} and p55^{fm}. Replacement of the hydroxyl group of the iminochrome ring with methoxyl resulted in a 35-fold loss of potency vs. c-Src. Several compounds of this series were tested for inhibition of growth in a number of human cancer cell lines, including HL-60, A-1, and HT-29. Compounds that showed the strongest inhibitory effect against p60^{c-src} were not as potent against these cell lines. The best compounds of this series inhibited these lines in the range of an IC_{50} of 3–7.5 μ M.

4.2.3. Leflunomide and metabolites. Leflunomide (see Section 2.2.4 and Fig. 2) is an immunosuppressant that is able to prevent autoimmune diseases and rejection of transplant allografts and xenografts. Earlier studies showed that it inhibited the tyrosine kinase activity of the EGFR (Mattar *et al.*, 1993). A study was carried out to characterize the inhibitory effects of leflunomide on Src family (p56^{lck} and p59^{fm})-mediated protein tyrosine phosphorylation (Xu *et al.*, 1995). Leflunomide was able to inhibit p59^{fm} and p56^{lck} activity *in vitro*. The IC_{50} values for p59^{fm} autophosphorylation and phosphorylation of an exogenous substrate were 125–175 μ M and 22–40 μ M, respectively, while the corresponding IC_{50} values for p56^{lck} were 160 μ M and 65 μ M, respectively. Leflunomide also demonstrated an ability to inhibit protein tyrosine phosphorylation induced by anti-CD3 monoclonal antibody in Jurkat cells. The IC_{50} values for total intracellular tyrosine phosphorylation ranged from 5 to 45 μ M, with the IC_{50} values for the ζ chain and PLC isoform γ 1 being 35 and 44 μ M, respectively. Leflunomide also inhibited Ca^{2+} mobilization in Jurkat cells stimulated with anti-CD3 antibody. Distal events of anti-CD3 monoclonal antibody stimulation, namely, IL-2 production and IL-2 receptor expression on human T lymphocytes, were also inhibited by leflunomide. Additionally, leflunomide was able to inhibit IL-2 stimulated tyrosine phosphorylation in CTLL-4 cells. These data collectively demonstrate the ability of leflunomide to inhibit tyrosine kinase activity *in vitro*, and suggest that inhibition of tyrosine phosphorylation events may be the mechanism by which leflunomide functions as an immunosuppressive agent.

A follow-up study was carried out by the same group on the active metabolite of leflunomide, A77 1726, to define its relative contribution toward the inhibition of tyrosine kinases and dihydroorotate dehydrogenase (Xu *et al.*, 1996). Studies were carried out in the murine leukemia cell line LSTRA, a leukemia T-cell line that over-expresses the Src family kinase p56^{lck} and has elevated levels of tyrosine-phosphorylated intracellular proteins. A77 1726 reduced

the intracellular levels of tyrosine-phosphorylated proteins with relatively high IC_{50} values ranging from 50 to 100 μ M. It also inhibited p56^{lck} activity in LSTRA membrane preparations and immunoprecipitates. The IC_{50} values for inhibition of immunoprecipitated p56^{lck} autophosphorylation and exogenous substrate histone 2B were 80 and 40 μ M, respectively. The antityrosine phosphorylation activity of A77 1726 was not affected by uridine. A77 1726 inhibited LSTRA cell growth and proliferation (IC_{50} = ca. 10–30 μ M), and the inhibition could be reversed by the addition of exogenous uridine, suggesting that the antiproliferative activity of A77 1726 may be due to inhibition of *de novo* pyrimidine nucleotide synthesis. *In vitro* enzyme assays confirmed that A77 1726 directly inhibited the activity of dihydroorotate dehydrogenase.

4.2.4. Thiol-reactive agents. A series of halogenated naphthoquinones based on the jugalone structural lead were synthesized as inhibitors of PI-3 kinase (Frew *et al.*, 1995). Within a series of 28 synthesized analogues, most compounds were weak inhibitors of this enzyme. Inhibition was time dependent and could be prevented by endogenous thiol. PKC and c-Src tyrosine kinase were also only weakly inhibited. The best inhibitor against c-Src was Compound 8 with an IC_{50} of 4.1 μ M.

The Parke-Davis group reported recently on a small series of 2,2'-diselenobis(1H-indoles) that were evaluated against a range of tyrosine kinases (Showalter *et al.*, 1997). Compounds of this class displayed broad inhibitory activity with an IC_{50} of 0.9–>100 μ M vs. EGFR, 3.4–>50 μ M vs. PDGFR, and 0.4–6.7 μ M vs. v-Src. The most active compound against v-Src was Compound 9 with an IC_{50} of 0.4 μ M. Interestingly, the corresponding disulfur congener, Compound 10 (Palmer *et al.*, 1995), was considerably less potent against v-Src with an IC_{50} of 6.7 μ M. However, in whole-cell studies, both compounds displayed equal potency for growth inhibition of Swiss 3T3 mouse fibroblasts with an IC_{50} of 5.5 μ M. Enzyme kinetic studies showed that both classes of compounds displayed primarily noncompetitive inhibition with respect to either ATP or peptide substrate. Dithiothreitol caused a general decrease in inhibition of the EGFR and v-Src tyrosine kinases by both the diselenium and disulfur series, with reversal of enzyme inhibition occurring less readily within the diselenium series.

References

- Ahn, S. C., Kim, B. Y., Park, C. S., Lee, H. S., Suh, P. G., Ryu, S. H., Rho, H. M., Rhee, J. S., Mheen, T. I. and Ahn, J. S. (1995) Inhibition of PDGF-induced phosphoinositide-turnover by glucopiericidin A. *Biochem. Mol. Biol. Int.* 37: 125–132.
- Alonso, G., Koegl, M., Mazurenko, N. and Courtneidge, S. A. (1995) Sequence requirements for binding of src family tyrosine kinases to activated growth factor receptors. *J. Biol. Chem.* 270: 9840–9848.
- Bando, K., Kannuki, S., Rokutan, K., Shoumo, M. and Matsumoto, K. (1995) Effects of trapidil and suramin on growth factor-induced calcium response and tyrosine phosphorylation in human glioma cells. *Neurol. Med. Chir. (Tokyo)* 35: 631–638.
- Bilder, G. E. and Rojas, C. (1996) Inhibitors of the platelet-derived

- growth factor receptor tyrosine kinase. *J. Cardiovasc. Drug Rev.* 14: 380-399.
- Blankley, C. J., Boschelli, D. H., Doherty, A. M., Hamby, J. M., Klutchko, S. and Panek, R. L. (1996a) Pyrido[2,3-d]pyrimidines as protein tyrosine kinase-mediated cell proliferation inhibitors. World Patent Application WO 9634867, November 7.
- Blankley, C. J., Doherty, A. M., Hamby, J. M., Panek, R. L., Schroeder, M. C., Showalter, H. D. H. and Connolly, C. (1996b) 6-Aryl pyrido[2,3-d]pyrimidines and naphthyridines for inhibiting protein tyrosine kinase-mediated cellular proliferation. World Patent Application WO 9615128, May 23.
- Bolen, J. B., Penhallow, R. C. and Burkhardt, A. L. (1997) Signal transduction by the src family of tyrosine protein kinases. In: *Encyclopedia of Cancer*, pp. 1657-1668, Bertino, J. (ed.) Academic Press, San Diego.
- Bornfeldt, K. E. (1996) Intracellular signaling in arterial smooth muscle migration versus proliferation. *Trends Cardiovasc. Med.* 6: 143-151.
- Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Mueller, M., Regenass, U. and Lydon, N. B. (1995) Selective inhibition of the platelet-derived growth factor signal transduction pathway by a protein-tyrosine kinase inhibitor of the 2-phenylaminopyrimidine class. *Proc. Natl. Acad. Sci. USA* 92: 2558-2562.
- Buchdunger, E., Zimmermann, J., Mett, H., Meyer, T., Mueller, M., Druker, B. J. and Lydon, N. B. (1996) Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res.* 56: 100-104.
- Channugam, P., Feng, L., Liou, S., Jang, B. C., Boudreau, M., Yi, G., Lee, J. H., Kwon, H. J., Beppu, T., Yoshida, M., Xia, Y., Wilson, C. B. and Hwang, D. (1995) Radicol, a protein tyrosine kinase inhibitor, suppresses the expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipo-polysaccharide and in experimental glomerulonephritis. *J. Biol. Chem.* 270: 5418-5426.
- Chen, C., Mattar, S. G., Hughes, J. D., Pierce, G. F., Cook, J. E., Ku, D. N., Hanson, S. R. and Lumsden, A. B. (1996) Recombinant mitotoxin basic fibroblast growth factor-saporin reduces venous anastomotic intimal hyperplasia in the arteriovenous graft. *Circulation* 94: 1989-1995.
- Claesson-Welsh, L. (1994) Platelet-derived growth factor receptor signals. *J. Biol. Chem.* 269: 32023-32026.
- Dahring, T. K., Lu, G. H., Hamby, J. M., Batley, B. L., Kraker, A. J. and Panek, R. L. (1997) Inhibition of growth factor-mediated tyrosine phosphorylation in vascular smooth muscle by PD 089828, a new synthetic protein tyrosine kinase inhibitor. *J. Pharmacol. Exp. Ther.* 281: 1446-1456.
- Dow, R. L. (1996) 5,10-Dihydropyrimido[4,5-b]quinolin-4(1H)-ones tyrosine kinase inhibitors. World Patent Application WO 9628444, September 19.
- Dow, R. L., Bechle, B. M., Chou, T. T., Goddard, C. and Larson, E. R. (1995) Selective inhibition of the tyrosine kinase pp60src by analogs of 5,10-dihydropyrimido[4,5-b]quinolin-4(1H)-one. *Bioorg. Med. Chem. Lett.* 5: 1007-1010.
- Dow, R. L., Koch, K. and Schulte, G. R. (1997) 4-Aminopyrazolo[3,4-d]pyrimidines and 4-aminopyrazolo[3,4-d]pyridines as tyrosine kinase inhibitors. US Patent 5593997, January 14.
- Edison, A. M., Barker, S. C., Kassel, D. B., Luther, M. A. and Knight, W. B. (1995) Exploration of the sequence specificity of pp60c-src tyrosine kinase. Minimal peptide sequence required for maximal activity. *J. Biol. Chem.* 270: 27112-27115.
- Faltynek, C. R., Schroeder, J., Mauvais, P., Miller, D., Wang, S., Murphy, D., Lehr, R., Kelley, M., Maycock, A., Michne, W., Miski, M. and Thunberg, A. L. (1995a) Damnacanthal is a highly potent, selective inhibitor of p56lck tyrosine kinase activity. *Biochemistry* 34: 12404-12410.
- Faltynek, C. R., Wang, S., Miller, D., Mauvais, P., Gauvin, B., Reid, J., Xie, W., Hoekstra, S., Juniewicz, P., Sarup, J., Lehr, R., Sawutz, D. G. and Murphy, D. (1995b) Inhibition of T lymphocyte activation by a novel p56lck tyrosine kinase inhibitor. *J. Enzyme Inhib.* 9: 111-122.
- Fredenhagen, A., Mett, H., Meyer, T., Buchdunger, E., Regenass, U., Roggo, B. E. and Petersen, F. (1995) Protein tyrosine kinase and protein kinase C inhibition by fungal anthraquinones related to emodin. *J. Antibiot.* 48: 1355-1358.
- Frew, T., Powis, G., Berggren, M., Gallegos, A., Abraham, R. T., Ashendel, C. L., Zalkow, L. H., Hudson, C., Gruszecka-Kowalik, E., Burgess, E. M., Benedetti-Doctorovich, V., Kerrigan, J. E., Lambropoulos, J., Merriman, R. and Bonjouklian, R. (1995) Novel quinone antiproliferative inhibitors of phosphatidylinositol-3-kinase. *Anticancer Drug Des.* 10: 347-359.
- Fry, D. W. (1994) Protein tyrosine kinases as therapeutic targets in cancer chemotherapy and recent advances in the development of new inhibitors. *Expert Opin. Invest. Drugs* 3: 577-595.
- Fry, D. W. and Bridges, A. J. (1995) Inhibitors of protein tyrosine kinases. *Curr. Opin. Biotechnol.* 6: 662-667.
- Fry, D. W. and Nelson, J. M. (1995) Inhibition of basic fibroblast growth factor-mediated tyrosine phosphorylation and protein synthesis by PD 145709, a member of the 2-thioindole class of tyrosine kinase inhibitors. *Anticancer Drug Des.* 10: 607-622.
- Gastl, G., Hermann, T., Steurer, M., Zmija, J., Gunsilius, E., Unger, C. and Kraft, A. (1997) Angiogenesis as a target for tumor treatment. *Oncology* 54: 177-184.
- Gazit, A., App, H., McMahon, G., Chen, J., Levitzki, A. and Bohmer, F. D. (1996) Tyrphostins. 5. Potent inhibitors of platelet-derived growth factor receptor tyrosine kinase: structure-activity relationships in quinoxalines, quinolines, and indole tyrphostins. *J. Med. Chem.* 39: 2170-2177.
- Giese, N. A. and Lokker, N. (1996) Method and compositions for inhibiting protein kinases. World Patent Application WO 9613259, May 9.
- Giese, N. A., Lokker, N., Laihelman, A. M. and Scarborough, R. M. (1996) Pharmaceutical pyrazole compositions useful as inhibitors of protein kinases. World Patent Application WO 9614843, May 23.
- Golomb, G. and Fishbein, I. (1997) Tyrphostins, inhibitors of protein tyrosine kinase, in restenosis. *Adv. Drug Delivery Rev.* 24: 53-62.
- Hamby, J. M., Connolly, C. J. C., Schroeder, M. C., Winters, R. T., Showalter, H. D. H., Panek, R. L., Major, T. C., Olsewski, B., Ryan, M. J., Dahring, T., Lu, G. H., Keiser, J., Amar, A., Shen, C., Kraker, A. J., Slintak, V., Nelson, J. M., Fry, D. W., Bradford, L., Hallak, H. and Doherty, A. M. (1997) Structure-activity relationships for a novel series of pyrido[2,3-d]pyrimidine tyrosine kinase inhibitors. *J. Med. Chem.* 40: 2296-2303.
- Hanke, J. H., Gardner, J. P., Dow, R. L., Changelian, P. S., Brissette, W. H., Weringer, E. J., Pollok, B. A. and Connelly, P. A. (1996) Discovery of a novel, potent, and src family-selective tyrosine kinase inhibitor. Study of lck- and fyn-T-dependent T-cell activation. *J. Biol. Chem.* 271: 695-701.
- Heldin, C.-H. and Westermark, B. (1991) Platelet-derived growth factor and autocrine mechanisms of oncogenic processes. *Crit. Rev. Oncol.* 2: 109-124.
- Hirth, K. P., Schwartz, D. P., Mann, E., Shawver, L. K., Keri, G., Szekely, I., Bajot, T., Haimichael, J., Orfi, L., Levitzki, A., Gazit, A., Ullrich, A., Lammers, R., Kabbinnavar, F. F., Slamon,

