

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-132584- -4	FECHA: 2015-12-17 13:49:39
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 8
ACT: 519 TRASLADOINFORME	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS R. OLARTE GARCIA

Asunto: Radicación: 14-132584- -4
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 15118
Folios: 8

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/IB2012/057554				
Fecha de Presentación Internacional	20/12/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N° 14-132584-00000-0000	18/Junio/2014
Dibujos:	Radicado N° 14-132584-00000-0000	18/Junio/2014
Reivindicaciones:	Radicado N° 14-132584-00000-0000	18/Junio/2014
	Radicado N° 14-132584-00003-0000	19/Marzo/2015
Prioridad:	US 61/579,231	22/Diciembre/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

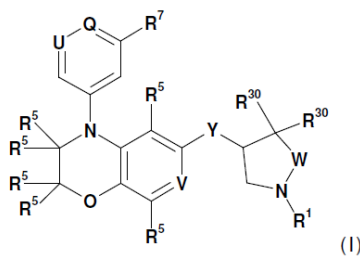
De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las

reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 15 del capítulo reivindicatorio (Radicado N°. 14-132584-00003-0000).

Título de la solicitud: "DERIVADOS DE DIHIDRO-BENZO-OXAZINA Y DIHIDRO-PIRIDO-OXAZINA".

El problema planteado en esta solicitud consiste en la necesidad de proporcionar nuevos inhibidores de PI3K que sean buenos fármacos candidatos, que se enlacen potentemente a PI3K mientras que muestran poca afinidad por otros receptores y muestren la actividad funcional como inhibidores.

Para resolver este problema técnico la solicitud en estudio proporciona compuestos de dihidro-benzooxazina y dihidro-pirido-oxazina de la fórmula (I) y/o a las sales farmacéuticamente aceptables y/o solvatos de los mismos.



El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 31/536 // 31/5365; C07D 413/14 // 498/04; A61P 19/02 // 33/06 // 35/00 // 37/08

4. De la solicitud de patente

Titulo

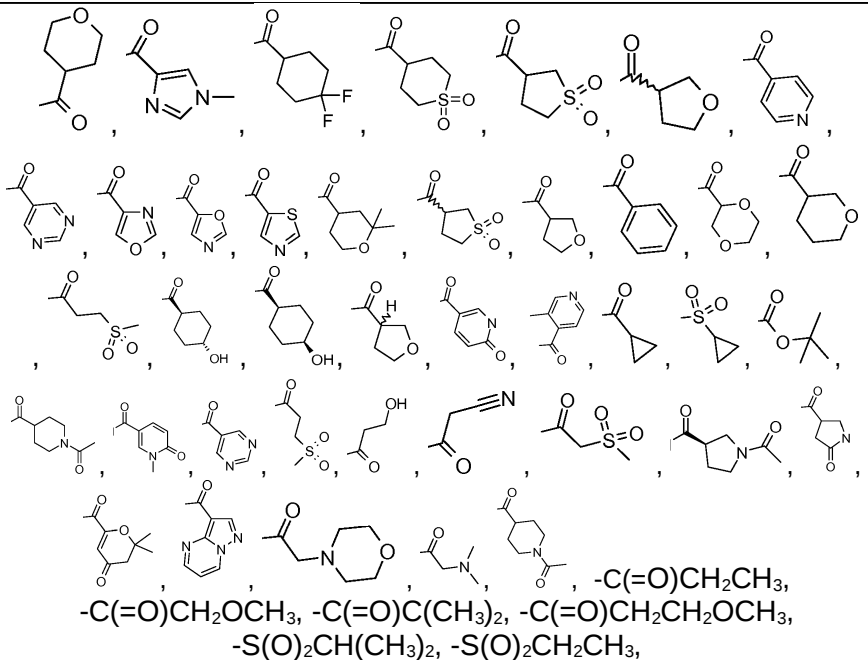
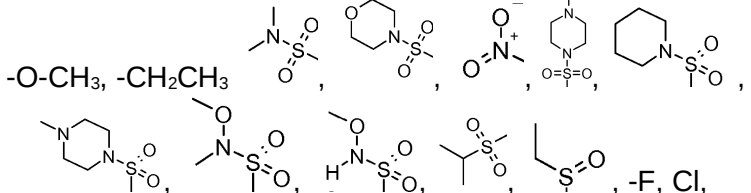
El título de la solicitud es general y ambiguo dado que se menciona de forma general el núcleo de dihidro-benzo-oxazina y dihidro-pirido-oxazina, el cual relaciona todos aquellos compuestos que comprenden dicho núcleo, sin embargo no todos ellos hacen parte del objeto de la solicitud. Se sugiere modificar el título de la solicitud, teniendo en cuenta que esta divulga y reclama compuestos los compuestos de la solicitud corresponden a derivados de 6-(tetrahidropirrolo-3-iloxi)-4-piridin-3-il-benzo-oxazina que actúan como inhibidores de PI3K.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

1- La reivindicación 1 no es clara ni concisa porque:

- Menciona de forma general sales farmacéuticamente aceptables de los compuestos reclamados, sin embargo la descripción no presenta sustento para dicha modalidad. Se sugiere que se elimine la referencia a modalidades no soportadas por la descripción. Lo mismo es aplicable para las reivindicaciones 2-8 y 10-13.
- La reivindicación 1 no es concisa porque las definiciones de R¹-R⁷ y Y, W son géneros funcionales, los cuales ofrecen una gran variedad de compuestos no sustentados por la descripción. Se sugiere restringir la solicitud a una razonable generalización del tipo de compuestos que ha sido sintetizado ó probado, es decir, que se encuentran debidamente sustentados.

Sustituciones	Soporte de los ejemplos presentados
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R¹	
R⁵	H, -D, F
R⁶	H, -Cl, -CF ₃ , -OCHF ₂ , -CH ₃ , -O-CH ₃ , -O-CH ₂ CH ₃ , -S(O) ₂ CH ₃ , -NH ₂ , -CH ₂ -O-CH ₃
R⁷	H, -CH ₃ , -CHF ₂ , -CH ₂ F, -NHCH ₃ , -N(CH ₃) ₂ , -CF ₃ , -CN, -S(O) ₂ CH ₃ , 

Lo mismo es aplicable a las reivindicaciones 2-7.

2-La reivindicación 9 no es clara porque al tratarse de un compuesto en la forma cristalina es necesario caracterizarlo por medio de:

- El patrón de rayos X en mono cristal, o
- El patrón de rayos X en polvo (DRXP), medido entre 5°, 2θ hasta 90°, 2θ; mencionando por lo menos los valores de 15 picos. O
- Las espectroscopías raman e IR,
- La espectroscopia RMN-C¹³, y
- Los métodos de análisis térmico: TGA, DTA y DSC.

Se sugiere a la solicitante incluir dichas características de acuerdo a las figuras 1 y 2.

3- La reivindicación 14 no es clara porque:

- El término “combinación” es ambiguo, ya que no permite comprender si el objeto reclamado se trata de un kit de partes (donde los principios activos que lo comprenden se encuentran en formulaciones independientes) o si se encuentran presentes como componentes de una única composición o formulación. Debido a que para la evaluación de estos dos tipos de productos se tiene en cuenta diferentes características esenciales, se sugiere a la solicitante que encabece la reivindicación de acuerdo a la modalidad que corresponda, sea un kit de partes o una composición, y que defina de manera adecuada sus características esenciales.
- El término “uno o más” no es limitativo por lo que no permite comprender el alcance de la materia reclamada. Lo mismo es aplicable a la reivindicación 15.
- Define los componentes de la composición en términos de función que cumplen de acuerdo a las expresiones “agentes terapéuticamente activos”, el cual no permite comprender los alcances de la materia reclamada. Se solicita definirlos

- de acuerdo a su estructura, nombre IUPAC o denominación común internacional y evitar la definición basada en función.
- Puesto que la descripción no presenta información sobre el desarrollo de productos que comprendan los compuestos reclamados en asociación con otros activos y se limita a mencionar de forma general la posibilidad de que estos compuestos sean administrados simultáneamente, antes o después de otros agentes, se considera que esta reivindicación no se encuentra sustentada en la reivindicación (Pág. 58, líneas 9-16).
- 4- La reivindicación 15 no es clara porque la expresión “*cantidad farmacéuticamente efectiva*” hace referencia a un resultado a alcanzar, y no establece una característica técnica que permita comprender los alcances de la materia reclamada. Se le recuerda a la solicitante que las características esenciales de una composición farmacéutica son los componentes y sus proporciones o cantidades, por lo que se sugiere que sean definidas en las reivindicaciones de este tipo de productos.

Se sugiere hacer las aclaraciones correspondientes.

5. Unidad de invención (Art 25 D 486)

La solicitud no cumple con el requisito de unidad de invención, porque se han identificado 2 grupos inventivos diferentes.

Los grupos inventivos están definidos por las siguientes características esenciales:

Grupo Inventivo I: Compuestos en los cuales V es N, reivindicaciones 1, y 6-15 parcialmente, 3 y 5 totalmente.

Grupo Inventivo II: Compuestos en los cuales V es CR⁵, reivindicaciones 1, y 6-15 parcialmente, 2 y 4 totalmente. (Divulgados en el documento D1)

Se le sugiere a la solicitante realizar una o más de las divisionales de acuerdo a los grupos de conceptos técnicos inventivos presentados en esta solicitud, esto claro, sin ampliar el objeto inicial de la invención.

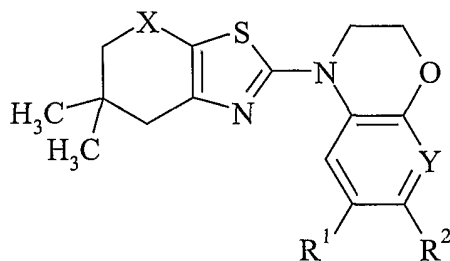
La solicitante también puede restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica. Esto se logra entre otras formas definiendo las características esenciales de la invención y mostrando las otras características particulares como dependientes, pero sin perjuicio de la concisión de las nuevas reivindicaciones tanto independientes como dependientes.

6. Determinación del Estado de la Técnica

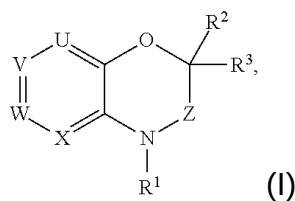
El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 22 de diciembre de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO 2008044022	“Fused thiazole derivatives as kinase inhibitors”	17/Abril/2008
D2	US 20110172217	“Ring-fused morpholine derivative having PI3k-inhibiting activity”	14/Julio/2011

El documento D1¹ divulga compuestos derivados de 5,5-dimetil-5,6-dihidro-1,3-benzotiazol-7(4H)-ona y 7,7-dimetil-5,6,7,8-tetrahidro-4H[1,3]tiazolo[5,4-c]azepin-4-ona, sustituidos en la posición por un grupo benzomorfolino de fórmula general I, que son inhibidores selectivos de PI-3 quinasa (PI3K) (Resumen).



El documento D2² divulga compuestos derivados de morfolino fusionado de formula (I) o sales farmacéuticamente aceptables que inhiben la actividad de la PI-3 quinasa (PI3K) (Pág. 2, Párr. [0051]-[0052]).



7. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art 18 D486)

Para el Grupo Inventivo II

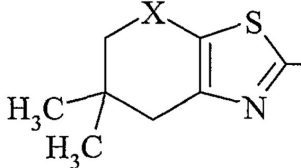
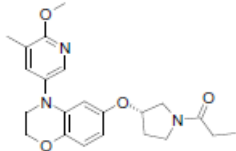
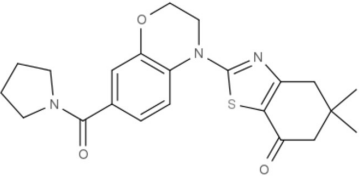
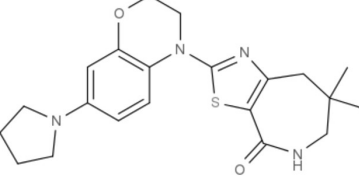
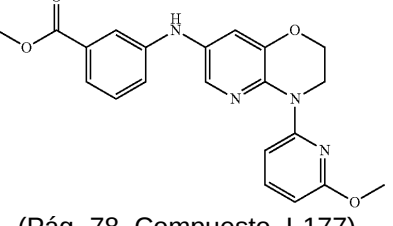
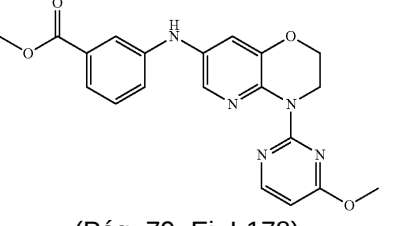
La reivindicación 1 consiste en: “Un compuesto de la fórmula (I) o una sal del mismo, en donde (...)” (Página 3 del Radicado N°. 14-132584-00003-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D3
Un compuesto de la fórmula (I) O una sal del mismo, en donde V es CR⁵ (I)	Donde Y es CH o N (Pág. 81, Reivindicación 1)	Donde U y W son =C(-R')- o =N-; Vy X son =C(-R'')- o =N-; Z es un enlace sencillo o -(CR⁴CR⁵)_n- (Pág. 129, Reivindicación 1)
Y se selecciona de O o NH	R ¹ y R ² representan H, halógeno, nitro, hidroxilo, C ₁₋₆ alquilo, arilo, heteroarilo, -NR ^a R ^b , -CONR ^a R ^b , -NR ^a COR ^c , -N(COR ^c) ₂ , -NR ³ SO ₂ R ^d , -CO ₂ R ^d or -OR ^e	R' y R'' son independientemente hidrógeno, halógeno, ciano, nitro, hidroxilo, carboxi, alquilo, alquenoilo, alquinoilo, arilo, cicloalquilo, cicloalquenoilo, heteroarilo, heterociclilo, amino, carbamoilo, alcoxi, ariloxi, cicloalquilo, heteroariloxi, heterocicliloxi, acilo,
W se selecciona de CH ₂ , o O	Donde R ^a y R ^b , cuando se toman junto al átomo de nitrógeno al	

1 http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2008044022A1&KC=A1&FT=D&ND=&date=20080417&DB=&&locale=en_EP

2 <https://www.google.com.co/patents/US20110172217?cl=en>

Características esenciales	D1	D3
	que se encuentran unidos representan un	alcoxicarbonilo, un grupo representado por -SO-R ^A , un
U se selecciona de N o CH Q se selecciona de N o CR ⁶ U y Q no son ambos N		R ¹ es seleccionado de un grupo que incluye arilo o heteroarilo sustituido o no sustituido
R ¹ se selecciona de fenilo, piridilo, pirimininyl, pirazinilo, piridazinilo, 1, 2,3-triazinilo, 1, 2,4- triazinilo, 1, 3,5-triazinilo o -XR ⁴	R ³¹ que representa H, alquilo C ₁ - C ₆ , hidroxialquilo, hidroxi[alcoxi]alquilo (En la fórmula IIC) (Pág. 83, Reivindicación 7)	No se menciona
R ⁵ se selecciona independientemente entre H, D, F o alquilo de 1 a 2 átomos de carbono	-H	-H
R ⁷ se selecciona de hidrógeno, halógeno, ciano, nitro, alquilo de 1 a 4 átomos de carbono, halo- alquilo de 1 a 4 átomos de carbono, alcoxilo de 1 a 4 átomos de carbono -, N(R ⁸) ₂ - sulfonil, alquilo de 1 a 4 átomos de carbono-sulfonil, alquilo de 1 a 4 átomos de carbono-sulfonil- amino, alcoxilo de 1 a 4 átomos de carbono - alquilo de 1 a 8 átomos de carbono, amino, N- alquilo de 1 a 8 átomos de carbono -amino, o N, N-di- alquilo de 1 a 8 átomos de carbono -amino; o R ⁶ y R ⁷ , juntos son CH = CH- CH = CH-	-CH ₃	No se menciona
Comparación de ejemplos específicos		
 Pág. 152, Ej. A13	 (Pág. 48, Ej. 18)  (Pág. 76, Ej. 88)	 (Pág. 78, Compuesto. I-177)  (Pág. 79, Ej. I-178)

El documento D1 se considera el estado de la técnica cercano a la solicitud definida en la reivindicación 1 porque divulga compuestos derivados de 5,5-dimetil-5,6-dihidro-1,3-benzotiazol-7(4H)-ona y 7,7-dimetil-5,6,7,8-tetrahidro-4H[1,3]tiazolo[5,4-c]azepin-4-ona, sustituidos en la posición por un grupo benzomorfolino de fórmula general I, que son inhibidores selectivos de PI3K (Resumen). Realizaciones preferidas de dichos

compuestos muestran un sustituyente de pirrolidina en el anillo de benzoxazina (Pág. 48, Ej. 18; Pág. 76, Ej. 88).

La diferencia entre el compuesto reclamado en la reivindicación 1 y los compuestos divulgados en el documento D1, consiste en la estructura del sustituyente heterocíclico sobre el átomo de nitrógeno del anillo de benzoxazina, dado que en los compuestos reclamados corresponde a un anillo aromático monocíclico, mientras que en los compuestos divulgados en el documento D1 corresponde a benzotiazol-7(4H)-ona o 7-dimetil-5,6,7,8-tetrahidro-4H[1,3]tiazolo[5,4-c]azepin-4-ona.

El efecto técnico asociado a la modificación del sustituyente arilo sobre el átomo de nitrógeno del anillo de morfolino, es el aumento en la potencia como inhibidores de PI3K, teniendo en cuenta que el documento D1 menciona de manera general valores de IC₅₀ menores o iguales a 50µM (Pág. 23, líneas 4-6) mientras que los valores proporcionados por la solicitud muestran que este parámetro se encuentra entre 0.5nM-500nM (Pág. 545, líneas 1-13).

Por lo tanto, el problema técnico objetivo que pretende resolver esta solicitud se puede considerar como la de proporcionar compuestos derivados de benzoxazina con potencia mejorada como inhibidores de PI3K.

Sin embargo, compuestos derivados de benzoxazina que presentan sustituyentes tipo arilo sobre el átomo de nitrógeno, se encuentran divulgados en el documento D2, el cual se refiere a compuestos que actúan como inhibidores de PI3K, así como composiciones que los comprenden (Resumen; compuestos I-177, I-178).

Por lo tanto, la persona del oficio normalmente versada en la materia, incluiría un sustituyente arilo monocíclico sobre el átomo de nitrógeno del anillo de benzoxazina de acuerdo a lo mostrado en el documento D2 en los compuestos enseñados en el documento D1, llegando así al objeto de la reivindicación 1, por lo que la materia allí reclamada se considera obvia. Más aun cuando sustituciones en esta posición en compuestos con actividad inhibitoria sobre PI3K son conocidas por la persona del oficio normalmente versada en la materia^{3,4}.

En consecuencia, la reivindicación 1 de acuerdo a lo divulgado en los documentos D1 y D2, carece de Nivel Inventivo.

Puesto que las reivindicaciones 2 a 15 no mencionan características inventivas adicionales, tampoco se pueden considerar inventivas.

8. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 2008044022	1-15	Nivel Inventivo
D2	US 20110172217	1-15	Nivel Inventivo

3 Lanni et al. Design and synthesis of phenethyl benzo[1,4]oxazine-3-ones as potent inhibitors of PI3Kinase. Bioorganic & Medicinal Chemistry Letters 17 (2007) 756–760. Disponible en <http://www.sciencedirect.com/science/article/pii/S0960894X06012534>.

4 Ameriks MK1, Venable JD. Small molecule inhibitors of phosphoinositide 3-kinase (PI3K) delta and gamma. Curr Top Med Chem. 2009;9(8):738-53. Disponible en <http://www.ncbi.nlm.nih.gov/pubmed/19689378>

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-12-22

Elaboró: Ruth Andrea Rodriguez
Revisó: Dayron Alexander Ramirez Reyes

INFORME DE BÚSQUEDA DE PATENTES PARA EL No de Radicación: 14-132584-00003						
CRITERIOS PARA LA BÚSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)						
Palabras clave utilizadas		"Dihydro-benzoxazine AND derivates" "Dihydro-pyridone-oxazole AND derivatives"				
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA		C07 413/14, C07D 498/04, A61K 31/536, A61K 31/5365, A61P 19/02, A61P 37/08, A61P 35/00. A61P 33/06				
BASES DE DATOS CONSULTADAS.						
Google patents		Espacenet		USPTO		Epoquenet
INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS (documentos patentes, literatura no patente etc...)						
Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
Europea	24/Junio/2014	WO 2008044022	1-15	Nivel Inventivo	X	
USPTO	No se ha realizado	NA	NA	NA	NA	NA
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
US 20110172217						
Lanni et al. Design and synthesis of phenethyl benzo[1,4]oxazine-3-ones as potent inhibitors of PI3Kinase. <i>Bioorganic & Medicinal Chemistry Letters</i> 17 (2007) 756–760.						
Ameriks MK1, Venable JD. Small molecule inhibitors of phosphoinositide 3-kinase (PI3K) delta and gamma. <i>Curr Top Med Chem.</i> 2009;9(8):738-53.						
BÚSQUEDA DE SECUENCIAS						
Número de depósito / patente y N° de secuencia % de identidad						
SEQ ID NO (de la solicitud)	NCBI	The Lens	EMBL-EBI	DDJB	Genome Quest	

CRITERIOS DE BÚSQUEDA / UNIDAD DE INVENCIÓN (Pantallazos)

Pantallazos Búsqueda

Dihydro-benzoxazine AND derivatives

Todos

Imágenes

Noticias

Videos

Más ▾

Herramientas de búsqueda

Cerca de 89,500 resultados (0.22 segundos)

Artículos académicos para Dihydro-benzoxazine AND derivatives

Ring-chain tautomerism of derivatives of o- ... - McDonagh - Mencionado por 74

Remarkably low catalyst loading in Brønsted acid ... - Rueping - Mencionado por 204

3-oxo-2, 3-dihydro-1, 4-benzoxazine derivatives - Pesson - Mencionado por 12

Dihydro-pyridone-oxazole AND derivatives

Todos

Imágenes

Noticias

Videos

Más ▾

Herramientas de búsqueda

Cerca de 396,000 resultados (0.27 segundos)

Oxazole synthesis - Organic Chemistry Portal

www.organic-chemistry.org/synthesis/.../oxazoles.sht... ▾ Traducir esta página

An improved one-pot van Leusen oxazole synthesis using tosylmethyl with Grignard reagents enables a facile preparation of 4-keto-oxazole derivatives. of electron-rich five-membered heterocycles and electron-poor pyridine oxides.

Heterocyclic Chemistry

https://www2.chemistry.msu.edu/.../heterocy.htm ▾ Traducir esta página

Finally, examples 4 through 7 illustrate reactions of 1,2- and 1,3-oxazole, ... Pyridine and its derivatives are weak bases, reflecting the sp² hybridization of the nitrogen. The dihydro-pyridine intermediate easily loses water or its equivalent by ...

Potent anticancer and antimicrobial activities of pyrazole ...

www.researchgate.net/.../282423702_Potent_antican... - Traducir esta página

14 nov. 2015 - Official Full-Text Publication: Potent anticancer and antimicrobial activities of pyrazole, oxazole and pyridine derivatives containing 1,2 ...

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M.; Zetterberg, F. 5-Alkyl-1,3-oxazole Derivatives of 6-Amino-nicotinic Acids 1.1 Use of pyridine derivatives as drug molecules multikilogram manufacture of ethyl 5-cyano-2-methyl-6-oxo-1,6-dihydropyridine-3-carboxylate and ethyl 6-.

[PDF] Biological Activities of Hydrazone Derivatives - MDPI.com

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17 ago. 2007 - methylene-hydrazide, a hydrazide derivative that is effective against S. aureus b]thieno-[2,3-d]pyridine-2-carbohydrazide were evaluated at 10 μM, ... - Substituted alkylidene/arylidene-6-phenylimidazo[2,1-b]thiazole-3-acetic acid hydrazides 20 or 4-yl-ethylidene-amino)-1,2-dihydropyridine-3,5-.

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PYRIDINES FROM VARIOUS PYRAN DERIVATIVES Pyran Conditions Pyridine V,O,—Al,O, pyridine (78%) 870 2~methoxy-5,G-dihydro-2H-pyran NH, steam, ... NH3 2,6-dimethyl-5H-oxazole 876 13,4-d]oxazol-4<3ne (4,5-c]pyridine-4—one ...

Structures of copper(II) complexes with dihydrobenzoxazine ...

TXTUS	SS Status	Results	Query
1		527	DIHYDROBENZOXAZIN?
2		356.442	DERIV?
3		7	1 AND 2
4		154	DIHYDROPYRIDON?
5		2	4 AND 2
6		0	3 AND 5
7		9	3 OR 5

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US2003134398

EPDOC / EPO

PN - US2003134398 A1 20030717

TI - Mitomycin biosynthetic gene cluster

AB - The invention provides a biosynthetic gene cluster for mitomycin, as well as methods of using gene(s) within the cluster to alter biosynthesis.

CCI - C12N15/52

UC - 435/69.1; 435/119; 435/191; 435/193; 435/252.3; 435/320.1; 536/23.2

IN - SHERMAN DAVID H [US]; MAO YINGQING [US]; VAROGLU MUSTAFA [US]; HE MIN [US]; SHELDON PAUL [US]

AP - US20010953348 20010912

PR - US20010953348 20010912

DT - *

Fig 1/117

Patent Application Publication Jul. 17, 2003 Sheet 1 of 117 US 2003/0134398 A1

FIG. 1

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US2004044080

EPDOC / EPO

PN - US2004044080 A1 20040304

TI - Treatment of dyspareunia with topically administered nitroglycerin formulations

AB - Methods and formulations for treating dyspareunia are provided. A pharmaceutical composition formulated so as to contain a therapeutically effective amount of nitroglycerin is administered to the vagina or vulvar area of the individual undergoing treatment. Preferred formulations are immediate release formulations in which at least 80% of the nitroglycerin in the formulation is released therefrom within 4 hours following administration. The formulations may contain one or more additional active agents, e.g., agents that are also useful to treat dyspareunia and/or potentiate the action of nitroglycerin. Such additional agents include vasoactive agents such as prostaglandins, phosphodiesterase inhibitors, androgens such as testosterone, estrogens such as estradiol, and selective modulators of estrogen and androgen receptors. A kit for a patient to use in the self-administration of the formulation is also provided.

CCI - A61K9/0034; A61K45/06; A61K31/00; A61K31/15; A61K31/21; A61K31/295; A61K31/48; A61K31/5377; A61K31/557; A61K31/5575; A61K31/5585; A61K31/56; B82Y5/00

CLC - A61K31/557, A61K2300/00, INV

UC - 51A/509; 51A/573

US 2004/044080 A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2004/044080 A1

Place et al. (43) Pub. Date: Mar. 4, 2004

(54) TREATMENT OF DYSPAREUNIA WITH TOPICALLY ADMINISTERED NITROGLYCERIN FORMULATIONS

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(21) Appl. No.: 10/407,858

(22) Filed: Apr. 4, 2003

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/905,458, filed on Jul. 13, 2001, now Pat. No. 6,593,313, which is a continuation of application No. 09/539,484, filed on Mar. 30, 2000, now Pat. No. 6,306,841, which is

a continuation of application No. 09/181,316, filed on Oct. 27, 1998, now abandoned, which is a continuation-in-part of application No. 08/959,064, filed on Oct. 28, 1997, now Pat. No. 5,877,216, and which is a continuation-in-part of application No. 08/959,067, filed on Oct. 28, 1997, now abandoned.

Publication Classification

(51) Int. Cl.⁷ A61K 31/21; A61K 31/557

(52) U.S. Cl. 514/573; 514/509

(57) ABSTRACT

Methods and formulations for treating dyspareunia are provided. A pharmaceutical composition formulated so as to contain a therapeutically effective amount of nitroglycerin is administered to the vagina or vulvar area of the individual undergoing treatment. Preferred formulations are immediate release formulations in which at least 80% of the nitroglycerin in the formulation is released therefrom within 4 hours following administration. The formulations may contain one or more additional active agents, e.g., agents that are also useful to treat dyspareunia and/or potentiate the action of nitroglycerin. Such additional agents include vasoactive agents such as prostaglandins, phosphodiesterase inhibitors, androgens such as testosterone, estrogens such as estradiol, and selective modulators of estrogen and androgen receptors. A kit for a patient to use in the self-administration of the formulation is also provided.

FIG. 1

Design and synthesis of phenethyl benzo[1,4]oxazine-3-ones as potent inhibitors of PI3Kinase γ

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Abstract—The Type 1 PI3Kinases comprise a family of enzymes, which primarily phosphorylate PIP2 to give the second messenger PIP3, a key player in many intracellular signaling processes [*Science*, **2002**, 296, 1655; *Trends Pharmacol. Sci.* **2003**, 24, 366]. Of the four type 1 PI3Ks, the γ -isoform, which is expressed almost exclusively in leukocytes [*Curr. Biol.*, **1997**, 7, R470], is of particular interest with respect to its role in inflammatory diseases such as rheumatoid arthritis (RA) and chronic obstructive pulmonary disease (COPD) [*Mol. Med. Today*, **2000**, 6, 347]. Investigation of a series of 4,6-disubstituted-4*H*-benzo[1,4]oxazin-3-ones has led to the identification of single-digit nanomolar inhibitors of PI3K γ , several of which had good cell based activity and were shown to be active in vivo in an asperic peritonitis model of inflammatory cell migration.