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1293

- D. J. and Tang, C. P. (1995) Treatment of platelet-derived growth factor-related disorders such as cancers using inhibitors of platelet-derived growth receptor. World Patent Application WO 9519169, July 20.
- Hori, H., Higashi, K., Ishiyama, T., Uramoto, M., Uehara, Y. and Oki, T. (1996) Structure of angelmicin B, a novel src signal transduction inhibitor. *Tetrahedron Lett.* 37: 2785-2788.
- Huang, C.-K. (1996) 2-Iminochromene derivatives as inhibitors of protein tyrosine kinase. World Patent Application WO 9640670, December 19.
- Huang, C.-K., Wu, F.-Y. and Ai, Y.-X. (1995) Polyhydroxylated 3-(N-phenyl) carbamoyl-2-iminochromene derivatives as potent inhibitors of tyrosine kinase p60c-src. *Bioorg. Med. Chem. Lett.* 5: 2423-2428.
- Jackson, C. L. and Schwartz, S. M. (1992) Pharmacology of smooth muscle cell replication. *Hypertension (Dallas)* 20: 713-736.
- Kelloff, G. J., Fay, J. R., Steele, V. E., Lubet, R. A., Boone, C. W., Crowell, J. A. and Sigman, C. C. (1996) Epidermal growth factor receptor tyrosine kinase inhibitors as potential cancer chemopreventives. *Cancer Epidemiol. Biomarkers Prev.* 5: 657-666.
- Kil, K.-S., Yum, Y.-N., Seo, S.-H. and Lee K.-T. (1996) Antitumor activities of hypericin as a protein tyrosine kinase blocker. *Arch. Pharmacol. Res.* 19: 490-496.
- Kovalenko, M., Roennstrand, L., Heldin, C.-H., Loubtchenkov, M., Gazit, A., Levitzki, A. and Boehmer, F. D. (1997) Phosphorylation site-specific inhibition of platelet-derived growth factor β -receptor autophosphorylation by the receptor blocking tyrphostin AG1296. *Biochemistry* 36: 6260-6269.
- Kown, H. J., Yoshida, M., Fukui, Y., Horinouchi, S. and Beppu, T. (1992) Potent and specific inhibition of p60v-src protein kinase both in vivo and in vitro by radicicol. *Cancer Res.* 52: 6926-6930.
- Levitzki, A. (1996) Src as a target for anticancer drugs. *Anticancer Drug Des.* 11: 175-182.
- Lindner, V. and Reidy, M. A. (1993) Expression of basic fibroblast growth factor and its receptor by smooth muscle cells and endothelium in injured rat arteries. An en face study. *Circ. Res.* 73: 589-595.
- Lindner, V., Giachelli, C. M., Schwartz, S. M. and Reidy, M. A. (1995) A subpopulation of smooth muscle cells in injured rat arteries expresses platelet-derived growth factor-B chain mRNA. *Circ. Res.* 76: 951-957.
- Liu, M., Liu, J., Buch, S., Tanswell, A. K. and Post, M. (1995) Antisense oligonucleotides for PDGF-B and its receptor inhibit mechanical strain-induced fetal lung cell growth. *Can. Am. J. Physiol.* 269: L178-L184.
- Lou, Q., Leftwich, M. E. and Lam, K. S. (1996) Identification of GIYWHHY as a novel peptide substrate for human p60c-src protein tyrosine kinase. *Bioorg. Med. Chem.* 4: 677-682.
- Lou, Q., Leftwich, M. E., McKay, R. T., Salmon, S. E., Rychetsky, L. and Lam, K. S. (1997) Potent pseudosubstrate-based peptide inhibitors for p60c-src protein tyrosine kinase. *Cancer Res.* 57: 1877-1881.
- Mattar, S. G., Hanson, S. R., Pierce, G. F., Chen, C., Hughes, J. D., Cook, J. E., Shen, C., Noe, B. A., Suwyn, C. R., Scott, J. R. and Lumsden, A. B. (1996) Local infusion of FGF-saporin reduces intimal hyperplasia. *J. Surg. Res.* 60: 339-344.
- Mattar, T., Kochhar, K., Bartlett, R., Bremer, E. G. and Finnegan, A. (1993) Inhibition of the epidermal growth factor receptor tyrosine kinase activity by leflunomide. *FEBS Lett.* 334: 161-164.
- McLeod, H. L., Brunton, V. G., Eckardt, N., Lear, M. J., Robins, D. J., Workman, P. and Graham, M. A. (1996) In vivo pharmacology and antitumor evaluation of the tyrphostin tyrosine kinase inhibitor RG13022. *Br. J. Cancer* 74: 1714-1718.
- Miller, D., Wang, S., Reid, J., Xie, W., Gauvin, B., Kelley, M., Sarup, J., Sawutz, D. G., Miski, M., Dolle, R. E. and Faltynek, C. R. (1995) Approach to the discovery of novel, selective inhibitors of p56lck tyrosine kinase: identification of non-hydroxylated chromones as p56lck inhibitors. *Drug Dev. Res.* 34: 344-352.
- Missbach, M. (1996) Pyrrolo[2,3-d]pyrimidines and their use. World Patent Application WO 9610028, April 4.
- Missbach, M., Green, J. R. and Hall, T. J. (1996) Synthetic inhibitors of the protein tyrosine kinase pp60c-src inhibit bone resorption in vitro. *J. Bone Miner. Res.* 11: S398.
- Miyaji, K., Tani, E., Nakano, A., Ikemoto, H. and Kaba, K. (1995) Inhibition by 5'-methylthioadenosine of cell growth and tyrosine kinase activity stimulated by fibroblast growth factor receptor in human gliomas. *J. Neurosurg.* 83: 690-697.
- Mohammadi, M., McMahon, G., Sun, L., Tang, C., Hirth, P., Yeh, B. K., Hubbard, S. R. and Schlessinger, J. (1997) Structure of the tyrosine kinase domain of fibroblast factor receptor in complex with inhibitors. *Science* 276: 955-960.
- Myers, M. R., Persons, P. E., Ly, C. Q. and Spada, A. P. (1995) Pyrrolo[3,4-g]quinoxaline compounds which inhibit PDGF receptor protein tyrosine kinase. US Patent 5476851, December 19.
- Myers, M. R., Setzer, N. N., Spada, A. P., Zulli, A. L., Hsu, C.-Y. J., Zilberstein, A., Johnson, S. E., Hook, L. E. and Jacoski, M. V. (1997) The preparation and SAR of 4-(anilino), 4-(phenoxy), and 4-(thiophenoxy)-quinazolines: inhibitors of p56lck and EGF-R tyrosine kinase activity. *Bioorg. Med. Chem. Lett.* 7: 417-420.
- Nishi, T., Budde, R. J. A., McMurray, J. S., Obeyesekere, N. U., Safdar, N., Levin, V. A. and Saya, H. (1996) Tight-binding inhibitory sequences against pp60c-src identified using a random 15-amino-acid peptide library. *FEBS Lett.* 399: 237-240.
- Ochs, H. D. (1996) The molecular basis of primary immunodeficiency disorders. In: *Intravenous Immunoglobulin Research and Therapy, Proceedings of the Fourth International Symposium on Intravenous Immunoglobulin Therapy, Interlaken, Switzerland, June 1996*, pp. 175-191, Kazatchkine, M. D. and Morell, A. (eds.) Parthenon Publishing, New York.
- Palmer, B. D., Rewcastle, G. W., Thompson, A. M., Boyd, M., Showalter, H. D. H., Sercel, A. D., Fry, D. W., Kraker, A. J. and Denny, W. A. (1995) Tyrosine kinase inhibitors. 4. Structure-activity relationships among N- and 3-substituted 2,2'-dithio-bis(1H-indoles) for in vitro inhibition of receptor and nonreceptor protein tyrosine kinases. *J. Med. Chem.* 38: 58-67.
- Panek, R. L., Lu, O. H., Klutchko, S. R., Batley, B. L., Dahring, T. K., Hamby, J. M., Hallak, H., Doherty, A. M. and Keiser, J. A. (1997) In vitro pharmacological characterization of PD 166285, a new broadly active protein tyrosine kinase inhibitor. *J. Pharmacol. Exp. Ther.*, in press.
- Patrick, D. R. and Heimbrook, D. C. (1996) Protein kinase inhibitors for the treatment of cancer. *Drug Discov. Today* 1: 325-330.
- Pillay, I., Nakano, H. and Sharma, S. V. (1996) Radicol inhibits tyrosine phosphorylation of the mitotic src substrate Sam68 and retards subsequent exit from mitosis of src-transformed cells. *Cell Growth Differ.* 7: 1487-1499.
- Reidy, M. A. and Bendeck, M. P. (1997) The development of arterial lesions: a process controlled by multiple factors. *Fundam. Clin. Cardiol.* 28: 55-67.

- Ross, R., Raines, E. W. and Bowen-Pope, D. F. (1986) The biology of platelet-derived growth factor. *Cell* 46: 155-169.
- Saltiel, A. R. (1995) Signal transduction pathways as drug targets. *Sci. Am. Sci. Med.* 2: 58-67.
- Sawutz, D. G., Bode, D. C., Briggs, M., Reid, J. R., Canniff, P., Caldwell, L., Faltynek, C. R., Miller, D., Dunn, J. A., de Garavilla, L., Guiles, J. W., Weigelt, C., Michne, W., Treasurywala, A. M. and Silver, P. J. (1996) In vitro characterization of a novel series of platelet-derived growth factor receptor tyrosine kinase inhibitors. *Biochem. Pharmacol.* 51: 1631-1638.
- Shimokado, K., Umezawa, K. and Ogata, J. (1995) Tyrosine kinase inhibitors inhibit multiple steps of the cell cycle of vascular smooth muscle cells. *Exp. Cell Res.* 220: 266-273.
- Showalter, H. D. H., Sercel, A. D., Leja, B. M., Wolfangel, C. D., Ambrosio, L. A., Elliott, W. L., Fry, D. W., Kraker, A. J., Howard, C. T., Lu, G. H., Moore, C. W., Nelson, J. M., Roberts, B. J., Vincent, P. W., Denny, W. A. and Thompson, A. M. (1997) Tyrosine kinase inhibitors. 6. Structure-activity relationships among N- and 3-substituted 2,2'-diselenobis(1H-indoles) for inhibition of protein tyrosine kinases and comparative in vitro and in vivo studies against selected sulfur congeners. *J. Med. Chem.* 40: 413-426.
- Shugar, D. (1995) Protein kinase inhibitors—potential chemotherapeutic agents. *Acta Biochim. Pol.* 42: 405-418.
- Sicheri, F., Moarefi, I. and Kuriyan, J. (1997) Crystal structure of the src family tyrosine kinase hck. *Nature* 385: 602-609.
- Soriano, P., Montgomery, C., Geske, R. and Bradley, A. (1991) Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 64: 693-702.
- Spada, A. P. and Myers, M. R. (1995) Small molecule inhibitors of tyrosine kinase activity. *Expert Opin. Ther. Pat.* 5: 805-817.
- Talamonti, M. S., Roh, M. S., Curley, S. A. and Gallick, G. E. (1993) Increase in activity and level of pp60^{c-src} in progressive stages of human colorectal cancer. *J. Clin. Invest.* 91: 53-60.
- Twamley-Stein, G. M., Pepperkok, R., Anson, W. and Courtneidge, S. A. (1993) The src family tyrosine kinases are required for platelet-derived growth factor-mediated signal transduction in NIH 3T3 cells. *Proc. Natl. Acad. Sci. USA* 90: 7696-7700.
- Uckun, F. M. (1996) Immunoconjugates comprising tyrosine kinase inhibitors. World Patent Application WO9606116, February 29.
- Uehara, Y., Li, P.-M., Fukazawa, H., Mizuno, S., Nihel, Y., Nishio, M., Hanada, M., Yamamoto, C., Furumai, T. and Oki, T. (1993) Angelimicins, new inhibitors of oncogenic src signal transduction. *J. Antibiot.* 46: 1306-1308.
- Wang, W., Chen, H. J., Schwartz, A., Cannon, P. J., Stein, C. A. and Rabbani, L. E. (1996) Sequence-independent inhibition of in vitro vascular smooth muscle cell proliferation, migration, and in vivo neointimal formation by phosphorothioate oligodeoxynucleotides. *J. Clin. Invest.* 98: 443-450.
- Weemink, P. A. O., Verheul, E., Kerkhof, E., van Veelen, C. W. M. and Rijksen, G. (1996) Inhibitors of protein tyrosine phosphorylation reduce the proliferation of two human glioma cell lines. *Neurosurgery* 38: 108-114.
- Xu, W., Harrison, S. C. and Eck, M. J. (1997) Three-dimensional structure of the tyrosine kinase c-src. *Nature* 385: 595-602.
- Xu, X., Williams, J. W., Bremer, E. G., Finnegan, A. and Chong, A. S.-F. (1995) Inhibition of protein tyrosine phosphorylation in T cells by a novel immunosuppressive agent, leflunomide. *J. Biol. Chem.* 270: 12398-12403.
- Xu, X., Williams, J. W., Gong, H., Finnegan, A. and Chong, A. S.-F. (1996) Two activities of the immunosuppressive metabolite of leflunomide, A77 1726. Inhibition of pyrimidine nucleotide synthesis and protein tyrosine phosphorylation. *Biochem. Pharmacol.* 52: 527-534.
- Yamaki, H., Nakajima, M., Seimiya, H., Saya, H., Sugita, M. and Tsuruo, T. (1995) Inhibition of the association with nuclear matrix of pRB, p70 and p40 proteins along with the specific suppression of c-myc expression by geldanamycin, an inhibitor of src tyrosine kinase. *J. Antibiot.* 48: 1021-1026.
- Yokoyama, A., Okabe-Kado, J., Uehara, Y., Oki, T., Tomoyasu, S., Tsuruoka, N. and Honma, Y. (1996) Angelmicin B, a new inhibitor of oncogenic signal transduction, inhibits growth and induces myelomonocytic differentiation of human myeloid leukemia HL-60 cells. *Leuk. Res.* 20: 491-497.
- Zhen, X., Bonjour, J.-P. and Caverzasio, J. (1997) Platelet-derived growth factor stimulates sodium-dependent Pi transport in osteoblastic cells via phospholipase C γ and phosphatidylinositol 3'-kinase. *J. Bone Miner. Res.* 12: 36-44.
- Zimmermann, J., Buchdunger, E., Mett, H., Meyer, T., Lydon, N. B. and Traxler, P. (1996) (Phenylamino)pyrimidine (PAP) derivatives: a new class of potent and highly selective PDGF-receptor autophosphorylation inhibitors. *Bioorg. Med. Chem. Lett.* 6: 1221-1226.
- Zimmermann, J., Buchdunger, E., Mett, H., Meyer, T. and Lydon, N. B. (1997) Potent and selective inhibitors of the abl-kinase: phenylaminopyrimidine (PAP) derivatives. *Bioorg. Med. Chem. Lett.* 7: 187-192.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

Radicación No. 01 12068

Clasificación Internacional (IPC): A 61 K ³¹/404

Concepto Técnico No. 3529

Patente de Invención ☒ Patente de Modelo de Utilidad ☐

Vía Nacional ☒

Vía PCT (fase nacional) ☐ Cap. I ☐ Cap. II ☐

Como resultado del examen de fondo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

1.1. Descripción: Folios: 8 a 106

1.2. Reivindicaciones: Folios: 239 a 256
Folios: 989 a 993 Memorial de 11/04/2005
Folios: 1014 a 1016 Memorial de 22/01/2007
Folios: 1032 a 1034 Memorial de 29/05/2007
Folios: 1106 y 1107 Memorial de 01/06/2009

1.3. Prioridad: US60/182.710 de 15/02/2000
US60/216.422 de 06/07/2000
US60/243.532 de 27/10/2000

1.4. Respuesta del solicitante: Folios: 987 a 993 Memorial de 11/04/2005
Folios: 1007 a 1012 Memorial de 22/01/2007
Folios: 1025 a 1034 Memorial de 29/05/2007
Folios: 1046 a 1062 Memorial de 29/10/2007
Folios: 1097 a 1231 Memorial de 01/06/2009

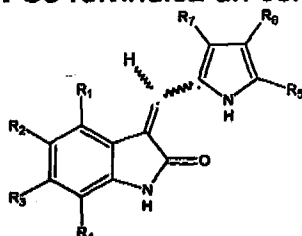
2. Objeto de la invención

Título de la solicitud:

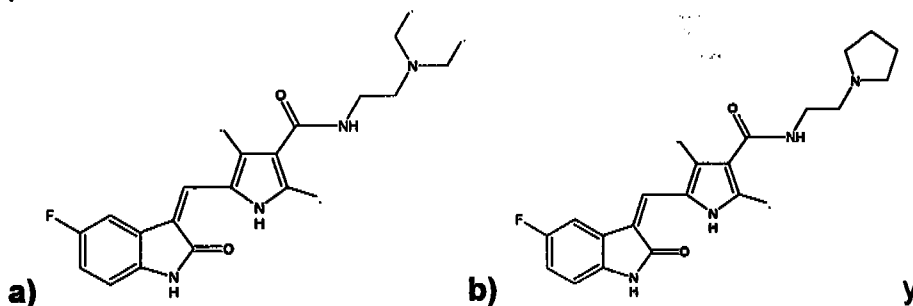
Indolinonas sustituidas con pirroles inhibidores de proteinquinasas

Reivindicaciones

Reivindicaciones 1 a 7: Se reivindica un compuesto de fórmula (I).



En particular se reivindican los compuestos:



c) La sal L-malato de la (2-dietilaminoetil)amida del ácido 5-(flúor-2-oxo-1,2-dihidroindol-3-ilidenmetil)-2,4-dimetil-1H-pirrol-3-carboxílico

Reivindicación 8: Se reivindica una composición farmacéutica caracterizada porque comprende un compuesto de la reivindicación 1, 5 ó 7 o una sal farmacéuticamente aceptable del mismo y un vehículo o excipiente farmacéuticamente aceptable.

Sustento Descriptivo

La presente solicitud tiene como objeto compuestos derivados de 2-indolinona sustituidos con 3-pirroles que se identifican por su capacidad de modulación de las protein-quinasas (PK) y porque presentan la siguiente fórmula estructural: ???

El interesado señala como parte del problema técnico que respecto del cáncer, dos de las hipótesis más importantes para explicar la proliferación celular excesiva que conduce al desarrollo de tumores se relacionan con funciones reguladas por la PK. Se sugiere que el crecimiento de células malignas resulta de una ruptura en los mecanismos que controlan la división y/o diferenciación celular. Se ha demostrado que los productos proteicos de una cantidad de proto-oncogenes están involucrados en las vías de transducción de señales que regulan el crecimiento y la diferenciación celular. Estos productos proteicos de proto-oncogenes incluyen los factores de crecimiento extracelulares, los receptores de factores de crecimiento transmembrana PTK (RTK), las PTK citoplasmáticas (CTK) y las STK citosólicas.

La presente solicitud hace un esfuerzo por identificar moléculas con actividad inhibidora de las protein-quinasas para el tratamiento del cáncer mediante el diseño de compuestos con estructura derivada de las 2-indolinonas sustituidas con pirroles.

- Una vez catalogada la invención ésta se clasificó en la sección: A 61 K ³¹/404 de la **Clasificación Internacional de Patentes**.

3. Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 15/02/2000, se procedió a efectuar la búsqueda de documentos relacionados en el estado de la técnica con fecha de publicación anterior a ésta.

Nº	Documento	Título	Fecha de Publicación
D1	WO9807695	"Indolinone combinatorial libraries and related products and methods	26/02/1998

		for the treatment of disease"	
D2	WO9850356	"2-Indolinone derivatives as modulators of protein kinase activity"	12/11/1998

Anterioridades D1 y D2 en los Folios 1063 a 1081

4. Respuesta a los argumentos del solicitante:

Mediante memorial de 01/06/2009 el señor solicitante argumenta lo siguiente:

- "D1 revela muchas estructuras genéricas, cada una de las cuales presenta sustituyentes opcionales que pueden ser combinados en innumerables combinaciones (sic). A manera de ilustración, adjunto al presente memorial (como ANEXO 1) un reporte de SciFinder, un servicio de Chemical Abstracts Service (CAS), que extrae compuestos de las publicaciones y proporciona su estructura química, en donde se evidencia que SciFinder encuentra 1325 ejemplos específicos en D1, en donde la mayoría de indolinonas allí reveladas no se encuentran sustituidos por pirrol sino por fenilo u otro grupo. Así las cosas, para un persona medianamente versada en la materia es difícil, a partir de la simple lectura de D1, determinar cuáles son los compuestos allí revelados".

"Es evidente que sólo una pequeña fracción de los compuestos revelados en D1 están sustituidos por pirrol (ver anexo 1), y de estos, muchos presentan estructuras de anillos fusionados y/o unidos a la posición equivocada del anillo de pirrol. De la pequeña cantidad de ejemplos que indolinonas (sic) sustituidas por pirrol de D1, los únicos sustituyentes en el anillo de pirrol son H, alquilo, halógeno, éster o alquiléster.

En las estructuras genéricas de D1 en las que el grupo unido en la posición 3 de la indolinona es usualmente un anillo A (ver página 36), la amida se encuentra enunciada entre un sinnúmero de posibles sustituyentes (aunque no una alquilaminoamida como en la presente invención); sin embargo, cuando a se limita a pirrol, los únicos sustituyentes posibles, aún en la estructura genérica, son hidrógeno, alquilo, halógeno, éster y alquiléster".

De acuerdo con los argumentos del señor solicitante, las enseñanzas del documento D1 no son suficientes para desvirtuar el nivel inventivo de la materia reivindicada se por el hecho de que en su divulgación no se encuentran algunas especies químicas ejemplificadas que de manera general han sido contempladas dentro de las modalidades de la fórmula Markush. Esta situación no es consecuente con la práctica recurrente en materia de patentes de acudir a la simbología Markush para definir el contenido y alcance de un grupo inventivo, asociado a una serie de compuestos químicos que comparten una estructura anular.

Al respecto es preciso señalar que no porque sea necesario realizar los correspondientes reemplazos sobre la estructura anular que agrupa un concepto inventivo se deja de ser acreedor a un posible derecho de exclusividad sobre la materia allí definida, tanto en su contenido como en su alcance estructural y funcional, obviamente partiendo del presupuesto de que dicho concepto inventivo y unificador cumple con los requisitos de patentabilidad.

Al parecer el señor solicitante se encuentra de acuerdo con la utilización de la práctica Markush sólo para el evento de la definición de la materia en un pliego reivindicatorio, situación que permite entrever la forma en que ha definido su invención, ya que ha recurrido a la práctica Markush para definir la materia pretendida. Sin embargo, no considera divulgación suficiente del estado del arte la materia que revelan los documentos de patente.

Para esta Superintendencia, es claro que el argumento esbozado por el señor solicitante resulta a todas luces contradictorio e inconsecuente con la forma como ha reivindicado su invención desde el momento en que presentó el primer pliego reivindicatorio ante esta administración y con la forma como divulga la invención en la memoria descriptiva de la solicitud.

Como se ha señalado en repetidas oportunidades, una persona versada en la materia técnica se encontraba en capacidad de derivar los elementos característicos de los compuestos reivindicados a partir de las enseñanzas del estado del arte, toda vez que los compuestos revelados por cada uno de los antecedentes D1 o D2 conservan los elementos funcionales de la estructura 2-indolinona sustituida en posición 3 por metilen-pirrol sustituido, así como su actividad farmacológica. Y contrario a las afirmaciones del señor recurrente el anexo SciFinder emitido por el Chemical Abstracts Service (CAS) lo que permite concluir es el profundo contenido y alcance de la invención revelada por el documento de patente WO9807695, sin embargo, su extensión no resulta oponible para que sea descartada como documento y arte próximo a la materia reivindicada.