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The type 1 phosphoinositide 3-kinases (PI3Ks) are heterodimeric enzymes which reside downstream of multiple cell surface receptors.³ Upon receptor ligation these enzymes are activated through the association of their specific adaptor proteins with phosphorylated intracellular domains of tyrosine kinase and growth factor receptors or β,γ -subunits released from G-protein coupled receptors.⁶ Once activated they phosphorylate phosphatidylinositol 4,5 bisphosphate (PIP2) in the 3 position to give the potent second messenger phosphatidylinositol 3,4,5 triphosphate (PIP3).^{3,14} This results in the engagement of a multitude of intracellular signal transduction pathways culminating in effects included cell migration, protein synthesis, actin polymerization, and cell proliferation.^{10,14}

There are four different isoforms of the type 1 PI3Kinase, and increasing evidence that these different isoforms have specialized functions within specific

cells.^{11,12} For instance, while the α and β isoforms are ubiquitously expressed, the γ and δ isoforms show much restricted expression patterns. PI3K γ is primarily restricted to granulocytes, monocytes, and macrophages while the δ isoform is mainly found in T and B cells.^{2,9} The migration and accumulation of these cells result in swelling of the joints, which in turn suggests a viable target for inflammation.^{6,9}

PI3K γ knockout mice⁸ were injected ip with 10^7 CFU of *Escherichia coli* and demonstrated significantly impaired recruitment of both neutrophils and macrophages to the peritoneum.¹ This model of *E. coli*-induced cell influx was therefore used to test the in vivo efficacy of multiple PI3Kinase inhibitors when administered by oral gavage. Wortmannin⁴ (3 mg/kg) potently inhibited cell influx by 79.06% (Fig. 1). LY294002¹³ (30 mg/kg) inhibited only 26.86%. Compound **I** (100 mg/kg) inhibited cell influx by 53.84% (Fig. 1). These results strongly indicate that a specific inhibitor of PI3K γ will have therapeutic value for the treatment of chronic inflammatory diseases such as rheumatoid arthritis (RA) and chronic obstructive pulmonary disease (COPD).^{11,12}

To identify chemical matter as substrate for a PI3K γ inhibitor program, a high-throughput screen (HTS)

Keywords: PI3Kinase; Phosphoinositide 3-kinase; Rheumatoid arthritis; Peritonitis; Inflammatory; Granulocytes; Monocytes; Intracellular; PI3K γ ; Inhibitor.

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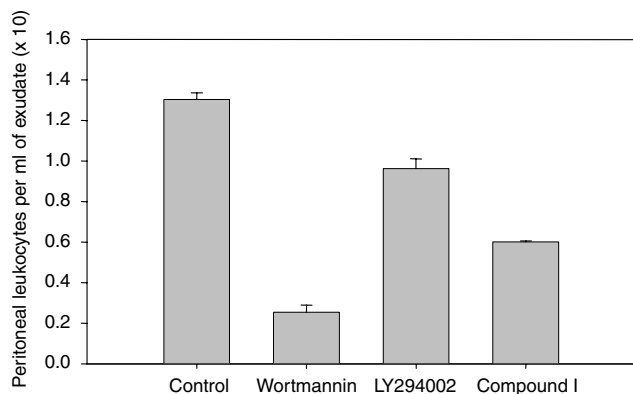


Figure 1. Effects of PI3Kinase inhibitors on a model of *E. coli*-induced peritoneal inflammation. Wortmannin (3 mg/kg), LY294002 (30 mg/kg), and Compound I were administered to mice by oral gavage. One hour later, the mice were injected ip with *E. coli* bacteria. Twenty-four hours later, peritoneal lavage was performed, and infiltrating cells counted. Data are averages + SEM for multiple experiments using five mice per group: control ($n = 76$), wortmannin ($n = 9$), LY294002 ($n = 2$) and Compound I ($n = 3$).

was conducted on the Pfizer corporate compound collection. A simple rhodanine, Compound I (Fig. 2), was identified as a potent inhibitor of PI3K γ and served as the starting point for our program. We describe herein, our chemistry efforts around increasing the enzymatic and cell based potency in this series along with in vivo results in a cell migration model.

PI3K γ activity was assessed by incubation of baculoviral co-expressed regulatory and catalytic subunits (p101 and p110) with lipid micelles prepared from phosphatidylethanolamine containing phosphatidylinositol 4,5 bisphosphate (PIP₂) at a molar ratio of 8:1 (approximately 25 μ M final PIP₂). Recombinant G-protein $\beta\gamma$ -subunits were used to activate the enzyme complex, and the reaction mix contained 20 μ M ³²P-ATP. Reactions were terminated by addition of cold 75 mM H₃PO₄ and the mixture filtered through glass fiber filter plates and washed with H₃PO₄. ³²P-PIP₃ on the filters was then monitored by scintillation counting.⁷ Compounds of interest were included in the assay and ability to inhibit formation of ³²P-PIP₃ expressed as percentage uninhibited value. IC₅₀ values were determined by incubation of the compound at various concentrations (typically a 10 point half-log dose distribution) and curve fitting by standard means (GraphPad Prism). While not routinely evaluated, representative compounds from this class were shown to be competitive with ATP in that relative IC₅₀ values increased with increasing ATP concentrations.

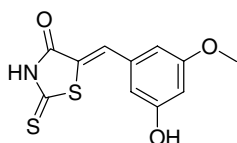
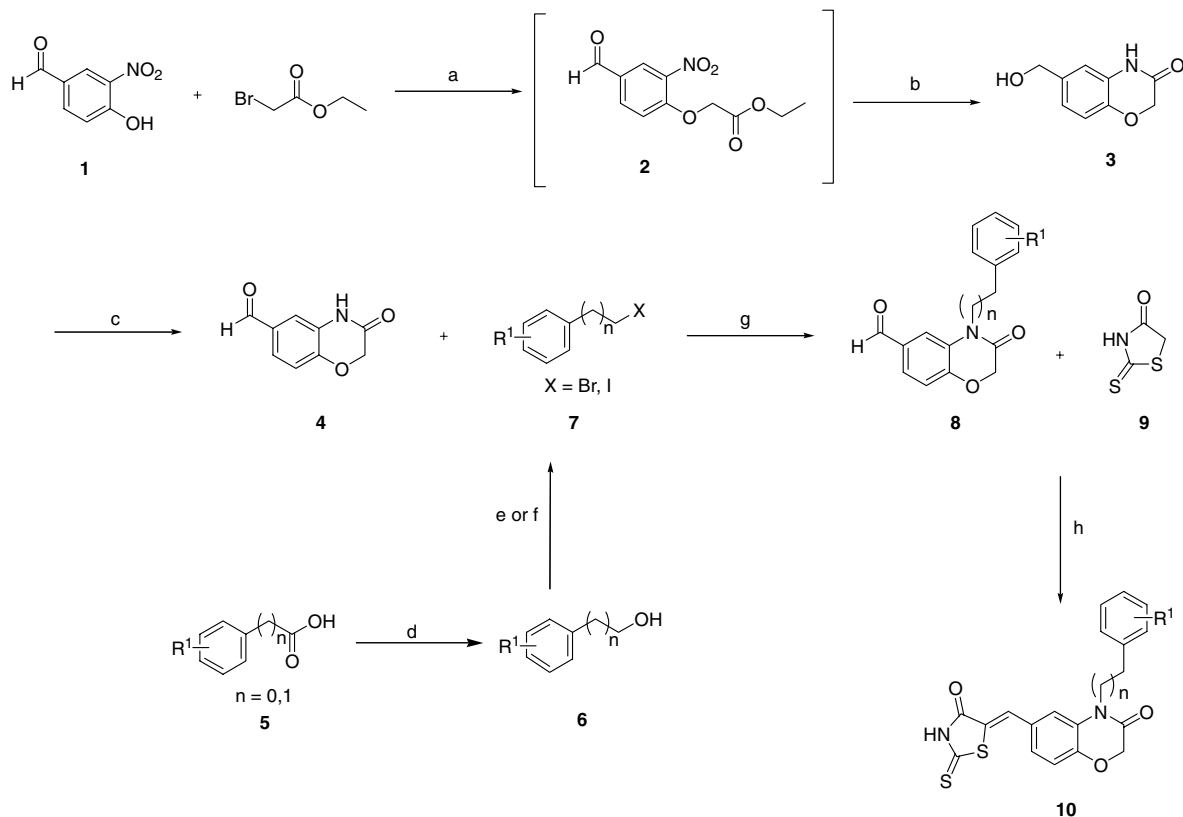


Figure 2.

For determination of compound activity in a cellular setting, the ability to influence stimulated superoxide production in human neutrophils was employed. Activation of this response by the chemotactic peptide *n*-formyl MetLeuPhe has been demonstrated to be dependent on PI3K γ .¹⁴ Briefly, compounds are incubated with human neutrophils (purified from fresh peripheral blood) in a salt solution containing 120 μ M cytochrome C. Cells are stimulated by addition of *n*-formyl MetLeuPhe and production of superoxide anion O₂⁻ is monitored by following reduction of cytochrome C at A₅₅₀ nm. Preliminary experiments demonstrated that O₂⁻ production by this procedure (change in A₅₅₀ nm) was abolished by inclusion of superoxide dismutase. IC₅₀ values were determined in a manner similar to the enzyme procedure.

A mouse peritonitis model was employed as a rapid screen to test the efficacy of potential PI3Kinase inhibitors in vivo. Groups of five mice were dosed by oral gavage with the control oral vehicle (0.5% hydroxypropylmethylcellulose/0.25% Tween 80), the positive control PI3Kinase inhibitors wortmannin at 3 mg/kg, or LY294002 at 30 mg/kg, or test compounds at 100 mg/kg. One hour later, the mice were injected ip with *E. coli* bacteria (250 μ l at 7.5×10^7 CFU/mL). The bacteria induce a robust, PI3Kinase dependent leukocyte migration into the peritoneum that peaks 24–48 h after injection. Twenty-four hours after *E. coli* administration, the mice were anesthetized and injected ip with 5 mL HBSS/2% FCS, the contents of the peritoneum massaged, and the fluid collected. The cells from this lavage were counted and subjected to differential stain (Data are averages + SEM for multiple experiments using five mice per group). Typical recovery is $1\text{--}2 \times 10^6$ cells/mL of lavage fluid, comprised of roughly equivalent numbers of neutrophils and macrophages.

The preparation (Scheme 1) of the benzoxazine–aldehyde template (4) begins with commercially available 4-hydroxy-3-nitro-benzaldehyde (1) by alkylation with ethyl bromoacetate to yield (4-formyl-2-nitro-phenoxy)-acetic acid ethyl ester (2).¹⁵ Subsequent hydrogenation over RaNi gave 6-hydroxymethyl-4H-benzo[1,4]oxazin-3-one (3)¹⁶ which was re-oxidized with PDC to yield template (4).¹⁷ Initial investigation of structure–activity relationships (SAR) involved N-substitution of template (4) with a variety of alkyl and benzyl groups followed by conversion to the rhodanine analog. A number of benzyl-substituted analogs were synthesized by either using the commercially available benzyl bromides (7, $n = 0$) or reducing benzoic acids (5, $n = 0$) to the corresponding alcohols with BH₃–SMe₂. The alcohols were then converted to the benzyl bromide (7) using carbon tetrabromide and triphenylphosphine, and used in the alkylation of the benzoxazine template (4) in respectable yields. Knoevenagel condensation of rhodanine (9) with aldehydes provided the target compounds (10a–i).⁵ Binding data showed that *N*-benzyl substitution improved the potency of PI3K γ binding versus the unalkylated benzoxazine product (PI3K γ IC₅₀ = 78.0 nM). The unsubstituted *N*-benzyl analog 10a was potent at PI3K γ (IC₅₀ = 23.0 nM,



Scheme 1. Reagents and conditions: (a) NaH, THF, reflux; (b) RaNi, MeOH, 96%; (c) PDC, CH₂Cl₂, 81%; (d) Me₂S:BH₃, THF, 76%; (e) CBr₄, PPh₃, CH₂Cl₂; or (f) I₂, PPh₃, Imidazole, 75–90%; (g) K₂CO₃, PTC, CH₃CN, 26–70%; (h) C₂H₈N₂·2(CH₃CO₂H), MeOH, 52–80%.

Table 1.

Compound	<i>n</i>	R ¹	IC ₅₀ γ (nM)	SUOX IC ₅₀ (nM)	Mouse peritonitis % inhibition
Wortmannin	—	—	19.0	19.00	
LY294002	—	—	1720	1720	
Compound I	—	—	77.7 (73.2–82.5, <i>n</i> = 4)	1320 (245–7110, <i>n</i> = 4)	
10a	0	H	23.0 (13.2–39.9, <i>n</i> = 2)	30,000	
10b	0	4-Methyl	29.5 (15.4–56.2, <i>n</i> = 2)	30,000	
10c	0	4- <i>t</i> -Butyl	2.42 (0.390–15.1, <i>n</i> = 2)	19,900	
10d	0	4-Chloro	7.16 (0.939–54.6, <i>n</i> = 2)	670	
10e	0	4-Cyano	36.1 (26.8–48.7, <i>n</i> = 2)	1570	
10f	0	4-Trifluoromethyl	27.5 (17.6–42.8, <i>n</i> = 4)	20,000	
10g	0	3-Methyl	43.7 (10.3–186, <i>n</i> = 2)	30,000	
10h	0	3,4-Dimethyl	2.59 (0.179–37.6, <i>n</i> = 2)	19,900	
10i	1	H	24.5 (18.9–31.7, <i>n</i> = 2)	10,320	
10j	1	3,5-Dimethyl	2.65 (1.43–4.89, <i>n</i> = 2)	1620 (90.9–28,800, <i>n</i> = 2)	
10k	1	3,5-Dimethoxy	2.79 (1.13–6.93, <i>n</i> = 2)	840	
10l	1	4-Chloro	4.76 (0.021–1090, <i>n</i> = 2)	363 (211–608, <i>n</i> = 3)	25.63
10m	1	4-Methoxy	6.57 (0.337–128, <i>n</i> = 2)	372 (233–595, <i>n</i> = 3)	43.69
10n	1	4-Methyl	8.83 (0.011–7370, <i>n</i> = 2)	1250 (1.64–74,000, <i>n</i> = 2)	48.15
10o	1	4-Bromo	2.34 (1.41–3.90, <i>n</i> = 4)	565	30.78
10p	1	3-Chloro	4.27 (0.001–35,500, <i>n</i> = 2)	424 (25.3–7110, <i>n</i> = 2)	41.27
10q	1	3,4-Dichloro	4.36 (0.184–104, <i>n</i> = 2)	285 (59.0–1360, <i>n</i> = 2)	35.25
10r	1	4- <i>t</i> -Butyl	33.2 (0.028–39,700, <i>n</i> = 2)	2140	
10s	1	4-Trifluoromethyl	1.92 (0.642–5.75, <i>n</i> = 2)	370 (0.379–27,600, <i>n</i> = 2)	36.94
10t	1	2,4-Dichloro	4.28 (0.047–388, <i>n</i> = 2)	610	
10u	1	3-Methyl	4.96 (1.07–23.0, <i>n</i> = 2)	1030	
10v	1	3-Methoxy	2.66 (1.35–5.27, <i>n</i> = 3)	375 (0.837–13,500, <i>n</i> = 2)	–13.97
10w	1	3,4-Difluoro	4.84 (0.011–2090, <i>n</i> = 2)	520	

Table 1), however, it was not active in the superoxide inhibition assay (SUOX IC₅₀ = 25,000 nM). Further substitution of the benzyl ring (Table 1) produced po-

tent analogs at PI3Kγ with the most potent analogs having of 4- and 3,4-substitution in the benzyl series. Most of the analogs synthesized in the benzyl series showed no

activity in the superoxide inhibition assay except for **10d** (R = 4-Cl, SUOX IC₅₀ = 670 nM). To further explore the SAR, a series of phenethyl analogs were synthesized in a similar manner to the benzoic acids. Commercially available phenyl-acetic acids (**5**) were reduced to the corresponding alcohols (**6**, *n* = 1) using BH₃–SMe₂ and the resultant alcohols were then converted to the primary phenethyl iodides (**7j–w**).^{9,18} The primary iodides were preferred over the primary bromides in the phase transfer alkylation of the template (**4**), affording modest yields of the N-substituted benzoxazine–aldehyde templates (**8j–w**).¹⁹ Knoevenagel condensation of rhodanine (**9**) with aldehydes (**8j–w**) provided the target compounds (**10j–w**).^{5,20} Table 1 summarizes the SAR around the phenethyl analogs. Various substitution of the phenyl ring gave potent analogs for PI3Kγ with limited SAR. The only analog that exhibited a loss of activity was the 4-*t*-butyl analog, **10r** (IC₅₀γ = 39.0 nM). *Para* substitution of the phenyl ring gave potent analogs in this series, especially **10o** (R = 4-Br, IC₅₀γ = 2.00 nM) and **10s** (R = 4-CF₃, IC₅₀γ = 2.00 nM). 2,4- and 3,4-substitution of the phenyl ring also gave potent PI3Kγ inhibition, for instance **10q** (R = 3,4-Cl, IC₅₀γ = 4.00 nM) and **10t** (R = 2,4-Cl, IC₅₀γ = 5.00 nM). Subsequent testing in cellular assays of superoxide inhibition showed that *para* substitution afforded good potency. Compound **10q** (R = 3,4-Cl, SUOX IC₅₀ = 285 nM) was the most potent compound in cell based assays. A few analogs were tested in the mouse peritonitis model with three of them (**10m**, **10n**, and **10p**) showing promising activity, while others exhibited insignificant inhibition. This series will be further evaluated by extending the alkyl linker and with various substitution of the phenyl ring.