Por otra parte, aún cuando los compuestos que presentan sustitución tipo pirrol constituyan una fracción de la divulgación que presenta el documento WO9807695 – D1-, esta situación no es argumento para que deje de ser considerado arte próximo y cercano a los compuestos reivindicados.

Aunque el señor solicitante desestime que el radical Ra que sustituye el grupo amino sobre la posición del anillo de pirrol sólo corresponda a $\text{CO}(\text{CH}_2)_2\text{COOH}$, arilo, alquilo, COOH , OH o NH_2 , para esta Superintendencia es clara la semejanza estructural de estos sustituyentes con el grupo carboxamido que sustituye la misma posición en los compuestos reivindicados. Así, aún cuando las enseñanzas del documento D1 sólo sean una fracción es evidente que a partir de la divulgación de los elementos estructurales que realiza este documento, una persona versada en la materia técnica se encontraba en capacidad de derivar los elementos característicos de los compuestos reivindicados, por lo que no se puede considerar que la materia cumple en estricto sentido con el requisito de altura inventiva.

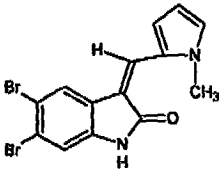
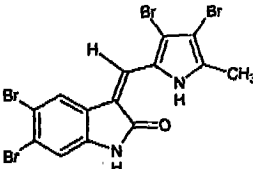
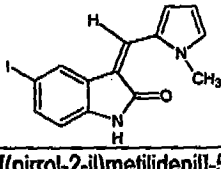
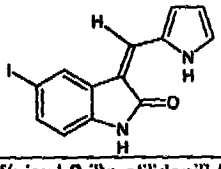
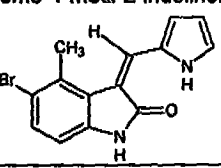
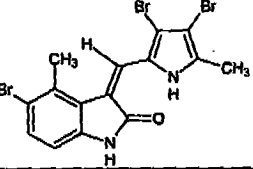
- *"Con respecto a la Tabla 13 de D1, los compuestos que presentan un sustituyente pirrol se encuentran ilustrados específicamente en 10717/04, 10717/A04, 10717/A11, 10717/B4, etc. Sin embargo, como se mencionó anteriormente, ninguno de dichos compuestos presenta una amida como sustituyente en el anillo de pirrol. Ahora, al evaluar la actividad de estos compuestos en la Tabla 14, se encuentra que los mismos no presentan actividad o presentan una actividad insipiente sobre FLK quinasa, y sólo una actividad moderada sobre quinasa EGFR o Met".*

"Por lo tanto, una persona medianamente versada en la materia, con la tarea de desarrollar un compuesto con actividad inhibitoria sobre VEGFR y PDGFR, no habría encontrado motivación alguna para seleccionar y modificar alguno de los compuestos de D1, que enseñan ser más activos sobre otro tipo de quinasas, y cuyo efecto sobre PDGFR es completamente desconocido. Particularmente, en vista de los datos presentados previamente, las indolinonas sustituidas con pirrol, con grupos éster como sustituyentes del anillo de pirrol (según D1), presentaron inhibición de PDGFR muy pobre".

Respecto de las afirmaciones del señor solicitante es preciso señalar que no sólo se encuentran tres ejemplos con sustituciones tipo pirrol sobre el anillo de 2-indolinona, existe otro grupo de compuestos frente a los cuales el señor solicitante ha omitido pronunciarse, toda vez que los datos de porcentaje de inhibición frente a Flk quinasa, EGFR y Met quinasa son significativos. Para ilustrar este hecho se remite al señor solicitante al contenido de la tabla que a continuación se presenta ya que los ejemplos 10717/B09, 10717/C09 y 10717/C11, es decir, el segundo grupo de compuestos con sustituyente de metilen-pirrol presentan porcentaje de inhibición

1265
 incluso del 77,1% sobre Flk quinasa en el caso de la estructura 10717/C09 o del 78,3% sobre EGFR y del 88,8% sobre Met quinasa en el caso de 10717/B09. Es decir, resultados considerables sobre los cuales el señor solicitante no se pronuncia, aún cuando de manera general desestima los resultados de las pruebas con base exclusivamente en los datos reportados para 10717/B09, 10717/C09 y 10717/C11.

Tablas 13 y 14. Estructura – actividad antecedente D1 (folios 1235 a 1237 de la solicitud)

CÓDIGO	NOMBRE	Flk quinasa % Inhibición	EGFR % Inhibición	Met quinasa % Inhibición
10717/A04	3-[(1-metilpirrol-2-il)metilidenil]-5,7-dibromo-2-indolinona 	-4,5	29,0	37,4
10717/A11	3-[(3,4-dibromo-2-metilpirrol-5-il)metilidenil]-5,7-dibromo-2-indolinona 	-5,8	59,9	64,8
10717/B04	3-[(1-metilpirrol-2-il)metilidenil]-5-yodo-2-indolinona 	-18,3	10,3	70,0
10717/B09	3-[(pirrol-2-il)metilidenil]-5-yodo-2-indolinona 	-40,7	78,3	88,8
10717/C09	3-[(pirrol-2-il)metilidenil]-5-bromo-4-metil-2-indolinona 	77,1	8,1	47,7
10717/C11	3-[(3,4-dibromo-2-metilpirrol-5-il)metilidenil]-5-bromo-4-metil-2-indolinona 	4,6	67,3	71,8

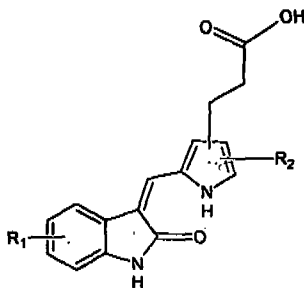
El receptor del factor de crecimiento epidermal EGFR es una proteínquinasa que posee un dominio intracelular y está sobre expuesto en una gran variedad de tumores sólidos, por lo que su inhibición es una medida de la actividad de los compuestos como agentes anticancerígenos. Y dado que el EGFR es indispensable en la proliferación celular e interviene en los procesos relacionados con la progresión del tumor como son adhesión celular, invasión y angiogénesis no entiende esta

Superintendencia cuál es el fundamento técnico sobre el cual basa el señor solicitante su argumentación, para afirmar que ésta no es una motivación suficiente para un experto en la materia para diseñar compuestos con características estructurales similares y con la expectativa de actividad anticancerígena, cuando la actividad del factor de crecimiento endotelial vascular VEGF depende de la actividad del receptor del factor de crecimiento epidermal y por ende, de una estructura con actividad inhibidora del EGFR necesariamente se espera una incidencia y efecto sobre el VEGF¹.

Careless
Argumentation

Por su parte, el documento D2 hace referencia a los resultados de las pruebas de inhibición sobre la proteínaquinasa FLK, PDGFR, EGFR y si bien es cierto dichos resultados de IC₅₀ no resultan tan alentadores debido a que en la mayoría de los casos son mayores a 100µM, existe evidencia de su actividad y esta evidencia fue confirmada en una gran variedad de estudios realizados durante la década del 90, los que aseguran la actividad de 3-[(3 o 4-carboxietilpirrol-2-il)metilidenil]indol-2-ones como inhibidores de los receptores de tirosin quinasas VEGF, FGF y PDGF.

Esta Superintendencia se refiere en particular a las publicaciones "Design, synthesis and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as Ihibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases²" de fecha 19/11/1999 y "Synthesis and biological evaluations of 3-substituted indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particular Receptor Tyrosine Kinases³" de fecha 09/06/1998. En el resumen de la primera publicación se indica que en caso de los compuestos indolin-2-ona sustituidas en posición 3 se han encontrado valores de IC₅₀ en el orden nanomolar, en particular frente a la inhibición de las tirosin quinasas PDGF-R, FGF-R y Flk-1. En especial se hace referencia al compuesto 1 que presenta valores de IC₅₀ de 20 y 30 nM frente a VEGF-R2 (Flk-1/KDR) y FGF-R1 respectivamente. Y en del compuesto 16f se publica un valor de IC₅₀ de 10nM frente al receptor de tirosin quinasa PDGF-R. Tanto el compuesto 1 como el compuesto 16f se derivan de la fórmula Markush que a continuación se presenta:



En consecuencia, una persona medianamente versada en la materia, con la tarea de desarrollar un compuesto con actividad inhibitoria sobre VEGFR y PDGFR, contaba con amplia información sobre las características estructurales de los núcleos de 2-indolinonas sustituidas en posición 3 con actividad anticancerígena ya que el documento D1 presenta una biblioteca amplia de compuestos con potente actividad

¹ Corey K. Goldman, et al. "Epidermal Growth Factor Stimulates Vascular Endothelial Growth Factor Production by Human Malignant Glioma Cells: A model of Glioblastoma Multiforme Pathophysiology". Molecular Biology of the Cell. Vol. 4, 121-133, January 1993.

² Li Sun, et al. "Design, synthesis and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as Ihibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases". Journal of Medicinal Chemistry, 1999, 42 (25), pp 5120-5130. November 19, 1999.

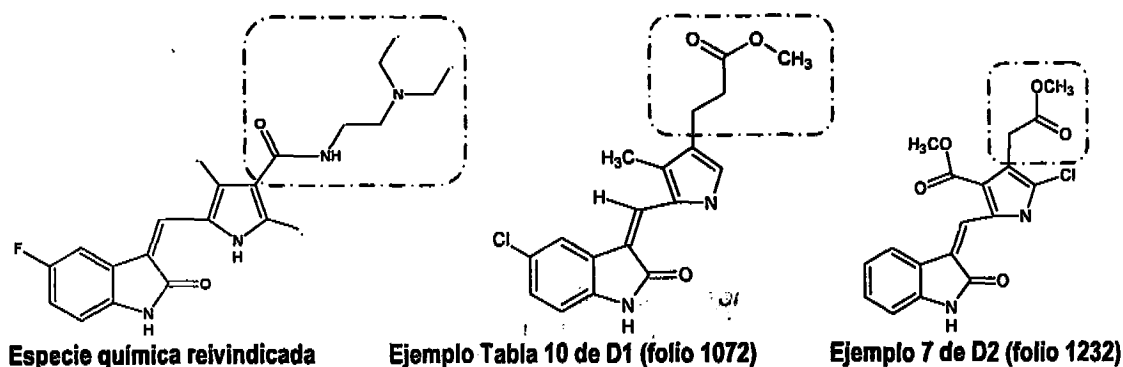
³ Li Sun, et al. "Synthesis and biological evaluations of 3-substituted indolin-2-ones: A Novel Class of Tyrosine Kinase Inhibitors That Exhibit Selectivity toward Particular Receptor Tyrosine Kinases". Journal of Medicinal Chemistry, 1998, 41 (14), pp 2588 - 2603. June 9, 1998.

sobre protein-quinasas y además de dicha publicación existía amplia bibliografía técnica que corroboraba los hallazgos de SUN y colaboradores (inventor relacionado en D1 e investigador que publicó sus hallazgos en el artículo: "Design, synthesis and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as Ihibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases").

De esta manera, los argumentos del señor solicitante sobre la motivación que encontraba una persona versada en ciencias farmacéuticas para diseñar compuestos como los reivindicados es clara y evidente a partir de los documentos del estado del arte D1 y D2, dado que los mismos inventores reiteraron sus resultados iniciales en artículos publicados en la revista Journal of Medicinal Chemistry, por lo que el resultado en términos de actividad que se atribuye el solicitante constituye un resultado obvio y no completamente desconocido como equivocadamente lo ha argumentado sino por el contrario, ha sido ampliamente estudiado.

- "De otra parte, aún cuando D2 revela compuestos que son estructuralmente similares a los de la presente invención, que corresponden a 3-((pirrol-2-il)metilideno)indolin-2-onas, D2 no proporciona enseñanzas o sugerencias respecto al uso de un grupo alquil-carboxamida sustituido en la posición 3 del anillo de pirrol".

Con relación a los argumentos del señor solicitante esta Superintendencia se permite señalar que en la divulgación del documento D2 existen varios ejemplos que permiten evidenciar la obviedad de las modificaciones que presenta el señor solicitante para el compuesto reivindicado (ver esquema comparativo), por lo que es contrario a la verdad que el anillo revelado en esta anterioridad no presente un sustituyente alquil-carboxilo sobre la posición 3 del anillo de pirrol.



Por otra parte, aún cuando se presentan sólo un par de ejemplos en D2 para ilustrar la sustitución de la posición 3 del anillo de pirrol, el señor solicitante olvida que en la definición que acompaña el pliego reivindicatorio de dicho antecedente se propone la opción de tener grupos C-amido en esa posición, por lo que sus argumentaciones tampoco se ajustan a la realidad del contenido divulgado en D2 (ver folio 1080, línea 28, en donde se establecen las connotaciones químicas y funcionales del radical R⁶ presente sobre el anillo de pirrol).

- "Nuevamente, deseo señalar que el concepto inventivo clave involucrado en la presente invención corresponde precisamente al grupo amida (R⁶) que sustituye el anillo de pirrol de los compuestos de la invención. Como lo demuestra la siguiente tabla, la presencia del grupo amida proporciona una mejor actividad, en comparación con compuestos que tienen sustituyentes diferentes en esa posición".

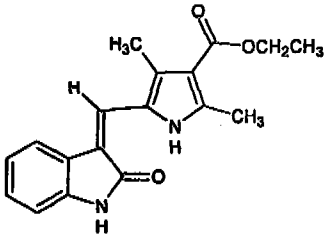
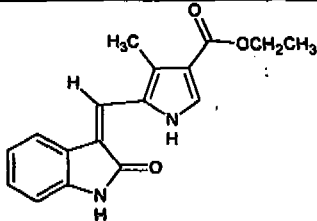
"En gracia de discusión, aunque los compuestos de la presente invención compartan un núcleo común con otros compuestos correspondiente a indolinonas sustituidas con pirrol, la sustitución de la posición 3 del anillo de pirrol en la presente invención (R⁶) resulta completamente novedosa e inventiva, pues compuestos similares del estado del arte, que a lo

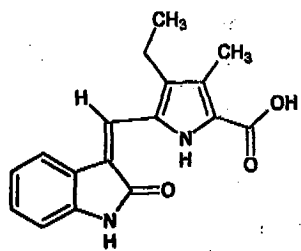
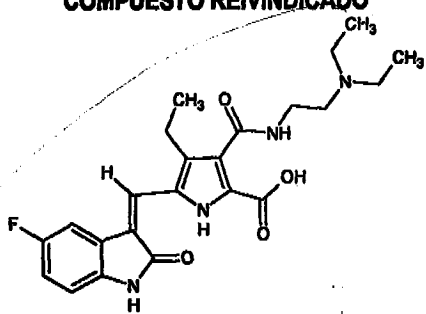
sumo presentan un grupo éster (-C(O)OR) como en los compuestos ilustrados en la anterior tabla, enseñan claramente que el reemplazo de dicho éster o alquil en la posición 3 por un grupo amida, proporciona un incremento significativo en la actividad, produciendo compuestos altamente útiles en la inhibición de ambas kinasas (4 órdenes de magnitud para PDGFR y 2 órdenes de magnitud para VEGFR2), lo que significa una importante ventaja en la terapia anticancerígena”.

Esta Superintendencia no comprende por qué en repetidas oportunidades el señor solicitante ha argumentado que precisamente el sustituyente carboxamida de la posición 3 sobre el anillo de pirrol es la causa y origen fundamental de la actividad anticancerígena de las estructuras de indolil-2-ona sustituidas en posición 3 por metilidenil-pirrol, cuando se ha comprobado en repetidas oportunidades que incluso estructuras que presentan como sustituyentes grupos metilo en dicha posición conservan la actividad anticancerígena, producto de la inhibición de la actividad de los receptores de tirosin-quinasas.

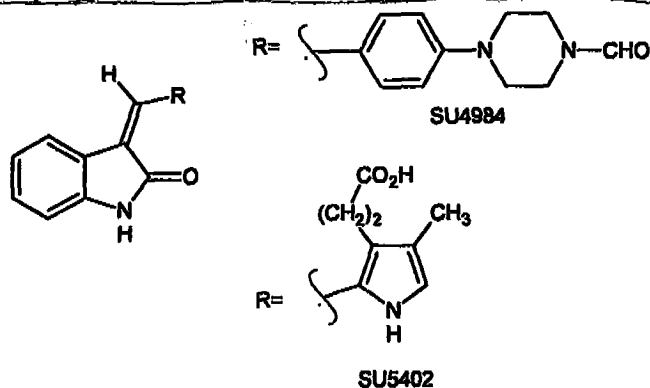
Tanto la publicación D1 como los artículos que sobre actividad han publicado los inventores de los compuestos allí revelados coinciden en afirmar que aún con sustituyentes éster se presenta la actividad inhibidora sobre los receptores de tirosin-quinasa PDGFR con concentraciones mínimas inhibitorias de orden nanomolar (10nM), como es el caso del compuesto 16f que responde a la estructura Markush de la publicación “Design, synthesis and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as lhibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases”². Y dado que el compuesto de referencia reivindicado por el solicitante, sobre el cual sustenta todo su argumento de nivel inventivo, presenta una concentración mínima inhibitoria (IC₅₀) de orden micromolar de 0,008 ±0,002, que traducida a nanomoles evidencia un resultado equivalente al revelado por las investigaciones de SUN y colaboradores, es decir, 8,0nM±2 ya que 1µM=1000nM, es claro que el argumento sobre la reducción en los órdenes de magnitud requeridos para alcanzar la actividad farmacológica no constituye un indicio de altura inventiva, toda vez que no es un efecto inesperado sino anticipado.

Ver la tabla que acompaña los memoriales de respuesta aportados por el señor solicitante en las fechas 29/10/2007 (folios 1049 y 1050) y 01/06/2009 (folio1102) que a continuación se transcribe en su totalidad:

Compuesto	PDGFRb IC ₅₀ (µM) ²	VEGFR2 IC ₅₀ (µM) ³
	>100	0,578 ± 0,035 (n=2)
	>100	0,465 ± 0,068 (n=2)

	>100	>10 (n=2)
COMPUESTO REIVINDICADO 	0,01	0,008±0,002 (n=5)

Otras publicaciones que forman parte de la bibliografía técnica relacionada con el tema, señalan que aún con estructuras como las que a continuación se presentan, se conserva la actividad inhibitoria de las protein-quinasas. Específicamente, en los estudios realizados por *Mohammadi y colaboradores*⁴ en el año 1997 se indica que las estructuras SU4984 y SU5402 inhiben las quinasas FGFr-1 con IC₅₀ de 10 a 20µM, por lo que es evidente que la actividad permanece incólume aún con modificaciones complejas sobre la estructura de la metiliden-indolil-2-ona.



Así las cosas, existe un gran número de publicaciones en revistas, derivadas de los estudios inicialmente revelados por los documentos D1 y D2, las que durante la década del 90 se dedicaron a constatar los resultados de actividad asociada al núcleo de las metiliden-indolil-2-onas sustituidas por pirrol reveladas inicialmente por SUN y colaboradores en D1 y por TANG y colaboradores en D2.

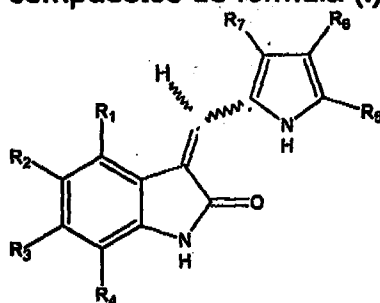
5. Análisis de patentabilidad (artículo 14 D. 486)

5.1 Nivel inventivo (artículo 18 D. 486)

El requisito de altura inventiva se evaluó por comparación entre los elementos característicos de los compuestos reivindicados y aquellos revelados por el estado del arte, empleando la aproximación problema-solución y a partir de las enseñanzas técnicas reveladas por los documentos D1 y D2.

⁴ Showalter, Hollis, et al. "Small Molecule Inhibitors of the Platelet-Derived Growth Factor Receptor, the Fibroblast Growth Factor Receptor, and Src Family Tyrosin Kinases". Pharmacol. Ther. Vol. 76, Nos. 1-3 pp. 55.71, 1997. Elsevier.

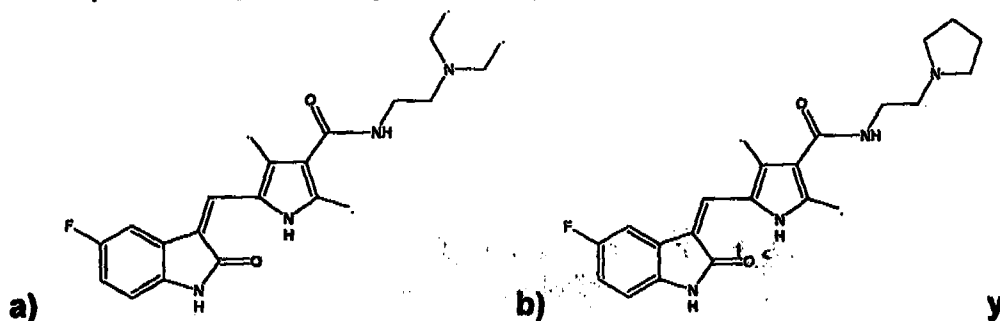
- Se reivindican compuestos de fórmula (I):



Para los cuales:

- R^1 es -H, alquilo C_{1-4} , $-(CH_2)_r R^{16}$ y $-C(O)NR^8 R^9$
- R^2 es -H, halo, fenilo, $-S(O)_2 NH$ fenilo y $-SO_2 NH$ piridinilo
- R^3 es -H, alquilo C_{1-4} , alcoxi C_{1-4} , fenilo, piridilo y $-C(O)R^{15}$
- R^4 es -H
- R^5 es -H y alquilo C_{1-4}
- R^6 es $-C(O)R^{10}$
- R^7 es -H, alquilo C_{1-4} y fenilo
- R^8 y R^9 se selecciona independientemente entre -H, alquilo C_{1-6} y fenilo
- R^{10} es $-N(R^{11})(CH_2)_n R^{12}$, donde n es 1, 2 ó 3
- R^{11} es -H
- R^{12} es alcoxi C_{1-4} , $-C(O)R^{15}$, pirrolidina, pirrolidin-2-ona, piperazina, imidazol-2-ona, piridin-2-ona, tetrahidropirimidin-2-ona, 2-oxo-azepan y $-NR^{13} R^{14}$
- R^{13} y R^{14} se seleccionan del grupo formado por -H, alquilo C_{1-4} , o R^{13} y R^{14} se pueden combinar para formar un grupo $-(CH_2)_4-$, $-(CH_2)_5-$, $-(CH_2)_2-O-(CH_2)_2-$ y $-(CH_2)_2 N(CH_3)(CH_2)_2-$
- R^{15} se selecciona del grupo formado por -H, hidroxi, alcoxi C_{1-4} y fenoxi
- R^{16} se selecciona del grupo formado por -OH, $-C(O)R^{15}$
- r es 2 ó 3

En especial, se pretende protección para las especies químicas:

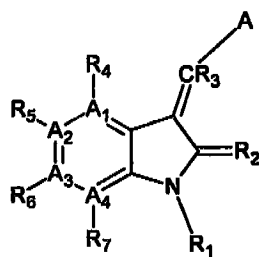


- La sal L-malato de (2-dietilaminoetil)amida de ácido 5-(flúor-2-oxo-1,2-dihidroindol-3-ilidenmetil)-2,4-dimetil-1H-pirrol-3-carboxílico.

- El estado del arte previo a la invención presenta los siguientes documentos:

Anterioridad D1:

La publicación D1 revela estructuras que presentan el núcleo de fórmula (III):



Para el que:

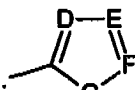
R_1 es H o alquilo

R_2 es oxígeno o azufre

R_3 es H

R_4 , R_5 , R_6 y R_7 se eligen de manera independiente entre H, alquilo, alcoxi, aril, ariloxi, alcaril, alcariloxi, halógeno, trihalometil, entre otros.

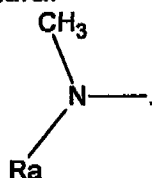
A_1 , A_2 , A_3 y A_4 se eligen de manera independiente entre C o N



A representa un anillo: donde D, E, F y G son átomos de nitrógeno, carbono o azufre

A es un anillo de cinco miembros de tipo heteroarilo seleccionado del grupo que consiste en: tiofeno, pirrol, pirazol, isoxazol, imidazol, 1,2,3-triazol, oxazol, tiazol, entre otros.