In conclusion, a series of benzoxazine–rhodanines have been developed as potent inhibitors of PI3Kγ in enzymatic and cell based assays. The most promising compounds were subsequently profiled in vivo in an aseptic peritonitis model of inflammatory cell migration and showed significant inhibition of neutrophil and monocyte migration to the infected area.

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- Wymann, M. P.; Zvelebil, M.; Laffargue, M. *Trends Pharmacol. Sci.* **2003**, 24, 366.
- Preparation of intermediate **1**: To a solution of 4-hydroxy-3-nitrobenzaldehyde (10.0 g, 59.8 mmol) in tetrahydrofuran (600 mL) and DMF (240 mL) were added dry sodium hydride (1.58 g, 65.82 mmol) and ethyl bromoacetate (7.30 mL, 65.82 mmol). The reaction mixture was refluxed for 24 h. The reaction was then concentrated and diluted with ethyl acetate (500 mL) and acidified to pH 2 with 1 N HCl. The organic layer was then washed with saturated sodium bicarbonate (2× 200 mL), sodium chloride (2× 200 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to yield a dark red oil. The oil was used in the next step without purification. ¹H NMR (d₆-DMSO) δ 1.18 (t, 3H), 4.15 (q, 2H), 5.14 (s, 2H), 7.49 (d, 1H), 8.11 (dd, 1H), 8.41 (s, 1H), 9.93 (s, 1H). MS: M^{−1} = 252 Da.
- Preparation of intermediate **2**: To a solution of intermediate **1** (6.40 g, 1.67 mmol) in methanol (100 mL) was added Raney nickel (3.0 g). The reaction was then pressurized under an atmosphere of hydrogen to 48 psi for 23 h. The solution was then filtered through a pad of Celite. The Celite cake was washed with methanol (200 mL) and concentrated under reduced pressure to yield a brown solid. ¹H NMR (d₆-DMSO) δ 4.36 (s, 2H), 4.50 (s, 2H), 5.12 (s, 1H), 6.62–6.86 (m, 3H). MS: M^{−1} = 178 Da.
- Preparation of intermediate **3**: To a solution of intermediate **2** (2.0 g, 11.2 mmol) in dichloromethane (80 mL) at room temperature was added pyridinium dichromate (6.31 g, 16.8 mmol). The reaction mixture was stirred for 24 h. The reaction mixture was then filtered through a pad of Celite. The Celite cake was washed with ethyl ether (100 mL) and ethyl acetate (100 mL). The solution was then concentrated under reduced pressure to yield a dark red solid. ¹H NMR (d₆-DMSO) δ 4.67 (s, 2H), 7.09 (d, 1H), 7.33 (s, 1H), 7.49 (d, 1H), 9.79 (s, 1H). MS: M^{−1} = 175 Da.
- Preparation of intermediate **7**: To a room temperature solution of PPh₃ (5.78 g, 22.0 mmol) and I₂ (5.59 g, 22.0 mmol) in dry CH₂Cl₂ (100 mL) after 10 min of stirring was added imidazole (2.50 g, 36.7). Following another 10 min of stirring 2-*m*-tolyl-ethanol (2.00 g, 14.7 mmol) was added and the reaction was monitored by TLC. The reaction was quenched with aqueous sodium metabisulfite and then extracted with Et₂O (3× 30 mL). The organics were dried over MgSO₄, filtered, and concentrated down under reduced pressure. The product was chromatographed with EtOAc/hexane (1:3) to yield 2.70 g of product. ¹H NMR (d₆-DMSO) δ 2.20 (s, 3H), 3.00–3.15 (m, 2H), 3.40–3.45 (m, 2H), 6.95–7.10 (m, 3H), 7.15–7.20 (m, 1H) ppm.
- Preparation of intermediate **8**: To a suspension of benzoxazine (0.500 g, 2.82 mmol) and K₂CO₃ (0.971 g,

7.05 mmol) in dry CH₃CN (100 mL) was added 1-(2-iodoethyl)-3-methyl-benzene (1.39 g, 5.64 mmol). Benzyltriethylammonium chloride (0.321 g, 1.41 mmol) was then added and the suspension was heated to reflux overnight with good stirring. The heat was turned off and the suspension was allowed to cool to room temperature. EtOAc was added and the reaction was extracted with 1 N HCl (3× 30 mL) and washed with brine (3× 30 mL). The organic layers were dried over MgSO₄, filtered, and concentrated down under reduced pressure. The product was chromatographed with EtOAc/hexane (1:3) to yield 0.520 g of product. ¹H NMR (*d*₆-DMSO) δ 2.20 (s, 3H), 2.75–2.85 (m, 2H), 4.15–4.20 (m, 2H), 4.70 (s, 2H), 6.90–7.00 (m, 3H), 7.10–7.18 (m, 2H), 7.55 (d, 1H), 7.62 (s, 1H), 9.85 (s, 1H) ppm. MS: M⁺ = 296 Da.

20. Preparation of **10g**: To a room temperature solution of 3-oxo-4-(2-*m*-tolyl-ethyl)-3,4-dihydro-2*H*-benzo[1,4]-oxazine-6-carbaldehyde (0.300 g, 1.02 mmol) in MeOH (20 mL) was added 2-thioxo-thiazolidin-4-one (0.135 g, 1.02 mmol) followed by ethylene diamine diacetate (0.183 g, 1.02 mmol). The suspension was allowed to stir overnight at room temperature. The suspension was filtered, then washed with MeOH (30 mL) and Et₂O (30 mL). The yellow product was placed in a drying oven under reduced pressure overnight to yield 0.221 g of product. ¹H NMR (*d*₆-DMSO) δ 2.20 (s, 1H) 2.90 (t, 3H), 4.15 (t, 3H), 4.70 (s, 2H), 7.005 (d, 1H), 7.15 (d, 1H), 7.18–7.20 (m, 4H), 7.40 (s, 1H), 7.70 (s, 1H) ppm. Microanalysis: C₂₁H₁₈N₂O₃S₂; calcd: C, 61.44; H, 4.42; N, 6.82; S, 15.62; found: C, 61.27; H, 4.30; N, 6.70; S, 15.75. MS: M⁺ = 411 Da.

Small Molecule Inhibitors of Phosphoinositide 3-Kinase (PI3K) δ and γ

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Abstract: In recent years, pharmaceutical companies have increasingly focused on phosphoinositide 3-kinases delta (PI3K δ) and gamma (PI3K γ) as therapeutic targets for the treatment of inflammatory and autoimmune diseases. All class I PI3-kinases ($\alpha/\beta/\gamma/\delta$) generate phospholipid second messengers that help govern cellular processes such as migration, proliferation, and apoptosis. PI3K δ/γ lipid kinases are mainly restricted to the hematopoietic system whereas PI3K α/β are ubiquitously expressed, thus raising potential toxicity concerns for chronic indications such as asthma and rheumatoid arthritis. Therefore, the challenge in developing a small molecule inhibitor of PI3K is to define and attain the appropriate isoform selectivity profile. Significant advances in the design of such compounds have been achieved by utilizing x-ray crystal structures of various inhibitors bound to PI3K γ in conjunction with pharmacophore modeling and high-throughput screening. Herein, we review the history and challenges involved with the discovery of small molecule isoform-specific PI3K inhibitors. Recent progress in the design of selective PI3K δ , PI3K γ , and PI3K δ/γ dual inhibitors will be presented.

Keywords: PI3K, inflammation, allergy, asthma, rheumatoid arthritis, COPD.

1. INTRODUCTION

The phosphoinositide 3-kinases (PI3Ks) comprise a family of enzymes that utilize both lipid and protein kinase activity to regulate numerous intracellular signal transduction pathways, which in turn coordinate a range of downstream cellular processes [1]. Based on sequence homology and lipid substrate specificity, the PI3K family is divided into three classes (I, II, and III). The class I PI3Ks have received the most attention from the scientific community and are further broken down into two subclasses, 1A and 1B, both of which catalyze the phosphorylation of phosphatidylinositol (4,5)-bisphosphate (PIP₂) to produce the signaling molecule phosphatidylinositol(3,4,5)-trisphosphate (PIP₃). The class IA isoforms, PI3K α , PI3K β , and PI3K δ , are primarily activated by protein tyrosine kinase-coupled receptors [2], whereas the sole class IB member, PI3K γ , is activated by G-protein coupled receptors (GPCRs), such as chemokine receptors [3]. PI3K α and β are ubiquitously expressed and play a key role in cell growth, survival, and proliferation. This is highlighted by the fact that genetic deletion of the catalytic subunit of either isoform results in embryonic lethality in mice [4,5]. Thus, due to the essential role of PI3K α and β in maintaining homeostasis, pharmacological inhibition of these molecular targets has been largely limited to cancer therapy [6-8]. In contrast to PI3K α and β , the expression patterns of PI3K δ and γ are mainly restricted to the hematopoietic system. PI3K γ is widely expressed in granulocytes, monocytes, and macrophages, while the δ isoform is also found in B and T cells. Mice containing knockouts for the genes encoding either PI3K δ or PI3K γ are viable and fertile but exhibit significant defects in both innate immunity as well as adaptive responses [9]. Given this phenotype, it is possible that selective inhibitors of PI3K δ and/or PI3K γ may prove useful in treating

inflammatory and autoimmune disorders [9-15]. This review summarizes the evolution of PI3K δ and PI3K γ inhibitors and focuses on chemotypes disclosed in peer-reviewed journals and patent applications through May 2009.

2. CHALLENGES AND STRATEGIES FOR DESIGNING SELECTIVE PI3K INHIBITORS

Due to the high sequence homology between the four class I PI3K catalytic domains and the conserved topology of the ATP-binding site across the human kinome, the design of small-molecule PI3K inhibitors that exhibit a sufficient level of isoform selectivity (δ and/or γ vs. α and β) and cross-kinase selectivity to enable oral treatment of chronic inflammatory diseases presents a formidable challenge [16]. To aid in this endeavor, crystal structures of the PI3K α and γ apoproteins have been elucidated [17,18], as well as numerous cocrystal structures for a range of ATP-competitive inhibitors bound to the latter isozyme [19-24]. These x-ray structures reveal a multidomain organization similar to traditional protein kinases, including an ATP-binding site between the N- and C-lobes of the catalytic domain. All of the known inhibitors whose binding mode has been determined by crystallography reside in this pocket and utilize a hydrogen bond acceptor to access a common hinge residue, Val882 (PI3K γ numbering). This is the same backbone amino acid that forms a hydrogen bond with N1 on the adenine ring of ATP. Although several structurally diverse inhibitors are able to engage in additional specific interactions with PI3K γ (i.e. Lys833, Asp964, Ile963, Ser860), all of the observed interactions are conserved among the class I PI3K isozymes. To further complicate matters, only three positions within the active site, corresponding to residues Lys802, Thr886, and Lys890 in PI3K γ , differ between all of the class I subtypes [25]. These residues are located on the periphery of the ATP-binding site, which suggests that rational design of isoform-specific PI3K inhibitors based on these differences may be difficult. However, it has recently been demonstrated that point

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mutations of two of the corresponding amino acids in PI3K α (His855 and Gln859) can negatively impact the potency of PI3K α inhibitors, whereas reciprocal mutations in PI3K β (Glu858 and Asp862) have no effect on the potency of PI3K β inhibitors [26]. Thus, it may be possible to optimize γ/α selectivity by designing inhibitors that stabilize interactions with Thr886 and Lys890 in PI3K γ , while destabilizing the corresponding interactions in PI3K α .

By targeting inactive enzyme conformations, allosteric sites, and gatekeeper residues, selective ATP-competitive inhibitors of protein kinases have been identified [27]. Despite the low overall sequence homology between lipid and protein kinases, similar strategies may allow for selective inhibition of lipid kinases as well. In the case of protein kinases, a 'gatekeeper' residue near the back of the hinge region frequently controls access to a pre-existing selectivity pocket [28]. For all of the class I PI3Ks, the gatekeeper amino acid is a conserved isoleucine. Site-directed mutagenesis of Ile848, the gatekeeper residue in PI3K α , to either glycine or alanine severely impairs enzymatic activity and does not improve sensitivity to known PI3K inhibitors [29]. However, Shokat *et al.* have disclosed two pyrazolopyrimidine kinase inhibitors that inhibit PI3K γ by projecting underneath the gatekeeper residue, Ile879 [23]. Several analogues of these inhibitors are also able to inhibit a variety of tyrosine kinases (Abl, Src, VEGF, etc.) by adopting a conformation that is accommodated through a rotation of the gatekeeper residue (threonine or valine) in this family of protein kinases. These same pyrazolopyrimidine inhibitors are inactive against a range of serine-threonine kinases, and the authors surmise that this is due to restricted movement of the larger gatekeeper residue (methionine or isoleucine) in many of these enzymes. Thus, it is possible that the isoleucine gatekeeper within the class I PI3Ks shows more conformational flexibility than the analogous amino acid in serine-threonine kinases and targeting this residue may provide an opportunity to improve selectivity against proteins from the Ser/Thr family of kinases.

Despite the numerous hurdles to identifying selective ATP-competitive kinase inhibitors, several isoform-selective class I PI3K inhibitors have been identified, and many of these will be discussed in the following sections. However, it should be noted that reported IC₅₀s for kinase inhibitors must be interpreted with caution when comparing values obtained by different research groups since many factors can influence the observed inhibitory concentration (i.e. variations in kinase construct, ATP concentration in the assay, K_m of ATP for the kinase of interest, kinetics of ligand binding, compound solubility, etc.). As a consequence, it is wise to further characterize the activity and selectivity of kinase inhibitors in pertinent cell-based assays, since these experiments can capture a range of dynamic signal transduction pathways involving numerous kinases [30].