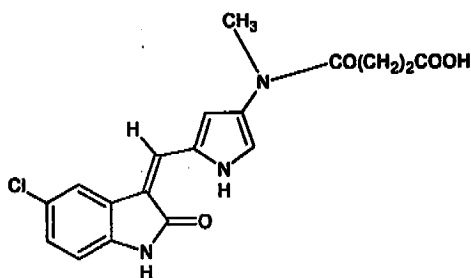
El anillo A se sustituye además en las posiciones 4, 5 y 6 por una amina que presenta la siguiente estructura:



Para la cual:

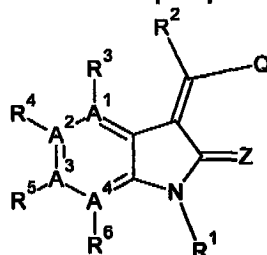
R_a es $\text{CO}(\text{CH}_2)_2\text{COOH}$, arilo, alquilo o contiene un grupo COOH , OH o NH_2

De esta manera, al sustituir dentro de la estructura los grupos más representativos se tienen compuestos que presentan los siguientes elementos característicos:

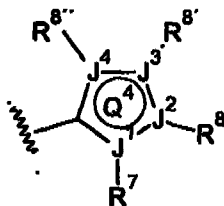


Anterioridad D2:

La publicación D2 revela estructuras que presentan el siguiente núcleo:



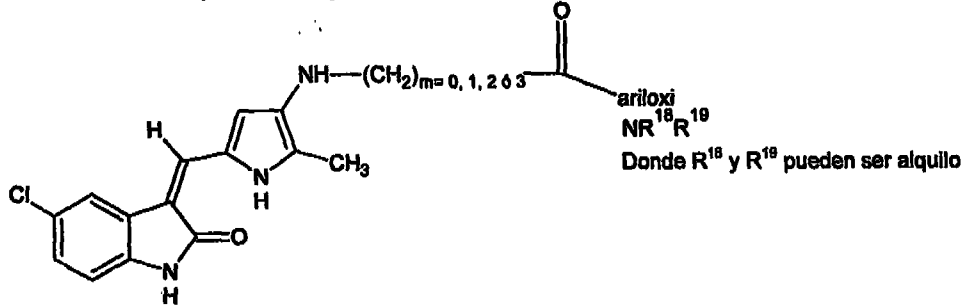
Para la cual:



Q es
Z es oxígeno
R¹ es -H
R² es -H
R³, R⁴, R⁵ y R⁶ es -H, alquilo, alcoxi, ariloxi, halo, entre otros.
A¹, A², A³ y A⁴ es carbono o nitrógeno y en el caso que es nitrógeno no existen R³, R⁴, R⁵ y R⁶

B¹ y B² son elegidos entre N y O
J¹ es O, N o S
J², J³ y J⁴ son elegidos de manera independiente del grupo que consiste entre carbono, nitrógeno, oxígeno y azúfre
R⁷, R⁸, R^{8'}, R^{8''}, R⁹, R^{9'}, R¹⁰, R^{10'}, R¹¹, R^{11'}, R¹², R^{12'} son elegidos de manera independiente entre H, alquilo, trihalometilo, N-sulfonamido, C-amido, N-carbamoil, O-carbamoil, NR¹⁸R¹⁹ (para el que R¹⁸ y R¹⁹ pueden ser elegidos de manera independiente entre H, alquilo, C-carboxi, carbonilo, acetilo, sulfonilo y puede estar combinado con un anillo heteroalícyclico de 5 ó 6 miembros), -W(CH₂)_mC(=Y)T (para el que W es elegido entre N, O y S; m es un número elegido entre 0, 1, 2 ó 3; Y es elegido entre O y S; T es un grupo -OH, alcoxi, ariloxi, O-carboxi, -NR¹⁸R¹⁹).

De esta manera al reemplazar los radicales por las connotaciones más representativas reveladas en el antecedente D2 se tienen compuestos con las siguientes características químicas y funcionales:



Análisis de diferencias técnicas:

Al comparar los elementos técnicos característicos de la fórmula 1 y de las especies químicas reivindicadas junto a las enseñanzas del estado del arte reveladas en D1 y D2 fue posible establecer las siguientes diferencias técnicas:

Tabla 1. Comparación de elementos técnicos.

Materia Reivindicada	D1 (WO9807695)	D2 (WO9850356)
Cíausula 1:		

Cláusulas 5 y 7:	Folio 1072 Tabla 10.	3-(2-acetil-3,4-dimetilpirrol-5-metilidenil)-2-indolinona (Ejemplo 2 folio 1232) 3-(2-cloro-4-metoxicarbonil-3-metoxicarbonilmetil-pirrol-5-metilidenil)-2-indolinona (Ejemplo 7 folio 1232) 3-[(3,4-dibromo-2-metilpirrol-5-il)metilidenil]-5,7-dibromo-4-metil-2-indolinona (Ejemplo 10717/H11 folio 1233)

Con respecto a D1 la diferencia estriba en que A corresponde a un grupo $-NCH_3R_a$ donde R_a es $-\text{CO}(\text{CH}_2)_2\text{COOH}$, arilo, alquilo o contiene un grupo $-\text{COOH}$, $-\text{OH}$ o $-\text{NH}_2$. Es decir, el grupo amido de la cadena lateral que se enlaza al anillo de pirrol lo hace por el átomo de nitrógeno y no por el carbono carbonílico.

Con respecto al documento D2 la diferencia estriba en que el sustituyente R^8 puede ser un grupo C-amido sin que incluya una sustitución adicional alquil-amino o alquil-heterociclo como en el caso de las especies reivindicadas en las cláusulas 5 y 7. O eventualmente puede ser $\text{NR}^{18}\text{R}^{19}$ donde R^{18} y R^{19} puede ser carbonilo y alquilo o un anillo heterocíclico de 5 ó 6 miembros, pero en este caso, el enlace al anillo de pirrol se efectúa por medio del átomo de $-\text{N}$ y no por el átomo de carbono del grupo carbonilo como en el caso del sustituyente R_6 de la fórmula Markush. R^8 en D2 también puede ser $-\text{W}(\text{CH}_2)_m\text{C}(=\text{Y})\text{T}$ donde W es $-\text{N}$, m es un número elegido entre 0, 1, 2 ó 3, Y es O y T es un grupo alcoxi, arilo, O-carboxi o $-\text{NR}^{18}\text{R}^{19}$. Es decir, en este caso también el enlace al anillo de pirrol se efectúa mediante el átomo de $-\text{N}$ y no por el átomo de carbono carbonílico.

Del problema técnico objetivo y el efecto técnico:

El problema técnico objetivo tiene como fundamento el diseño y desarrollo de estructuras químicas alternativas que presenten actividad como inhibidores de las quinasas PDGFRb y VEGFR2.

No obstante, el problema técnico parece haber sido resuelto por los compuestos revelados en los antecedentes en especial por los que enseña el documento D2 que presentan el sustituyente carbamoilo en la posición 3 del anillo de pirrol y de otros que se publican las Tablas 7 y 8 del folio 1075 que hacen referencia a la actividad de 2-indolinonas sustituidas en posición 3 por metilidenil-pirrol y cuyo sustituyente es de tipo carboxilo, como inhibidores de la actividad del grupo de quinasas relacionadas con el factor de crecimiento endotelial EGFR, y con el receptor del factor de crecimiento derivado de plaquetas PDGFR.

De igual manera, las enseñanzas reveladas por el antecedente D1 dejan entrever que estructuras que conservan el núcleo de indolinona sustituida por metiliden-pirrol y con radicales tipo carboxamido en la posición 3 del anillo de pirrol presentan actividad sobre el grupo de protein quinasas conformado por: Flk, FGFR, PDGFR y raf.

Así las cosas, al estudiar en conjunto o de manera independiente las enseñanzas de los antecedentes técnicos D1 y D2 es posible anticipar tanto los elementos estructurales y funcionales de los compuestos reivindicados como el efecto técnico atribuido a las estructuras, debido a que su actividad como anticancerígenos y moduladores de procesos inflamatorios en enfermedades como artritis, ha sido estudiada desde la década del 90.

Para ilustrar este hecho se remite al señor solicitante al contenido de la tabla 10 del antecedente D1 (folios 1072 y 1073) y de las tablas 7 y 8 del antecedente D2 (folio 1075) en las que se incorporan datos de concentración mínima inhibitoria IC₅₀ como medida de la actividad de los derivados de pirrol-metilen-indolinona sobre un grupo de enzimas pertenecientes a las protein quinasas. Así, en virtud de los conocimientos que revela el estado del arte más cercano a la invención era posible derivar las características de la materia reivindicada y en consecuencia una persona versada en ciencias farmacéuticas se encontraba en capacidad de diseñar moléculas como las reivindicadas, tomando como punto de partida las enseñanzas de los antecedentes D1 y/o D2, por lo que la materia reivindicada carece de altura inventiva y no constituye un salto cualitativo en la regla técnica.

Ahora bien, un experto en la materia sobre la base de la totalidad de los conocimientos recogidos en los documentos D1 y D2 habría reconocido la posibilidad de modificar el sitio de unión al radical carbamoilo sin que ello generara alteración alguna en la actividad farmacológica de las moléculas, ya que D2 presenta la opción de sustituir la posición 3 del anillo de pirrol exclusivamente por un grupo carboxamido enlazado a través del carbono carbonílico sin que se modifique la actividad, o eventualmente por un grupo carboxamido sustituido por alquilo o arilo, por lo que la diferencia técnica hallada no es evidencia de un efecto técnico inesperado.

Por las razones expuestas anteriormente esta dependencia reitera que el arte previo afecta la altura inventiva de la materia reclamada y por consiguiente existe incumplimiento de las disposiciones contempladas en el artículo 18 del ordenamiento jurídico andino.

6. Conclusión:

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Negar** las reivindicaciones: 1 a 8 (folios: 1106 a 1107)

Bogotá, D.C., 06/10/2009

CONCEPTO TECNICO No. 3529	EXAMINADOR TECNICO Código No. 202044
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REPÚBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCIÓN N° 53738

Por la cual se resuelve un recurso de reposición

Rad. PCT 01 012068

29 10 2

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
en ejercicio de sus facultades legales, especialmente de las conferidas en el
numeral 54 del artículo 3° del Decreto 1687 de 2010, y

CONSIDERANDO

PRIMERO: PRIMERO: Que, mediante Resolución N° 68274 del 29 de diciembre de 2009, la Superintendencia de Industria y Comercio denegó la solicitud de patente de invención a la creación denominada "INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE PROTEINQUINASAS", con fundamento en los artículos 14 y 18 de la Decisión 486 de la Comisión de la Comunidad Andina, al estimar que la misma no cumple con el requisito de nivel inventivo, por tratarse de moléculas con actividad inhibidor sugeridas por el arte previo.

SEGUNDO: Que, mediante escrito radicado en esta Entidad el 29 de enero de 2010, con el número 01 012068 00020 - 0000, el doctor Carlos Olarte, en su calidad de apoderado de la sociedad Sugén Inc. y Pharmacia & Upjohn Company, interpuso recurso de reposición contra la citada Resolución, con fundamento en los argumentos que a continuación se sintetizan:

El recurrente sostiene que el documento WO9807695 (en adelante llamado D1), no revela o sugiere compuestos correspondientes a un sistema de indolin-2-ona-3-pirrol, que a su vez esté sustituido en la posición 3 por un grupo amida sustituida, además afirma que en ninguna de las posibilidades sugeridas por D1 se considera incluir un grupo amida en la posición 3 de dicho anillo de pirrol. Adicionalmente, señala que la persona medianamente versada en la materia al incluir un grupo altamente electronegativo como sustituyente del anillo de pirrol y los electrones resonantes de la estructura se deslocalizan disminuyendo la densidad electrónica global del anillo. Por lo tanto, la diferencia estructural entre los compuestos de D1 y la presente invención, específicamente relacionada con la inclusión de una sustitución de un grupo electronegativo sobre su estructura, claramente conlleva a cambios en las propiedades moleculares.

En relación con la anterioridad WO9850356 (en adelante llamado D2), el apoderado afirma que, como lo reconoce el examinador, dicha anterioridad, si bien ella menciona como posible sustituyente del anillo de pirrol la inclusión de un grupo dicho C-amido, éste no puede estar sustituido adicionalmente por alquil amino o alquil-heterocíclico, como en el caso de las especies reclamadas. Igualmente, resalta que ninguno de los ejemplos ilustrados en D2 enseña el uso de un grupo C-amido (obviamente no sustituido) sobre la posición 3 del anillo de pirrol que se encuentre en la posición 3 del grupo indolin-2-ona.

Por otra parte, el apoderado argumenta que "es incomparable la sustitución amidica sustituida, a su vez por una dialquil-alquil-amina de la presente invención, con grupos alquil-carboxílicos o alquil-éster del arte previo". Y afirma que "los



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efectos electrónicos de un grupo amida sobre un anillo de pirrol disminuyen significativamente la densidad de electrones sobre dicho anillo, con consecuencias en las propiedades fisicoquímicas y aún biológicas de la molécula. Adicionalmente, no es lo mismo incluir en la molécula un extremo de carácter ácido (-COOH o -COOR), que un extremo de carácter básico (dietilamina -Reivindicación 5, o pirrolidina -Reivindicación 7), pues esto definitivamente modifica las interacciones ligando-receptor en el sitio de unión de la proteína. En conclusión, la configuración estructural específica de los compuestos de la presente invención así como sus efectos, no han sido sugeridas en el arte previo y por lo tanto no puede ser considerada obvia para una persona medianamente versada en la materia".

De otro lado, el recurrente hace referencia a la declaración juramentada de la Dra. Jean Cui (la cual adjunta como anexo 3); experta en la química medicinal de compuestos basados en un núcleo de indolinona-3-pirrol como los de la solicitud denegada y alega que *"la invención reivindicada ha permitido determinar que la sustitución amida sobre la posición R⁶ en los compuestos desarrollados, aumenta los efectos de inhibición de la quinasa en general, y de manera más importante mejoró la solubilidad y estabilidad metabólica, de manera dramática".* Igualmente, afirma que *"aunque el arte previo citado por el examinador haya revelado moléculas que comparten un núcleo estructural con las moléculas de la presente invención y se haya determinado su actividad general sobre enzimas de tipo quinasa, a partir de dichas enseñanzas no es posible deducir la utilidad final de dichas moléculas y menos aún la ventajas que proporcionarían variaciones novedosas e inventivas, no sugeridas en dicho arte previo".*

El recurrente considera que no hay enseñanzas en la anterioridad D1 que guíen a una persona medianamente versada en la materia al desarrollo específico de moléculas con la estructura específica (3-amido)-3-(pirrol)-3-indolin-2-ona. Es así como los ensayos de D1 corresponden a un screening farmacológico inicial cuyos resultados proporcionan datos de porcentajes (%) de inhibición, lo que significa que los ensayos fueron realizados con una concentración fija que, tal como lo manifiestan los mismos investigadores (ver Declaración del Dr. J. Cui), en la época de presentación de dicha solicitud correspondían a 10µM; por el contrario, los resultados comparativos que se proporcionan para la presente invención ilustran datos de IC₅₀, que para los compuestos reivindicados se encuentran en niveles del rango nanomolar, lo que significa por lo menos 3 órdenes de magnitud de potencia mejorada.

El apoderado alega que el cambio estructural en el sustituyente de la posición 3 del anillo de pirrol, modifica significativamente la actividad de moléculas de tipo 3-(pirrol)-3-indolin-2-ona; con lo anterior se demuestra que los compuestos reivindicados (i) proporcionan una potente inhibición sobre VEGFR2 y PDGFR y (ii) que la potencia multikinasa aumentada se debe principalmente a la presencia de un grupo amido (-C(O)NR'(CH₂)_nR, donde R es como se define en las reivindicaciones) en la posición R⁶ del anillo de pirrol. Igualmente, indica que ni las



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modificaciones estructurales en esta posición ni las ventajas del sustituyente amido fueron siquiera sugeridas por el arte previo.

El recurrente insiste en que la Dra. J. Cui en su declaración juramentada proporciona ejemplos adicionales en este sentido, donde compuestos que comparten el núcleo estructural, pero difieren en sus sustituyentes, proporcionan resultados diferenciales sobre tres enzimas quinasas (FLK1, el ortólogo en ratón de la VEGFR humana; Src; y CDK2, que es una serina-treonina quinasa).

Complementa lo anterior expresando que *"me permito llamar la atención de ese despacho a los resultados ilustrados en el artículo de Li Sun et al., J. Med. Chem, 46, 2003 1116-1119, que también son concluyentes demostrando que la inclusión del grupo amida en la posición 4 del anillo pirrol (R⁶), proporciona resultados ventajosos e inesperados (ver Tabla 1 del artículo en mención, anexado en la respuesta al tercer examen de fondo)"*.

De otro lado, el recurrente afirma que *"[e]l compuesto SU5416 (incluido en la divulgación general de D1 y D2, y la publicación Li Sun, J. Med. Chem, 1998), no tiene sustituyentes polares sobre el anillo de pirrol. Este compuesto mostró una potencia moderada contra VEGFR, una pobre solubilidad y pobre estabilidad metabólica, lo que resultó en una inadecuada farmacocinética in-vivo. El anillo de pirrol rico en electrones proporciona un compuesto que es fácilmente metabolizable, para que el (sic) la vida media resulta excesivamente reducida y por lo tanto con un potencial anticancerígeno limitado"*.

Además, hace énfasis en que *"[e]l compuesto SU6668 (cubierto en general por D1 y la publicación Li Sun, et al. J. Med. Chem, 1999-pie de página 5), que tiene un sustituyente alquil en la posición R⁶ (ácido propiónico), mostró una potencia prometedora contra VEGFR y PDGFR, sin embargo, éste compuesto sufre un alto 'clearance' (es removido rápidamente de la sangre) y tiene un perfil de unión a proteínas muy alto, por lo tanto, no proporcionó efectividad alguna en humanos. Lo anterior ilustra que una actividad in-vitro dada, no garantiza que un principio activo proporcione también una actividad útil in-vivo y aún menos que dicha molécula pueda formularse en un medicamento"*.

Finalmente, el recurrente señala que muchos países han concedido patente a solicitudes equivalentes a esta, entre ellos Perú y Ecuador que, como Colombia, son miembros de la CAN y se rigen por la misma legislación de propiedad industrial. Asimismo, afirma que se entiende que bajo lo establecido por el artículo 18 de la Decisión 486 Perú y Ecuador consideran que la invención aquí reclamada es inventiva.

TERCERO: Que, dentro del contexto antes descrito, esta Entidad procede a resolver el recurso de reposición interpuesto en los siguientes términos:



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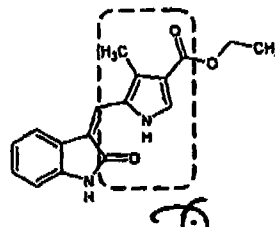
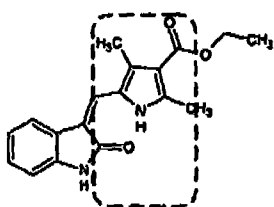
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En relación con los argumentos del recurrente sobre el nivel inventivo de la solicitud denegada frente a la anterioridad D1, este Despacho reitera que, tal y como se expuso en la Resolución de denegación, la persona medianamente versada en la materia se encontraba en capacidad de derivar los elementos característicos de los compuestos reivindicados a partir de las enseñanzas del estado del arte, toda vez que los compuestos revelados por cada uno de los documentos D1 o D2 conservan los elementos funcionales de la estructura 2-indolinona sustituida en la posición 3 por metilen-pirrol sustituido, así como su actividad farmacológica. Adicionalmente, la extensión del documento de patente WO9807695 no es un argumento relevante para que sea descartado como documento y arte próximo a la materia reivindicada. Si bien los compuestos que presentan sustitución tipo pirrol representan una proporción de la divulgación total contenida en D1, esto no se constituye en un argumento valedero para no seguir considerándolo como arte próximo a los compuestos reclamados.

Ahora bien, la anterioridad D1 enseña que el radical Ra sustituye el grupo amino sobre la posición del anillo de pirrol, el cual corresponde a $\text{CO}(\text{CH}_2)_2\text{COOH}$, arilo, alquilo, COOH , OH o NH_2 . Para este Despacho es evidente la similitud estructural de estos sustituyentes con el grupo carboxamido que sustituye la misma posición en los compuestos reivindicados, con lo cual se evidencia que, a partir de la divulgación de los elementos estructurales que realiza D1, una persona medianamente versada en la materia se encontraba en capacidad de derivar los elementos característicos de los compuestos reivindicados, por lo que no se puede considerar que la materia reivindicada cumple con el requisito de altura inventiva.

En relación con la afirmación del recurrente sobre la presencia de un grupo altamente electronegativo en el anillo pirrol que da lugar a una deslocalización que disminuye la densidad electrónica del anillo conllevando a cambios en las propiedades moleculares, cabe anotar que es un fenómeno que no sólo puede presentarse en los compuestos reclamados en la presente invención, sino que también puede tener lugar en los compuestos 1 y 2 de la tabla presentada por el recurrente (FI.1307) que corresponden al estado de la técnica, los cuáles contienen el grupo altamente electronegativo en el anillo pirrol. Sin embargo, dicha posible deslocalización electrónica no garantiza la actividad superior sobre PDGFR y VEGFR2 en los compuestos 1 y 2 de las tablas referidas, según lo demostrado por el mismo solicitante:



Compuestos 1 y 2 tabla folio 1307



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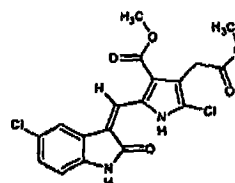
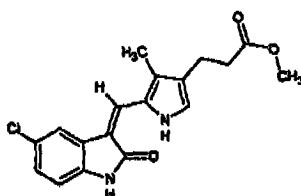
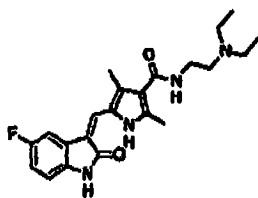
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Dichos compuestos, a pesar de presentar un grupo altamente negativo en el pirrol (el cuál se encuentra encerrado en el recuadro), no logran la marcada inhibición sobre los dos receptores ni una potencia multiquinasa aumentada según lo expresa el recurrente (FI.1308). En consecuencia, dicha deslocalización en los derivados de indolinonas no sustenta la afirmación del apoderado, en cuanto a que la misma da lugar a cambios moleculares con efectos como: (i) la potencia inhibitoria; (ii) el perfil de selectividad sobre quinasas y (iii) sus propiedades farmacéuticas; por el contrario, la presencia de dicha estructura en las anterioridades desvirtúa lo afirmado por el recurrente. Es por ello que no es posible extrapolar la actividad farmacológica de un compuesto específico como el de la reivindicación 5 a todos los contenidos en la fórmula general Markush de la reivindicación 1, puesto que como se demostró, la presencia de un grupo altamente electronegativo sobre el anillo pirrol no necesariamente puede dar lugar a compuestos inhibidores potentes de VEGFR2 y PDGFR.

Por otra parte, contrario a lo afirmado por el recurrente, en la divulgación del documento D2 existen varios ejemplos que permiten evidenciar la obviedad de las modificaciones que reclama la presente invención para el compuesto solicitado:



Especie química reivindicada Ejemplo Tabla 10 de D1 (folio 1072) Ejemplo 7 de D2 (folio 1232)

En consecuencia, el compuesto revelado en la anterioridad D2 sí presenta un sustituyente alquilcarboxilo sobre la posición 3 del anillo pirrol. Si bien es cierto que D2 presenta sólo dos ejemplos para ilustrar la sustitución de la posición 3 del anillo de pirrol, el recurrente olvida que en la definición que acompaña el pliego reivindicatorio de dicho antecedente se propone la opción de tener grupos C-amido en esa posición (FI.1080, línea 28, en donde se establecen las connotaciones químicas y funcionales del radical R8' presente sobre el anillo de pirrol).

Respecto al argumento según el cual *"es incomparable la sustitución amidica sustituida, a su vez por una dialquil-alquil-amina de la presente invención, con grupos alquil-carboxílicos o alquil-ester del arte previo"*, esta Entidad estima que los efectos que podrían presentarse por una posible deslocalización electrónica en el anillo de pirrol, también pueden tener lugar en compuestos de las anterioridades D1 y D2 como los de la tabla relacionada en el folio 1307 (compuestos 1 y 2 correspondientes a las anterioridades), y que ello no garantiza como consecuencia afinidad y elevada potencia sobre los receptores PDGFR y VEGFR2, por lo tanto no es posible atribuir en forma general la actividad del compuesto a una eventual deslocalización electrónica desde el nitrógeno del anillo pirrol. Cabe aclarar que las modificaciones en la estructura y componentes de una molécula tienen efecto

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sobre las interacciones ligando receptor como bien lo afirma el apoderado, es por ello que no es aceptable afirmar que por el hecho de que un compuesto posea una actividad específica, todos los demás compuestos que se encuentren comprendidos en una estructura Markush, como la presentada en la reivindicación 1, sean igualmente una solución al problema técnico.

Ahora bien, a la luz de la información proporcionada por el recurrente, es claro que solamente los compuestos específicos contenidos en las reivindicaciones 5 y 7, para los cuales el apoderado ha aportado información en la descripción, evidencian actividad sobre los receptores PDGFRb y VEGFR2. Sin embargo, como ya se ha señalado, la actividad demostrada para los compuestos específicos no puede extrapolarse a las demás variaciones reclamadas que no contienen los grupos dietilamina o pirrolidina, o que no son básicos como cuando R12 es alcoxi (C₁-C₄) o C(O)R₁₅ (donde R₁₅ puede ser OH, dando lugar a un grupo carboxilo de carácter ácido), ya que esto conllevaría una modificación de la interacción ligando receptor en el sitio de unión a la proteína, como lo expone el recurrente.

De otro lado, en cuanto a las mejoras que presentan las estructuras reivindicadas, este Despacho encuentra que no existe dentro de la presente invención información o datos que sustenten una mejora en la solubilidad y estabilidad metabólica como lo argumenta el recurrente, razón por la cual estos aspectos no pueden considerarse como una eventual ventaja de los compuestos reclamados por parte del examinador.

Con respecto a los IC₅₀ presentados para el compuesto de la reivindicación 5, se reitera que tanto la anterioridad D1 como los artículos que sobre actividad han publicado los inventores de los compuestos allí revelados coinciden en afirmar que aún con sustituyentes ester se presenta la actividad inhibidora sobre los receptores de tirosinaquinasa PDGFR con concentraciones mínimas inhibitorias de orden nanomolar (10 nM), como es el caso del compuesto 16f que responde a la estructura Markush de la publicación "*Desing, synthesis and evaluations of substituted 3-[(3- or 4-carboxyethylpyrrol-2-yl)methylidenyl]indolin-2-ones as inhibitors of VEGF, FGF, and PDGF Receptor Tyrosine Kinases*" (Fl. 1240), y dado que el compuesto reivindicado, sobre el cuál el apoderado sustenta su argumento sobre nivel inventivo, presenta una concentración mínima inhibitoria (IC₅₀) de orden micromolar de 0,008 ± 0,002, que traducida a nanomolares evidencia un resultado equivalente al revelado por las investigaciones de Li Sun y colaboradores, es decir, 8,0 nM ± 2, queda claro que el argumento sobre la reducción en los órdenes de magnitud requeridos para alcanzar la actividad farmacológica no constituyen un indicio de altura inventiva, toda vez que no es un efecto inesperado sino anticipado.