3. PI3K INHIBITORS DERIVED FROM NATURAL PRODUCTS

The steroidal furan wortmannin **1** (Fig. 1) was isolated in 1957 from *Penicillium wortmanni*, and its structure was fully elucidated through x-ray crystallography by researchers at

Sandoz laboratories [31]. Although the potent anti-inflammatory properties of **1** had been known for some time, it was not until 1993 that its molecular target was determined to be PI3K [32,33]. Further biochemical studies revealed that **1** functions as a mechanism-based inhibitor by forming an irreversible covalent adduct between C-20 of its strained, electrophilic furan ring and a lysine residue in PI3K that is normally involved in the phosphate transfer reaction [34]. In the case of PI3K γ , this residue is Lys833, and the covalent interaction can be clearly observed in the cocrystal structure of **1** bound to porcine PI3K γ (PDB ID: 1E7U) [22]. Several oxygen atoms in the steroidal skeleton also engage the enzyme through hydrogen bonds: the ketone oxygen of the D-ring interacts with Val882 in the hinge region of the ATP pocket; the A-ring carbonyl interacts with the Ser806 hydroxyl group; and both the B-ring ketone and the furan oxygen interact with Asp964. Since all of these residues are conserved within the class I PI3K family, it is not surprising that **1** is unable to discriminate between the four isozymes (IC₅₀s at 50 μ M ATP: PI3K α = 4.0 nM; β = 0.7 nM; δ = 4.1 nM; γ = 9.0 nM) [35]. At higher concentrations **1** also cross-reacts with myosin light-chain kinase (MLCK) and PI4K [36], as well as several PI3K-related kinases, such as mTOR, DNA-PK, ATM, and ATR [37].

Although compound **1** and related analogues have helped to define the role of PI3K in numerous intracellular signaling pathways, their development as viable therapeutic agents has been limited by poor aqueous solubility and stability (through hydrolytic ring opening of its furan), as well as high *in vivo* toxicity [38]. However, researchers at Lilly revealed that nucleophilic addition of certain secondary amines to C-20 of the labile furan ring provides stable, ring-opened vinylogous carbamates that retain activity against PI3K [39]. Further refinement of this series by Wipf and coworkers led to the identification of PX-866 (**2**, Fig. 1), which is currently being evaluated in phase I trials for the treatment of patients with advanced solid tumors [40,41].

The naturally occurring flavanoid quercetin **3** (Fig. 1) is a pan-kinase inhibitor with moderate affinity for PI3K (50 μ M ATP; PI3K α IC₅₀ = 3.8 μ M) [42]. Researchers at Lilly explored substitution around the chromone core of **3** and identified a morpholine moiety as a suitable replacement for the catechol ring [43]. The lead compound from this study, LY294002 (**4**, Fig. 1), exhibits similar PI3K inhibition (20 μ M ATP; PI3K α IC₅₀ = 1.4 μ M) as **3** but also demonstrates significantly improved cross-reactivity against off-target kinases (i.e. 50 μ M of **4** does not inhibit PI4K, PKC, PKA, MAPK, EGFR, or c-Src). Due to its chemical stability and non-covalent mechanism of action, **4** is often used in place of wortmannin to investigate the role of PI3K in various cellular processes. However, the results of these experiments should be carefully analyzed since recent studies have revealed that the overall selectivity of **4** towards class I PI3Ks, PI3K-related kinases, and numerous protein kinases may not be as high as originally thought [44].

Even though chromone **3** was used as a template to design **4**, cocrystal structures of each compound bound to PI3K γ reveal remarkably different modes of inhibition [22]. In the case of **3** (PDB ID: 1E8W), the carbonyl of the chromone ring is the hinge-binding element with Val882.

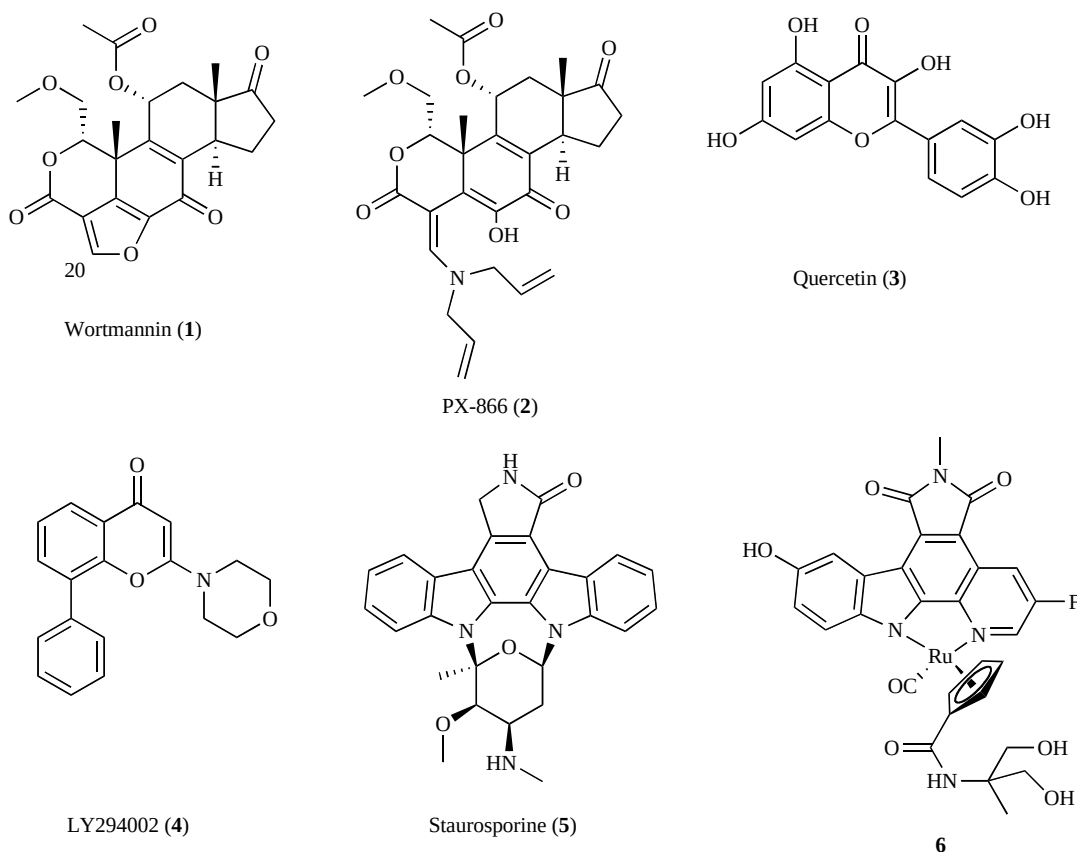


Fig. (1). Natural product PI3K inhibitors and related analogues.

The two flanking hydroxyl groups at the 3- and 5-positions of the chromone also make hydrogen bonds with residues 880-882 in the hinge region, and the 3'-hydroxyl group on the catechol ring interacts with Lys833. However, in the cocrystal structure of compound **4** (PDB ID: 1E7V), the ligand is flipped 180° with respect to **3** so that the morpholine ring can avoid a sterically disfavored interaction with Asp964. As a result, the chromone carbonyl of **4** forms a putative hydrogen bond with Lys833, while the oxygen atom of the morpholine interacts with Val882 and superimposes onto the carbonyl oxygen of **3**. Thus, the morpholine ring provides a unique opportunity to introduce a solubilizing group as a hinge-binder, and it is not surprising that numerous PI3K inhibitors based on the arylmorpholine scaffold have been disclosed (see section 4).

In addition to steroid **1** and flavanoid **3**, the alkaloid staurosporine (**5**, Fig. 1) is a natural-product inhibitor of PI3K (K_d for PI3K γ = 290 nM) [22]. A cocrystal structure of **5** bound to human PI3K γ (PDB ID: 1E8Z) revealed that the lactam of the oxindole mimics the adenine ring of ATP by forming a two-point interaction with the backbone of Val882 in the hinge region of the enzyme [21]. Unfortunately, this mode of binding is also accommodated by numerous protein kinases (there are 23 different kinase cocrystal structures with staurosporine deposited in the Protein Data Bank, www.rcsb.org/pdb). The broad-spectrum activity of **5** was recently confirmed in a 290-kinase panel (K_d < 1 μ M for 83% of the molecular targets) [45]. However, despite the promiscuous nature of **5**, Marmorstein *et al.* have modified

the natural product's heteroaromatic tetracyclic core in an effort to design PI3K-selective inhibitors. By replacing the sugar moiety of staurosporine with an organoruthenium scaffold, the bis-indole ring system with a pyridocarbazole, and the oxindole ring with a succinimide, the authors identified a potent PI3K γ inhibitor, **6** (10 μ M ATP; PI3K γ IC_{50} = 39 nM). This compound is 5-fold less active against PI3K α and demonstrates minimal inhibition (IC_{50} > 5 μ M) of five protein kinases (MST1, PAK1, BRAF, GSK3 α , and PIM1) from four major kinase families (STE, TKL, CMGC, and CAMK, respectively). A cocrystal structure of PI3K γ in complex with **6** (PDB ID: 3CST) suggests that the selectivity of the organoruthenium staurosporine analogue may be attributed to the N-methyl group of the succinimide moiety, which is within van der Waals contact distance of a tyrosine residue (Tyr867) that is conserved among the PI3K isoforms but not present in protein kinases. Additional interactions observed in the cocrystal structure include a hydrogen bond between the succinimide carbonyl and the backbone amide of Val882 in the hinge region. The phenol moiety of the ligand serves as a hydrogen bond donor for the carbonyl of Val882, while simultaneously functioning as a hydrogen bond acceptor for Asp884. In regards to the organoruthenium scaffold, the CO ligand is within bonding distance of both the main chain amide nitrogen and side chain hydroxyl of Thr887, while the dihydroxy-tert-butyl amide attached to the Cp ligand is directed towards a solvent channel. The authors suggest that this region of the molecule may provide an opportunity to improve isoform selectivity since the solvent exposed boundary of the ATP pocket contains the greatest

variation of amino acid residues among the class I PI3K enzymes.

4. PI3K δ INHIBITORS

Quinazolinones and Related Derivatives

Whereas the natural products discussed in the previous section display limited selectivity for the individual PI3K isoforms, the xanthine alkaloid theophylline **7** (Fig. 2) preferentially inhibits PI3K δ (IC_{50} s at 100 μ M ATP: PI3K δ = 75 μ M; α = 400 μ M; β = 400 μ M; γ = 1 mM) [46]. The N-methylated congener, caffeine **8**, also exhibits a similar profile. Although the 5-fold isoform selectivity displayed by **7** and **8** is encouraging, weak inhibition of PI3K δ limits their utility as leads for medicinal chemistry programs. However, researchers at ICOS have disclosed a series of quinazolinone PI3K δ -selective inhibitors that bear structural characteristics reminiscent of the xanthine alkaloids [47,48]. A well characterized compound from this series, IC87114 (**9**, Fig. 2) exhibits high *in vitro* selectivity (100- to 1000-fold) for PI3K δ over the other PI3K isozymes (IC_{50} s at 10 μ M ATP: PI3K δ = 0.13 μ M; α > 200 μ M; β = 16 μ M; γ = 61 μ M), does not inhibit several related lipid kinases (IC_{50} > 100 μ M for class II and class III PI3Ks, PI4Ks, PIKKs, PIPKs), and is remarkably clean against a panel of 36 diverse protein kinases (<20% inh at 10 μ M) [24,49,50]. This unique selectivity profile has enabled researchers to utilize **9** as a tool for deciphering the role of PI3K δ in leukocytes. For instance, Sadhu *et al.* demonstrated that pre-treatment of neutrophils with **9** blocked both fMLP- and TNF1 α -induced superoxide production and elastase exocytosis, whereas PMA and Fc γ R-stimulated superoxide generation were unaffected [51]. Further experiments using neutrophils treated with **9** revealed that PI3K δ also regulates the amplification of PIP₃ levels that lead to neutrophil polarization and directional migration [49]. Given the clear importance of PI3K δ in coordinating neutrophil activation and chemotaxis, it is encouraging that administration of **9** (32 mg/kg, ip) blocked TNF1 α -stimulated elastase exocytosis and neutrophil migration in a mouse air pouch model of inflammation [51]. Compound **9** was also effective in a neutrophil-dependent murine K/BxN serum transfer model of inflammatory arthritis [52]. In this model, mice containing a genetic deletion in either p110 δ or p110 γ showed a statistically significant reduction in paw swelling and histologic score (14 days after administration of autoreactive antibodies) compared to wild-type animals. Mice deficient in both p110 δ and p110 γ (p110 $\gamma\delta^{-/-}$) developed even less paw edema, and histological evaluation of joints from these animals revealed almost no sign of inflammation and bone or cartilage erosion. Administration of **9** (20 mg/kg, po TID) to wild-type mice resulted in decreased induction and progression of joint deterioration in a manner similar to p110 $\delta^{-/-}$ mice, whereas treatment of p110 $\gamma^{-/-}$ mice with **9** completely reversed disease pathology. Taken together, the results of genetic and pharmacological inhibition of PI3K δ and γ suggest that selective inhibitors of either isoform can help alleviate the symptoms of rheumatoid arthritis, but dual inhibition may provide superior clinical outcomes.

Mast cells release inflammatory mediators that play an important role in initiating and maintaining allergic respon-

ses [53]. Ali *et al.* have reported that bone marrow mast cells derived from mice expressing a loss-of-function allele for p110 δ (p110 δ^{D910A}) exhibit significant defects in SCF-stimulated *in vitro* proliferation, adhesion, migration, and allergen IgE-induced degranulation and cytokine release [54]. Furthermore, in a mast cell-dependent model of passive cutaneous anaphylaxis (PCA), p110 δ^{D910A} mice displayed a significant reduction (70%) in vascular permeability compared to wild-type animals from the same genetic background [55]. Treatment of wild-type mice with compound **9** (30 mg/kg, 1h before Ag challenge) diminished vascular permeability by 40%. Interestingly, mice containing a genetic deletion for p110 γ do not exhibit reduced allergic responses in this model, despite strong evidence that PI3K γ plays an important role in mast cell degranulation *in vitro* [55,56]. The ability of **9** to reduce vascular permeability *in vivo* was also observed in a mouse ovalbumin model of asthma and could be correlated with a decrease in expression of VEGF and hypoxia-inducible factor 1 α (HIF-1 α) [57]. Intratracheal administration of **9** (1 mg/kg, 1h before 1st OVA challenge, day 21, and 3h after last OVA challenge, day 23) to mice with OVA-induced bronchial asthma also reduced several markers of allergic airway inflammation (lung tissue eosinophilia, airway mucus production, peribronchial/perivascular inflammation score) and suppressed airway hyperresponsiveness to inhaled methacholine [58]. Thus, based on data from a variety of pre-clinical disease models, it appears that selective inhibition of PI3K δ may be an attractive strategy for the treatment of asthma in humans [59].

Although cancer therapies targeting the PI3K pathway have typically focused on inhibition of the α and/or β isoforms, PI3K δ is the only isozyme consistently expressed at a high level in blast cells from patients with acute myeloid leukemia (AML) [60]. Treatment of these malignant cells with **9** (10 μ M) completely blocked Flt-3-induced cell proliferation, whereas the same concentration of inhibitor had no effect on the proliferation of normal hematopoietic progenitor cells. This suggests that selective inhibition of PI3K δ may provide an opportunity to effectively treat patients with AML, while minimizing the toxicities associated with traditional oncolytics. In June 2009, Calistoga Pharmaceuticals (which formed as a spin out of ICOS' PI3K δ program [61]) reported that oral administration of CAL-101, a compound with 40- to 300-fold selectivity for delta over the other PI3K isoforms, resulted in partial clinical responses in 12 of 24 patients with advanced hematologic malignancies [62]. Partial response was defined as a decrease of at least 50% in tumor burden. CAL-101 was also utilized in a Phase I allergic rhinitis trial (100 mg BID for 7 days), but results from this investigation are still pending [63]. In addition to CAL-101, Calistoga plans to pursue cancer as an indication for CAL-120, a dual PI3K δ/β inhibitor and also initiate Phase I trials with CAL-263, a PI3K δ -selective inhibitor, for the treatment of patients with inflammatory diseases [62].

PIK-39 (**10**, Fig. 2) is a mercaptopurine analogue of **9** that displays a similar level of isoform selectivity for PI3K δ (IC_{50} s at 10 μ M ATP: PI3K δ = 0.18 μ M; α > 200 μ M; β = 11 μ M; γ = 17 μ M) [24]. Knight *et al.* have published a cocrystal structure of **10** bound to PI3K γ (PDB ID: 2CHW),

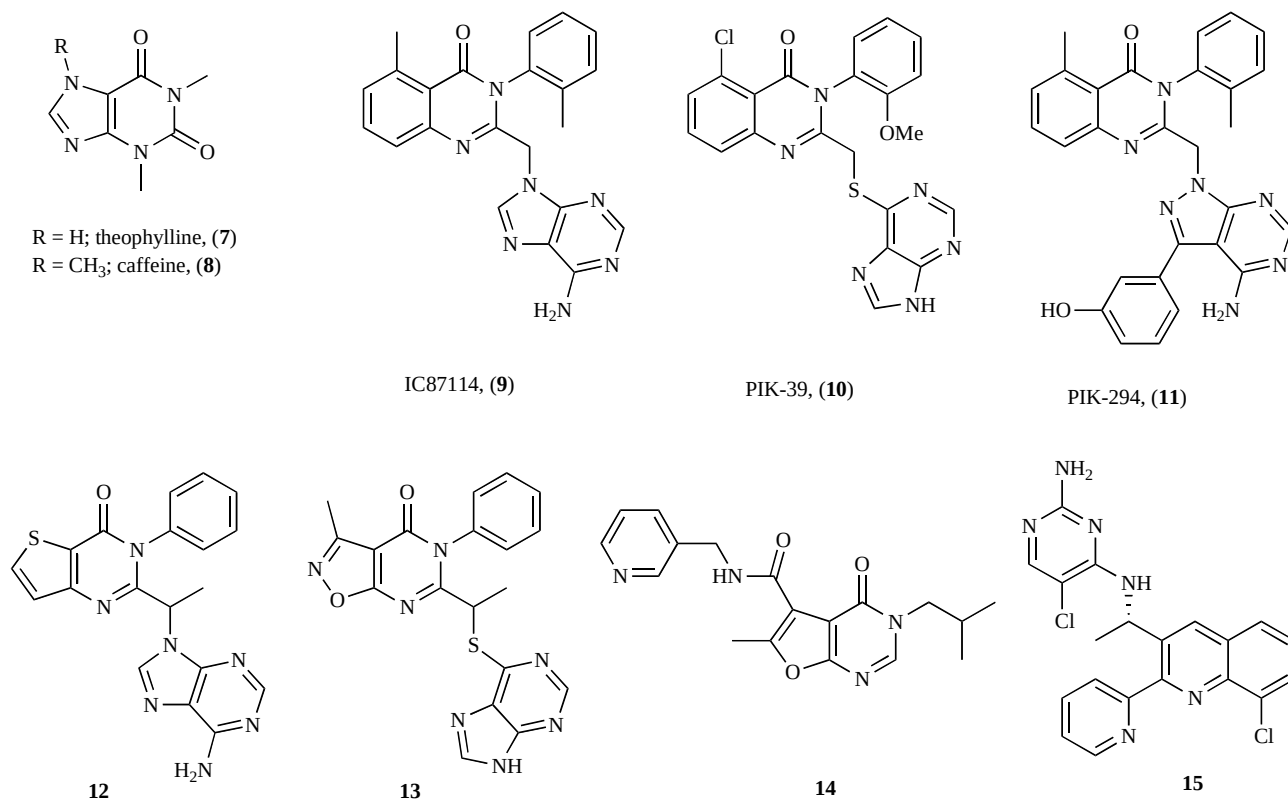


Fig. (2). Quinazolinones and related PI3K δ inhibitors.

and the inhibitor appears to adopt a non-planar orientation in which the mercaptopurine and quinazolinone rings are nearly orthogonal to each other [24]. In this binding mode, N3 and N9 of the purine ring form hydrogen bonds with the backbone amides of Glu880 and Val882, respectively, in the hinge region. In order to accommodate the bulky quinazolinone moiety, the enzyme must undergo a conformational change in the loop between Met804 and Trp812 and induce the formation of a hydrophobic cavity at the entrance to the kinase active site. Given the poor affinity of compound **10** for PI3K γ , there is probably a large energetic penalty associated with this reorganization, and the exquisite δ -selectivity of **10** may be attributed to differential conformational mobility in this region of the protein among the four class I isoforms. In support of this theory, site-directed mutagenesis of Met752 in PI3K δ (which corresponds to Met804 in PI3K γ) to an amino acid (valine or isoleucine) that would be expected to restrict the conformational flexibility of the hydrophobic loop results in a 100-fold loss in inhibitor potency [24]. Interestingly, the recently disclosed crystal structure of the PI3K α catalytic subunit (PDB ID: 2V1Y) reveals that the loop between residues 771 and 779 (which corresponds to residues 803 to 811 in PI3K γ) adopts a conformation that is distinct from the analogous hydrophobic loop in PI3K γ [64]. The authors docked **10** in the ATP pocket of PI3K α and observed that the ligand clashes with Met772 (the equivalent of Met804 in PI3K γ) and can not be accommodated by a change in the loop conformation. Thus, it appears that compound **10** may function in a similar manner to imatinib, by binding to a structurally dynamic region of the target enzyme and stabilizing a single

conformation [65]. It has been suggested that the 'Met804 down' conformation is the PI3K equivalent of the 'DFG out' orientation in protein kinases [66], and it remains to be seen whether targeting this motif will prove to be a general strategy for improving PI3K δ selectivity.

Despite the obvious differences in shape and hydrogen bonding orientation between a mercaptopurine and adenine ring, molecular modeling of quinazolinone **9** within PI3K γ predicts a similar binding mode to **10** [24]. Interestingly, neither of these inhibitors appears to occupy a "deep affinity pocket" in the back of the ATP-binding site. However, by converting the purine ring of **9** into an isosteric pyrazolo-pyrimidine, it was anticipated that the incorporation of substituents at C-3 would allow access to the desired region between Tyr867 and Asp964. Indeed, the introduction of a phenol moiety provided PIK-294 (**11**, Fig. 2), an inhibitor that is 100-fold more active against all of the PI3K isoforms than purine **9** (IC₅₀s at 10 μ M ATP: PI3K δ = 0.01 μ M; α = 10 μ M; β = 0.49 μ M; γ = 0.16 μ M) [24]. This compound retains the same level of PI3K δ -selectivity as **9** and suggests that quinazolinones **9-11** bind to all of the PI3K isoforms in a conserved manner. Knight *et al.* have filed several patent applications describing **11** and related pyrazolopyrimidines as PI3K inhibitors [67-69]. Eli Lilly has also disclosed a similar series of pyrimidinone-based PI3K δ inhibitors, of which adenine **12** (K_is for PI3K δ = 0.04 μ M; γ = 7.5 μ M; α = 58 μ M; β = 5.0 μ M) and mercaptopurine **13** (IC₅₀ = 1.2 μ M in fMLP-induced elastase exocytosis from neutrophils) are representative examples [70,71]. Researchers at the Chemical Diversity Research Institute have identified PI3K inhibitors comprised of a bicyclic pyrimidinone core, and

furanopyrimidinone **14** is reported to be a PI3K δ -selective inhibitor (IC_{50} s for PI3K δ = 1.2 μ M; γ = 71 μ M; α = 13 μ M; β = 80 μ M) [72]. Quinoline **15** is a PI3K δ inhibitor (IC_{50} = 105 nM) exemplified in a recent patent application from Amgen [73]. Although procedures for assessing inhibitor selectivity in numerous enzymatic and cellular assays are described, only data from a PI3K δ enzymatic assay is provided.

Pyrimidinylmorpholines

In 2006, Astellas Pharma reported a morpholino-thienopyrimidine **16** (Fig. 3), which was discovered through a high-throughput screening campaign to find PI3K α inhibitors [74]. In an effort to improve the poor physicochemical properties of **16**, scientists at Piramed and Genentech (both later acquired by Roche) subsequently identified indazole **17** [75]. Although this compound was originally described as a PI3K α -selective inhibitor, it was later revealed that **17** is also equipotent against PI3K δ with

10- to 20-fold selectivity over the β and γ isoforms (IC_{50} s at 1 μ M ATP: PI3K δ = 3 nM; α = 3 nM; β = 33 nM; γ = 75 nM) [76]. The compound exhibited acceptable oral bioavailability in several species (mouse = 77%, rat = 30%; dog = 71%; monkey = 20%) and also demonstrated a dose-dependent reduction of tumor growth in a human U87-MG glioblastoma xenograft model in mice. Due to this impressive preclinical profile, compound **17** has progressed into Phase 1 trials for the treatment of metastatic solid tumors [77] and non-Hodgkin lymphoma [78]. A cocrystal structure of **17** with PI3K γ (PDB ID: 3DBS) reveals a binding mode reminiscent of chromone **4**; the morpholine interacts with the hinge Val882 amide, while the indazole nitrogens are within close proximity to Tyr867 and Asp841. The piperazine portion of the molecule resides in the solvent exposed region, and the oxygens of the sulfonamide are within hydrogen bonding distance of the side chain of Lys802 and the backbone amide of Ala805. Related bicyclic and monocyclic-pyrimidinylmorpholines (example structures **18-24**) were disclosed in more recent Piramed/ Roche

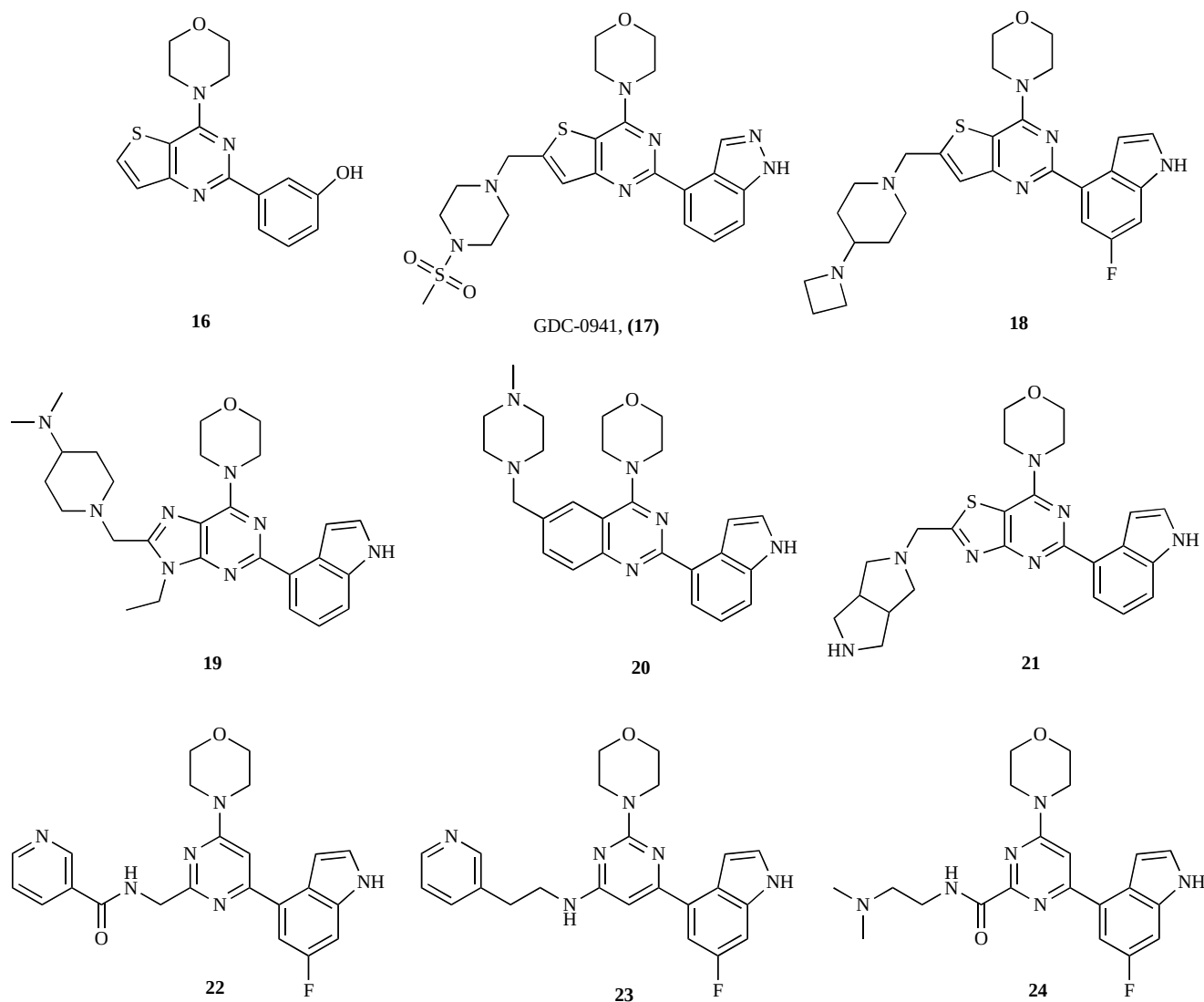


Fig. (3). Pyrimidinylmorpholine PI3K δ inhibitors.

patents [79-85]. While no isoform specific data is presented, it is stated that the compounds exhibit some selectivity for the p110 δ isoform.