Ahora bien, este Despacho encuentra que los argumentos expuestos en la declaración rendida por la Dra. Jean Cui, si bien ratifican la actividad del compuesto de la actual reivindicación 5 dentro del rango que se había divulgado en el estado



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de la técnica, no desvirtúan que la fórmula general de la reivindicación 1 haya sido evidente a partir de las enseñanzas contenidas en los documentos D1 y D2, que junto con los resultados divulgados por SUN y sus colaboradores en el Journal of Medicinal Chemistry entre 1998 y 1999, claramente indicaban la utilidad de las indolin-2-onas como inhibidores de la tirosina quinas al actuar sobre VEGF2 y PDGFR, siendo en consecuencia obvio para la persona medianamente versada en la materia en el momento de la invención buscar compuestos alternativos que compartan el núcleo indolin-2 ona sustituido en la posición 3 con un anillo pirrol.

En el caso particular, el hecho de que tiempo después de presentarse la solicitud denegada se determinaran desventajas de los compuestos divulgados por Li Sun en 1998, no le restituye el nivel inventivo a la presente invención, ya que al momento de invocarse la prioridad, la información existente en el estado de la técnica y con la cual contaba el solicitante antes de la invención direccionaba a la persona medianamente versada en la materia en forma inequívoca a los compuestos aquí reclamados.

En relación con que el cambio estructural en el sustituyente de la posición 3 del anillo de pirrol modifica significativamente la actividad de moléculas de tipo 3-(pirrol)-3-indolin-2-ona, esta Oficina aclara que es ampliamente reconocido que la inhibición de la proteinquinasa es una medida de la actividad de los compuestos como agentes anticancerígenos. Y dado que el EGFR es indispensable en la proliferación celular e interviene en los procesos relacionados con la progresión del tumor como son adhesión celular, invasión y angiogénesis, para esta Entidad esto es razón suficiente para que un la persona medianamente versada en la materia diseñe compuestos con características estructurales similares a los divulgados el estado de la técnica, con la expectativa de que pudiesen tener actividad anticancerígena, puesto que una estructura con actividad inhibidora del EGFR necesariamente da lugar a un efecto sobre el VEGF.

Por su parte, el documento D2 hace referencia a los resultados de las pruebas de inhibición sobre la proteinaquinasa FLK, PDGFR, EGFR por parte de los derivados de indolin-2-onas 3-sustituidos; si bien es cierto que dichos resultados de IC₅₀ no son muy ventajosos, existe evidencia de su actividad que fue confirmada en una gran variedad de estudios realizados durante la década de los 90'S, que aseguran la actividad de 3-[(3-o 4-carboxietilpirrol-2-il) metilidenil]indol-2-onas como inhibidores de los receptores de tirosin quinasas VEGF, FGF y PDGF.

Respecto a que "[e]l compuesto SU5416 (incluido en la divulgación general de D1 y D2, y la publicación Li Sun, J. Med. Chem, 1998), no tiene sustituyentes polares sobre el anillo de pirrol...", esta Entidad indica que no existe dentro de la solicitud denegada información o datos que sustenten una mejora en la solubilidad y estabilidad metabólica como lo argumenta el recurrente; por tal motivo, estos aspectos no pueden considerarse como una eventual ventaja de los compuestos reclamados por parte del examinador, ya que no fueron sustentados en el proceso.



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Asimismo, frente a los compuestos SU5416 y SU668, este Despacho reitera que la información contenida en el estado de la técnica en el momento de la solicitud era suficiente para que la persona medianamente versada en la materia desarrollara los compuestos aquí reclamados, conservando la estructura 3-[(3-o 4-carboxietilpirrol-2-il) metilidenil]indol-2-onas, cuya actividad como inhibidores de los receptores de tirosin quinasas VEGF, FGF y PDGF ya se había corroborado para la época. Igualmente, como lo refiere la Doctora Cui en su declaración, el ejemplo 80 de la reivindicación 5 ha demostrado ser un agente anticancerígeno seguro y efectivo (Fl. 1376). Pero, dicha afirmación no se extrapola a los demás compuestos de la solicitud, ya que no existe información técnica que lo sustente. Igualmente, como lo afirma la Doctora Cui *"la simple identificación de un compuesto con una estructura no tiene significado a menos que se demuestre ser biológicamente activo y se demuestre no tener efectos adversos"* (Fl. 1373). Bajo los anteriores argumentos, este Despacho no encuentra nivel inventivo en los compuestos enmarcados en la estructura general Markush de la reivindicación 1 y sus dependientes.

Es así como, esta Oficina hace énfasis en que los compuestos reclamados en la fórmula general Markush de la reivindicación 1 son obvios para la persona medianamente versada en la materia a la luz de la información contenida en el estado de la técnica. Sin embargo, y teniendo en cuenta los argumentos y resultados presentados por el solicitante, este Despacho encuentra que los compuestos específicos de las reivindicaciones 5 y 7 poseen ventajas farmacológicas según la información divulgada por Li Sun et al., J. Med. Chem, 46, 2003 1116-1119 (Fis. 1228 a 1231) allegada por el recurrente.

A pesar que dichos resultados no se encuentran contenidos en la solicitud original, se ha revisado el documento y se encontró que los sustituyentes específicos para la fórmula (I) $n = 2$, y R_{12} = dietilamina o pirrolidina, poseen una actividad ventajosa sobre VEGF-R2 y PDGF-R. Esta especificidad sobre los receptores para dos compuestos que mantuviera un IC_{50} en el rango ηM con $8\eta M$ para el compuesto de la reivindicación 5 y de $6\eta M$ para el compuesto de la reivindicación 7, es algo que la persona medianamente versada en la materia no hubiese podido derivar de la información contenida en el estado del arte. Por lo anterior, los compuestos de las reivindicaciones 5 y 7 se consideran inventivos.

En cuanto a que muchos países han concedido patente a solicitudes equivalentes a ésta, este Despacho aclara que en el análisis de patentabilidad se encontraron documentos relevantes que afectan parcialmente el nivel inventivo del objeto estudiado, tal como se demostró durante la actuación administrativa. Asimismo, sobre el examen de patentabilidad realizado por distintas oficinas extranjeras, es importante manifestar que en esta materia rige el principio de la independencia de las patentes, respecto a lo cual el Tribunal de Justicia de la Comunidad Andina ha sostenido: *"El sistema de otorgamiento de patentes que se adoptó en la Comunidad Andina se encuentra soportado en la actividad autónoma e*



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independiente de las Oficinas Competentes en cada País Miembro. Dicha actividad, que aunque de manera general se encuentra regulada por la normativa comunitaria, deja a salvo la mencionada independencia..." (Proceso 51-IP-2008).

Finalmente, si bien es cierto que los requisitos de patentabilidad pueden ser similares de un país a otro, también lo es que la normatividad que regula la materia puede variar entre ellos. Se reitera, cada oficina es autónoma para decidir sobre la patentabilidad de una creación, teniendo en cuenta el examen de patentabilidad por ella realizado, sin olvidar la normatividad vigente aplicable al caso.

En ese orden de ideas, se sostiene que el objeto de la solicitud en estudio no cumple con los requisitos de patentabilidad respecto a las reivindicaciones 1 a 4, 6 y 8 (Fls. 1106 y 1107) y se revoca en cuanto a la denegación del privilegio solicitado para las reivindicaciones 5 y 7 (Fl. 1107).

Con fundamento en las anteriores consideraciones, el Superintendente de Industria y Comercio,

RESUELVE

ARTÍCULO PRIMERO: REVOCAR PARCIALMENTE el Artículo Primero de la Resolución No. 68274 del 29 de diciembre de 2009 en el sentido de conceder la patente a las reivindicaciones 5 y 7, por lo expuesto en la parte motiva de esta providencia. En consecuencia, el Artículo Primero de la Resolución No. 68274 del 29 de diciembre de 2009 quedará así:

"ARTÍCULO PRIMERO: Otorgar el privilegio de patente de invención a la solicitud que entró en fase nacional en virtud del Tratado de Cooperación en Materia de Patentes (PCT), para la creación denominada:

**"INDOLINONAS SUSTITUIDAS CON PIRROLES INHIBIDORAS DE
PROTEINQUINASAS"**

Clasificación IPC: A 61 K 31/404.

Reivindicaciones: 5 y 7 **Folio:** 1107.

Titular: SUGEN INC. Y PHARMACIA & UPJOHN COMPANY.

Domicilio: California, Estados Unidos.

Inventores: Peng Cho Tang, Todd Miller, Xiaoyuan Li, Li Sun, Chung Wei Chen, Shahzad Shirazian, Congxin Liang, Tomas Vojkovsky, Asaad S. Nematalla.



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Industria y Comercio
SUPERINTENDENCIA

REPÚBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCIÓN Nº 53738

Por la cual se resuelve un recurso de reposición

Rad. PCT 01 012068

29 10 2

Prioridad No.: US 60/182.710 Fecha: 15 de febrero del 2000 País: US
Prioridad No.: US 60/216.422 Fecha: 6 de Julio de 2000 País: US
Prioridad No.: US 60/243.532 Fecha: 27 de Octubre de 2000 País: US

Vigente desde: 15 de febrero del 2001 Hasta: 15 de febrero del 2021."

ARTICULO SEGUNDO: CONFIRMAR en lo demás el Artículo Primero de la Resolución N°. 68274 del 29 de diciembre de 2009 esto es, denegar el privilegio de patente a las reivindicaciones 1 a 4, 6 y 8 de la presente solicitud.

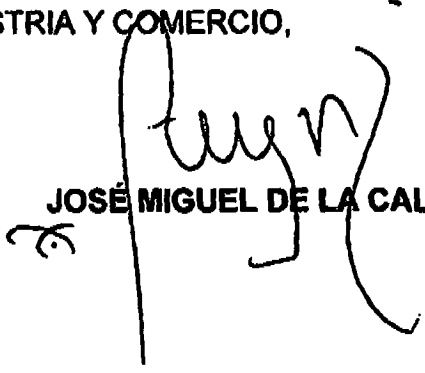
ARTICULO TERCERO: Notificar personalmente el contenido de la presente Resolución al doctor Carlos Olarte, en su calidad de apoderado de la sociedad Sugem Inc. y Pharmacia & Upjohn Company, o a quien haga sus veces, entregándole copia de la misma y advirtiéndole que contra ella no procede recurso alguno, por lo cual queda agotada la vía gubernativa.

NOTIFIQUESE Y CUMPLASE

Dada en Bogotá, D. C., a los

30 SET. 2010

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,


JOSÉ MIGUEL DE LA CALLE RESTREPO

Doctor
CARLOS OLARTE
Calle 100 N° 8A-37
Torre A, Piso 10
World Trade Center
Bogotá D. C.

202066

Superintendencia de Industria y Comercio

Otorga la Patente de Invención a:

Pugen, Inc.

California, Estados Unidos de America

Pharmacia & Up John Company

California, Estados Unidos de America

A la Creación denominada:

"Indolinas sustituidas con pirroles inhibidoras de proteínquinasas"

Inventor(es):

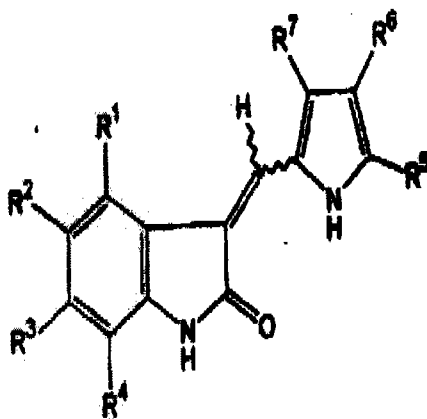
*Peng Cho Tang, Todd Miller, Xiaoyuan Li, Li Sun, Chung Wei Chen,
Shahrad Piraxian, Longxin Liang, Tomas Vojtkovsky, Asaad P. Nematalla*

Certificado No. 29102

Clasificación Internacional: A61K 31/404
de Patentes

2 Reivindicaciones
Vigente Desde: 15/02/2001
Hasta: 15/02/2021

Prioridad No. US 60/182.710 15/02/2000
US 60/216.422 06/07/2000
US 60/243.532 27/10/2000



Que la solicitud de Patente de Invención No. 112068 cumple con los requisitos previstos en las disposiciones legales vigentes, Resolución No. 53738 de 30/09/2010, en testimonio de ello se estampa la firma del funcionario competente y el sello de esta Entidad.

Superintendente de Industria y Comercio