5. PI3K δ / γ DUAL INHIBITORS

Morpholinothiazoles

The cocrystal structure of chromone **4** bound to PI3K γ served as a template for pharmacophore modeling and screening of commercial compound databases by scientists at UCB [86]. This resulted in the identification of a 2-morpholino fused thiazole **25** (Fig. 4) with pan-PI3K activity (IC_{50} s: PI3K α = 1.3 μ M; β = 0.69 μ M; δ = 0.70 μ M; γ = 3.5 μ M). Structure-activity relationship studies demonstrated the importance of dimethyl substitution on the cyclohexanone ring. Additionally, a complete loss in activity was observed when the morpholine was replaced by a piperidine, suggesting that the binding mode in the active site of PI3K γ was similar to that of morpholine **4**. Further evaluation of the core led to the identification of lactam **26** with greater than 10-fold improvement in activity at PI3K α , γ , and δ (IC_{50} s: PI3K α = 59 nM; β = 1006 nM; δ = 18 nM; γ = 31 nM) [86,87]. The *in vivo* potency of **26** was assessed utilizing a U87-MG xenograft model in nude mice. After a

single dose (200 mg/kg, po), this compound demonstrated a 65% decrease in pAKT at 1h and a 32% decrease at 6h in an *ex vivo* analysis of the tumor. Furthermore, dosing of **26** (100 mg/kg, po BID) resulted in a 31% reduction in tumor growth (*T/C* value) after 15 days. An x-ray cocrystal structure of **25** with PI3K γ (PDB ID: 3DPD) prompted a second publication from UCB describing the effects of fusing the morpholine to an aryl ring, thus allowing for an edge-face interaction with Trp812 while maintaining the hinge interaction of the morpholine oxygen with Val882 [88]. Compounds containing the aforementioned benzomorpholine were synthesized in both the ketone and lactam series. The presence of the 6-phenyl benzomorpholine in the lactam series significantly improved activity against both the PI3K γ and PI3K δ isoforms, providing greater than 50-fold selectivity over PI3K β (IC_{50} s for **27a**: PI3K α = nd; β = 3.7 μ M; δ = 0.05 μ M; γ = 0.08 μ M). The analogous compound in the ketone series (**27b**) showed improved affinity for only PI3K δ (IC_{50} s: PI3K α = 2.7 μ M; β = 8.0 μ M; δ = 0.08 μ M; γ = 0.85 μ M). Further optimization of the aryl substituent resulted in N-methylpyrazole **28** (IC_{50} s: PI3K α = 0.30 μ M; β = 0.54 μ M; δ = 0.03 μ M; γ = 0.08 μ M) and pyridazine **29** (IC_{50} s: PI3K α = 0.29 μ M; β = 0.34 μ M; δ = 0.05 μ M; γ = 0.05 μ M), with 5- to 15- fold selectivity for the γ/δ isoforms over α/β [88,89]. Due to their high oral exposure and low

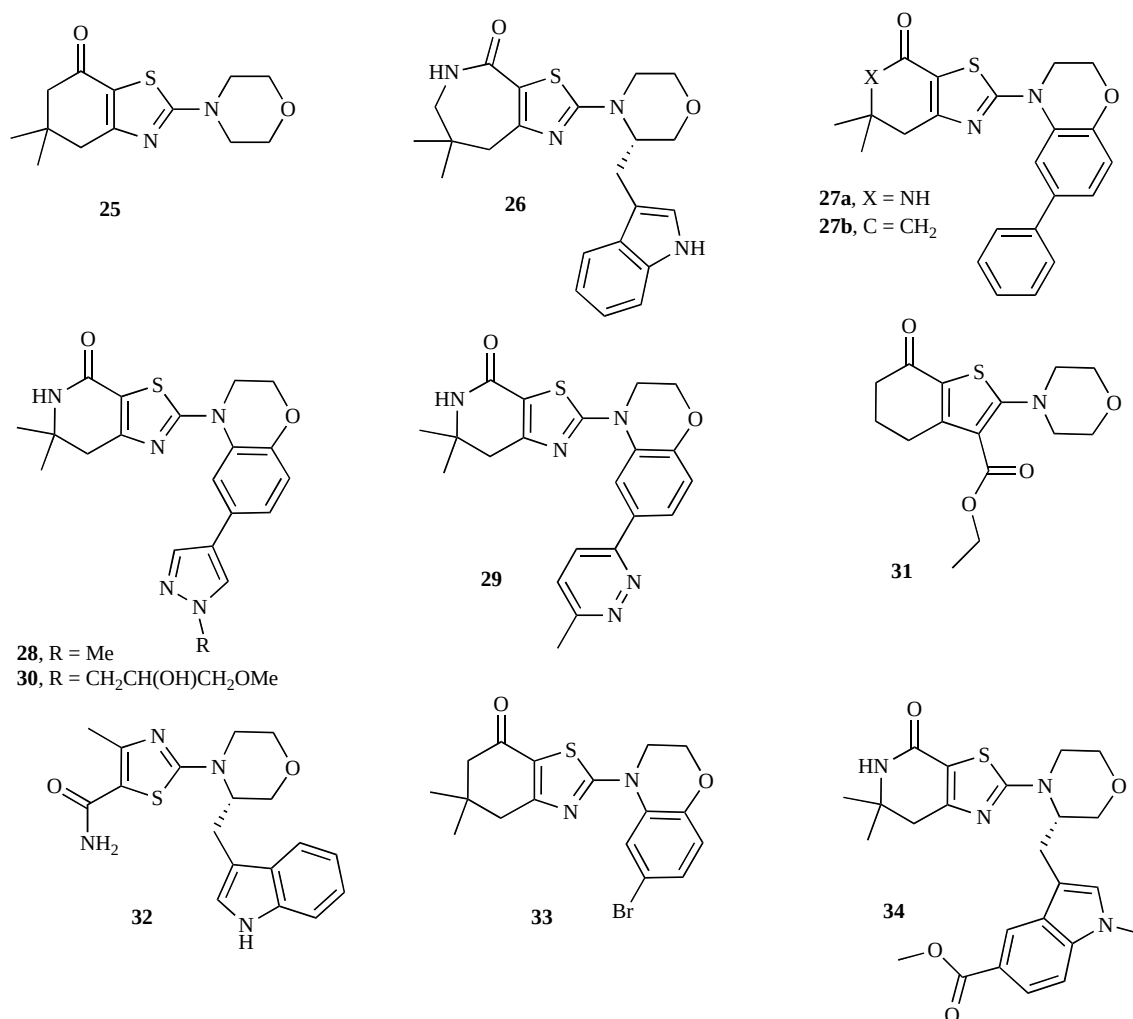


Fig. (4). 2-Morpholinothiazole PI3K δ / γ inhibitors.

clearance, these compounds were examined in a T-cell activation model in rats. Inhibition of IL-2 release into the blood after activation of T-cells by anti-CD3 was measured after oral dosing to give ED₅₀s of 25 and 15 mg/kg for **28** and **29**, respectively. Further improvements in *in vivo* efficacy were attributed to the increased cellular activity and reduced protein binding of pyrazole **30**, which demonstrated an ED₅₀ of 5 mg/kg in the same CD3-induced IL-2 release study in Lewis rats [90]. Additional compounds from this class have been revealed by UCB in the patent literature, although no activity data was disclosed (**31-34**, Fig. 4) [91-93].

Pteridines

With the primary goal of identifying compounds that could be used to treat acute myocardial infarction by inhibiting VEGF-induced vascular leakage, researchers at TargeGen prepared a library of compounds containing an ATP-binding element (i.e. aminoheterocycles) fused to a phenol moiety [94]. It was anticipated that the phenol would mimic a common structural motif of flavanoids, which are known to have vascular protective properties, and the aminoheterocycle would bind to any of the numerous kinases involved in the complex signal transduction pathways that regulate vascular permeability. Although phenols are often considered a metabolic liability in drug leads, a quick onset of action and rapid clearance may be a desirable property in therapies that treat acute indications such as myocardial infarction. A Miles assay, in which compound is injected into rats prior to sequential administrations of Evans blue and VEGF, was used as an *in vivo* screen to evaluate the ability of the library compounds to inhibit VEGF-induced vascular leakage. In general, compounds containing an aminopyrimidine or pyridinopyrazine scaffold showed the most efficacy, and TG100-115 (**35**, Fig. 5) was selected as a lead. Subsequent profiling against a panel of 140 kinases revealed that compound **35** is a PI3K γ / δ dual inhibitor (IC₅₀s at 10 μ M ATP: PI3K γ = 83 nM; δ = 235 nM; α = 1300 nM; β = 1200 nM). Remarkably, **35** did not significantly inhibit any of the other 136 protein kinases tested (<50% inh at 1 μ M). Additional structure-activity relationship studies indicated that the phenol attached to the 6-position of the pteridine is required for activity, whereas the aromatic ring in the 7-position is not essential. However, removal of this substituent had a detrimental effect on the PI3K isoform selectivity of **36** (IC₅₀s: PI3K γ = 50 nM; δ = 24 nM; α = 165 nM; β = 215 nM) [95]. The position of the meta-hydroxyl

group also appears to be important, since des-hydroxy and para-hydroxy analogues of **35** and **36** were all 30-fold less active against PI3K γ . An indole ring can serve as a bioisostere for the phenol in **36**, but not without a substantial loss in selectivity against PI3K β (IC₅₀s for **37**: PI3K γ = 85 nM; δ = 64 nM; α = 1200 nM; β = 107 nM). Molecular modeling studies indicate that the superior isoform selectivity of **35** relative to **36** and **37** may be attributed to decreased rotational freedom of the aromatic rings attached to the pteridine core [95].

In addition to attenuating VEGF-induced vascular permeability *in vivo*, compound **35** demonstrated a cardio-protective effect in a rodent model of myocardial infarction [95,96]. In this disease model, occlusion of the left anterior descending coronary artery creates an ischaemia/reperfusion (I/R) injury to the rat myocardium. A single bolus dose of **35** (0.5 mg/kg, i.v.) delivered at 60 minutes post-reperfusion provided a 50% reduction in infarct area after 22 hours. This cardioprotective effect was also reproduced in a porcine model of myocardial infarction. Due to the encouraging pre-clinical efficacy of **35**, TargeGen initiated a Phase I/II clinical trial in 2005 to evaluate an intravenous delivery of this compound to patients who suffer a heart attack and undergo angioplasty to restore blood flow [97]. No results of the study have been reported, and a recent publication from TargeGen revealed that intranasal administration of **35** was effective in murine models of asthma and COPD, suggesting that TargeGen may be considering an alternate indication(s) for this compound [98].

6. PI3K γ INHIBITORS

Thiazolidinones

Serono has published extensively on a series of PI3K γ inhibitors comprised of vinylthiazolidinedione and vinylrhodanine scaffolds (**38-40**, Fig. 6) [20, 99-101]. The original leads for these series were identified using a proprietary substructure analysis of low affinity inhibitors derived from a PI3K γ high-throughput screening campaign [20]. Since several thiazolidinediones (also called glitazones) are well established hypoglycemic agents [102], promising compounds were frequently counterscreened against various peroxisome proliferator-activated receptors (PPAR α , γ , δ) early in the discovery program. Subsequent optimization through synthetic chemistry provided AS-252424 (**38**), which exhibits greater than 300-fold selectivity for PI3K γ

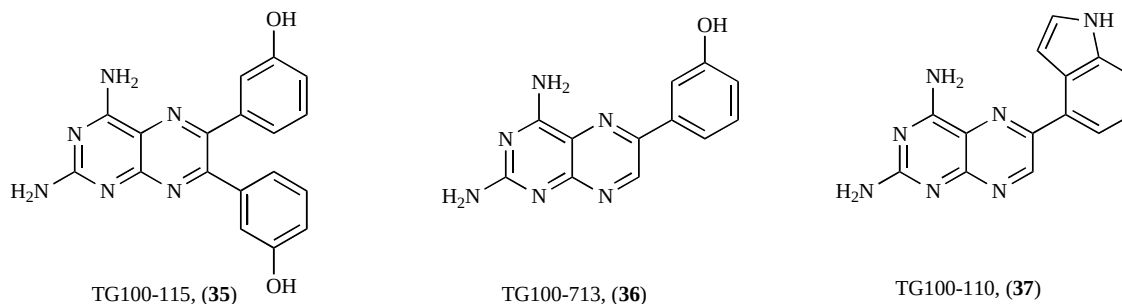


Fig. (5). Pteridine-based PI3K δ / γ dual inhibitors.

over the class I isozymes (IC_{50} s: $PI3K\gamma = 33$ nM; $\alpha = 935$ nM; $\beta = 20000$ nM; $\delta = 20000$ nM). Compound **38** is also remarkably clean against a panel of serine/threonine and tyrosine kinases ($IC_{50} > 10$ μ M for 78/79), with the notable exception of casein kinase 2 ($IC_{50} = 20$ nM). Structure-activity relationship studies demonstrated that the 4-fluorine substituent on the aromatic ring is not important for $PI3K\gamma$ activity but does provide a 3-fold improvement in selectivity against $PI3K\alpha$. A cocrystal structure of **38** bound to $PI3K\gamma$ revealed two specific protein-ligand interactions: 1) a salt-bridge between the side chain amine of Lys833 and the nitrogen atom of the thiazolidinedione ring, and 2) a hydrogen bond between a backbone NH of Val882 and the 2-hydroxy group on the phenol ring [20]. Despite the high clearance (2.3 L/kg*h) and short oral half-life ($t_{1/2} = 1$ h) of **38** in rats, oral dosing (10 mg/kg) of this compound in a mouse model of thioglycollate-induced peritonitis resulted in a 35% reduction of neutrophil recruitment. This is nearly identical to the phenotype observed in $PI3K\gamma$ -deficient mice [21].

Although compound **38** provides proof that $PI3K\gamma$ -selective inhibitors can be identified, poor pharmacokinetic properties limit its utility in preclinical disease models. In order to improve bioavailability, the phenol moiety was replaced with a quinoxaline ring to provide AS-605240 (**39**, Fig. 6). This compound exhibits 8- to 40-fold selectivity for $PI3K\gamma$ over the other class I $PI3K$ isoforms (IC_{50} s: $PI3K\gamma = 8$ nM; $\alpha = 60$ nM; $\beta = 270$ nM; $\delta = 300$ nM) and minimal cross-reactivity against protein kinases (3 of 38 >50% inhibition at 1 μ M) [21]. A cocrystal structure of quinoxaline **39** with $PI3K\gamma$ (PDB ID: 2A5U) revealed a similar binding mode as furan **38**, except instead of the phenol oxygen serving as a hinge-binding element, N1 of the pyrazine ring forms a hydrogen bond with Val882. More importantly, the pharmacokinetic profile of **39** is sufficient to enable its use in

rodent disease models. For instance, therapeutic dosing (begun once disease was established) of **39** (50 mg/kg, po, BID) in a murine α CII-induced arthritis model significantly reduced clinical and histological signs of joint inflammation [21]. Similarly, in a mouse model of collagen-induced arthritis (CIA), **39** prevented arthritis progression when administered at disease onset (semitherapeutic dosing) and reversed disease symptoms (i.e. synovial inflammation, cartilage erosion, paw thickness) when dosed therapeutically [21]. Histological analysis also revealed that both dosing protocols reduced neutrophil infiltration into arthritic joints by 50%. In addition to rheumatoid arthritis, **39** has demonstrated efficacy in murine models of atherosclerosis [103] and lupus [104]. Clearly, thiazolidinedione **39** has proven to be a valuable chemical probe for assessing the pharmacology associated with inhibition of $PI3K\gamma$. The results of these *in vivo* experiments, together with data from studies involving $PI3K\gamma$ knockout mice, suggest an important role for $PI3K\gamma$ in the pathophysiology of inflammation and autoimmune disease.

In a recent patent application from TargeGen, quinoxaline **41** (Fig. 6) and related thiazolidinediones are described as $PI3K\gamma/\alpha$ dual inhibitors (i.e. IC_{50} s for **41**: $PI3K\gamma = 15$ nM; $PI3K\alpha = 11$ nM) [105]. In 2004, researchers at Warner-Lambert (now Pfizer) disclosed a series of benzoxazin-3-one and benzoxazine thiazolidinones (**42-43**, Fig. 6), several of which inhibit $PI3K\gamma$ [106,107]. Although activity against other $PI3K$ isoforms was not reported in these patent applications, subsequent characterization of PIK-124 (**42**) revealed cross-reactivity towards $PI3K\alpha$ and $PI3K\delta$ (IC_{50} s at 10 μ M ATP: $PI3K\gamma = 54$ nM; $\alpha = 23$ nM; $\beta = 1100$ nM; $\delta = 340$ nM) [24]. Compound **42** also displays strong affinity for two class II $PI3K$ enzymes, $PI3KC2\alpha$ ($IC_{50} = 0.14$ μ M) and $PI3KC2\beta$ ($IC_{50} = 0.37$ μ M). However, despite the poor selectivity of **42** towards lipid kinases, this

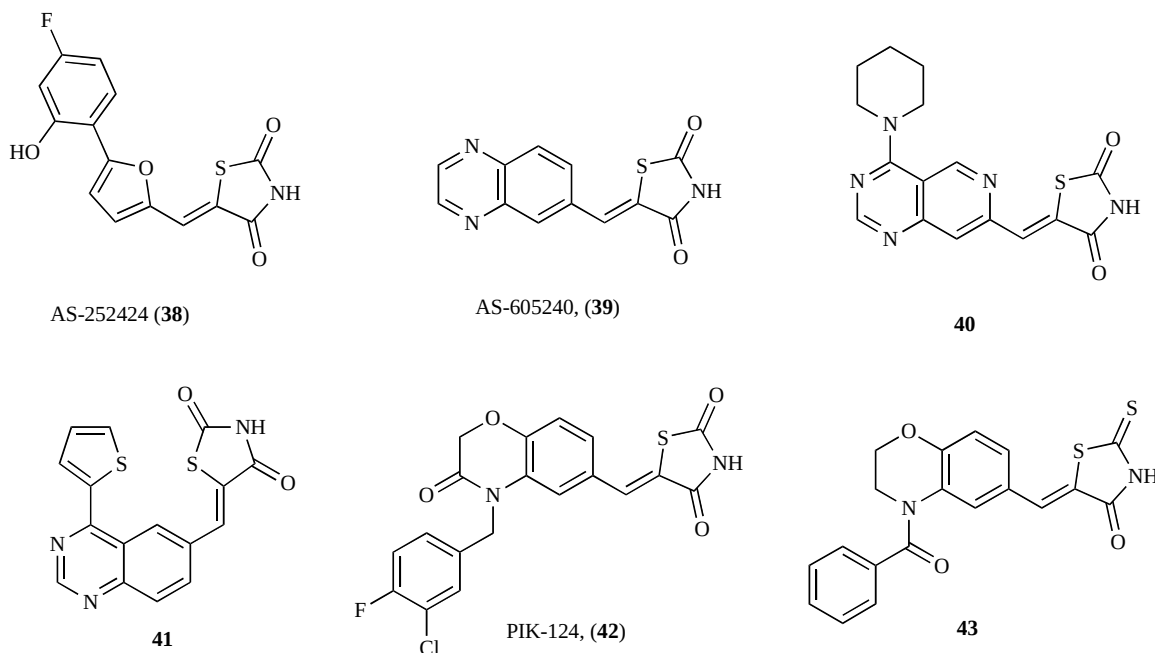


Fig. (6). Thiazolidinone $PI3K\gamma$ inhibitors.

compound failed to demonstrate any significant activity (<20% inhibition at 10 μ M) against a panel of 36 protein kinases. Pfizer later disclosed that rhodanine-based compounds structurally related to **42** exhibited sub-micromolar activity in a cellular assay measuring inhibition of superoxide formation by human neutrophils following stimulation with fMLP [108]. Furthermore, several of these inhibitors displayed *in vivo* efficacy (>40% inhibition of leukocyte infiltration) when dosed in an E. coli-induced peritoneal inflammation mouse model at 100 mg/kg po.

2-Aminothiazoles

Novartis first reported the 2-aminothiazole **44** (Fig. 7) as a selective PI3K γ inhibitor in 2003 [109]. Since that time, over 10 patents and publications have emerged from Novartis, thereby establishing their presence as one of the leaders in the development of PI3K γ inhibitors. Compound **44** was initially reported to have a PI3K γ IC₅₀ of 2 nM [110] and was later shown by Knight *et al.* to inhibit all of the class 1 PI3K isoforms (IC₅₀s at 10 μ M ATP: PI3K γ = 16 nM; α = 39 nM; β = 590 nM; δ = 120 nM), as well as PI4KIII β (IC₅₀

= 19 nM) [24]. The cocrystal structure of **44** bound to PI3K γ (PDB ID: 2CH7) displays hydrogen bonding interactions between the 2-acetamidothiazole and both the backbone amide NH and carbonyl of Val882. The sulfonamide makes a third hydrogen bond with Asp964 [24]. Novartis continued to expand on this series of compounds in several patent applications spanning 2005-2008 [110-114]. Isoform selectivity data was provided for the urea **45** (IC₅₀s: PI3K γ = 16 nM; α = 3018 nM; δ = 626 nM) [110]. Interestingly, this scaffold was also utilized to generate PI3K δ inhibitors [115]. As disclosed in WO08000421, compound **46** potently inhibits PI3K δ (IC₅₀ = 5 nM) and also attenuates B-cell proliferation *in vitro* (IC₅₀ = 15 nM). The most recent patent application from Novartis describes select 5-phenyl-2-aminopyrimidines with PI3K γ activity, as exemplified by sulfonamide **47** (PI3K γ IC₅₀ = 4 nM) [116].

Boehringer Ingelheim has focused their efforts on tricyclic 2-aminothiazoles [117-119] and most recently disclosed a series of thiazolyl-dihydro-indazoles (**48**, Fig. 8) with activity against PI3K γ (IC₅₀s < 600 nM) [120]. Additional 2-aminothiazoles have been reported by Vertex

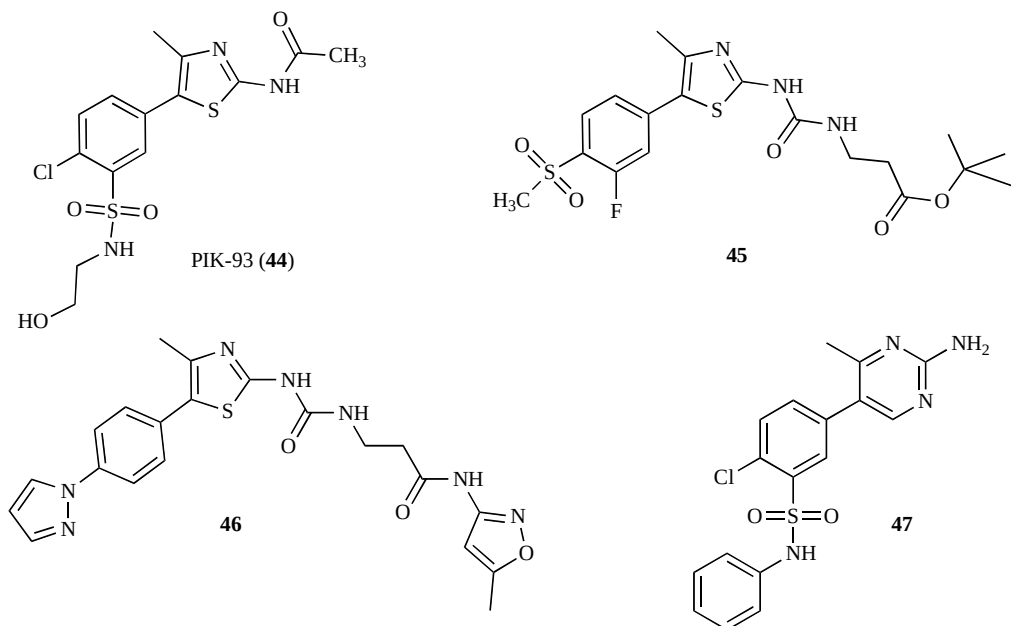


Fig. (7). PI3K γ inhibitors from Novartis.

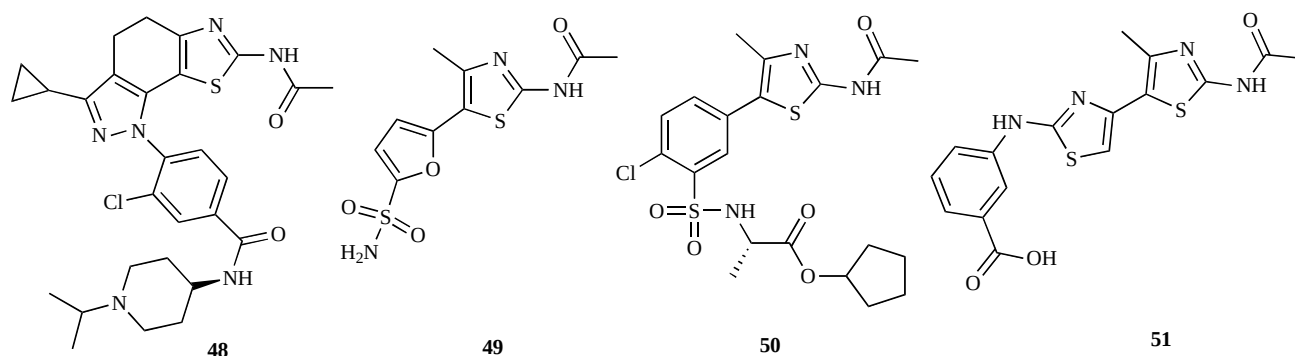


Fig. (8). 2-Aminothiazole PI3K γ inhibitors (Boehringer Ingelheim, Vertex, Chroma, Serono).

(49) and Chroma (50) with activities in a PI3K γ enzymatic assay of $IC_{50} < 0.10 \mu M$ and $0.10 < IC_{50} < 1.0 \mu M$, respectively [121,122]. Serono has also described aminothiazoles as PI3K γ inhibitors, of which bis-thiazole **51** is shown as a representative example [123,124].

Miscellaneous 2-Aminoheterocycles

In addition to the aforementioned 2-aminothiazoles, a variety of 2-aminoheterocycles have been explored as PI3K inhibitors. Specifically, Boehringer Ingelheim [125], Pfizer [126], Bayer [127], and Novartis [128] have all disclosed fused 2-aminopyrimidine scaffolds with limited activity data for PI3K γ in their respective patents. The structures of examples 1 from the corresponding patent applications are shown in Fig. 9 (52-55). Quinoxaline **56**, developed by Serono, is reported to inhibit PI3K γ with an IC_{50} of 56 nM and demonstrate activity in blocking SCF-induced AKT phosphorylation in human peripheral blood mononuclear cells ($IC_{50} = 1.19 \mu M$). Inhibition of IgM-induced AKT phosphorylation in mouse bone marrow mast cells was also reported at an IC_{50} of 21 nM for **56**. Additionally, dosing of **56** (30 mg/kg po) in a murine passive cutaneous anaphylaxis model resulted in an 84% inhibition of vascular permeability [129].

Warner-Lambert has described a series of amidotetrazole PI3K inhibitors, including the cycloheptyloxy derivative **57** (Fig. 10), which exhibits a PI3K γ IC_{50} of 0.197 μM [130]. This compound elicited anti-inflammatory activity at 30 mg/kg QD in a murine collagen induced arthritis model, resulting in a reduction in paw edema by 52% and clinical score by 35%. Compound **57** also demonstrated an ID_{50} of 16.3 mg/kg in a streptococcal cell wall-induced edema model in rat. Unfortunately, glucose dysregulation at high doses in a rat toxicology study precluded further development of **57** [131]. This compound was later stated to be a non-specific inhibitor of the PI3Ks, although data detailing the affinities for the class 1 isoforms is not available. Earlier patent applications from Warner-Lambert also disclose benzo-furanyl and benzothiophene amidotetrazoles as inhibitors of PI3K. For example, **58** and **59** inhibited PI3K γ activity with IC_{50} s of 0.69 and 0.25 nM, respectively [132-134].

Finally, both Novartis and Cellzome have divulged various 6,5-fused heterocycles in the recent patent literature. While Cellzome [135,136] has focused on triazoles (**60**, Fig. 11), a 2009 Novartis patent application discloses multiple heterocycles [137]. Compounds **61-68** are active at PI3K γ with IC_{50} s of 60, 7, 12, 27, 884, and 640 nM, respectively. Imidazolopyridazines **67** (IC_{50} s: PI3K γ = 241 nM; α = 206

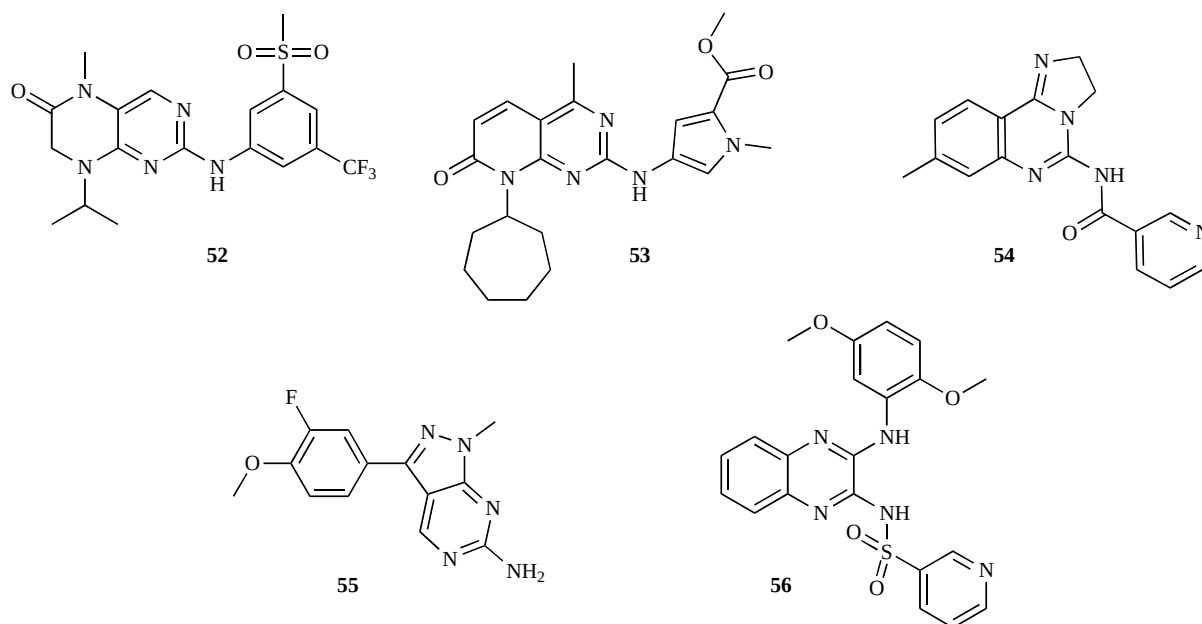


Fig. (9). 2-Aminoheterocycles as PI3K γ inhibitors.

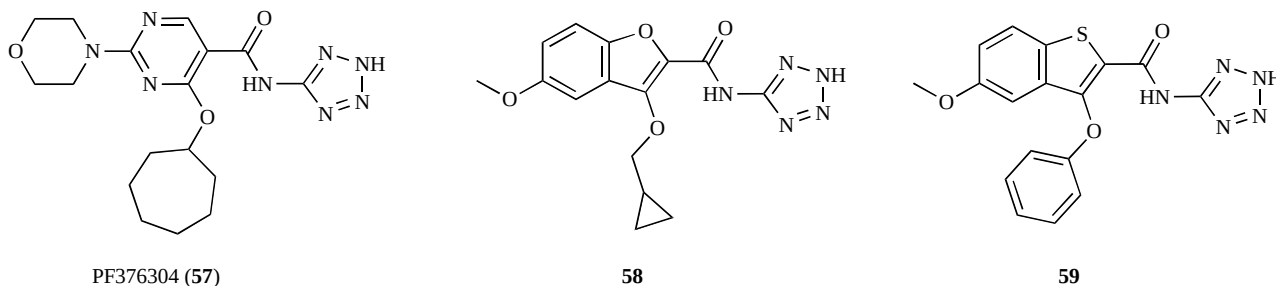


Fig. (10). Amidotetrazole PI3K γ inhibitors.

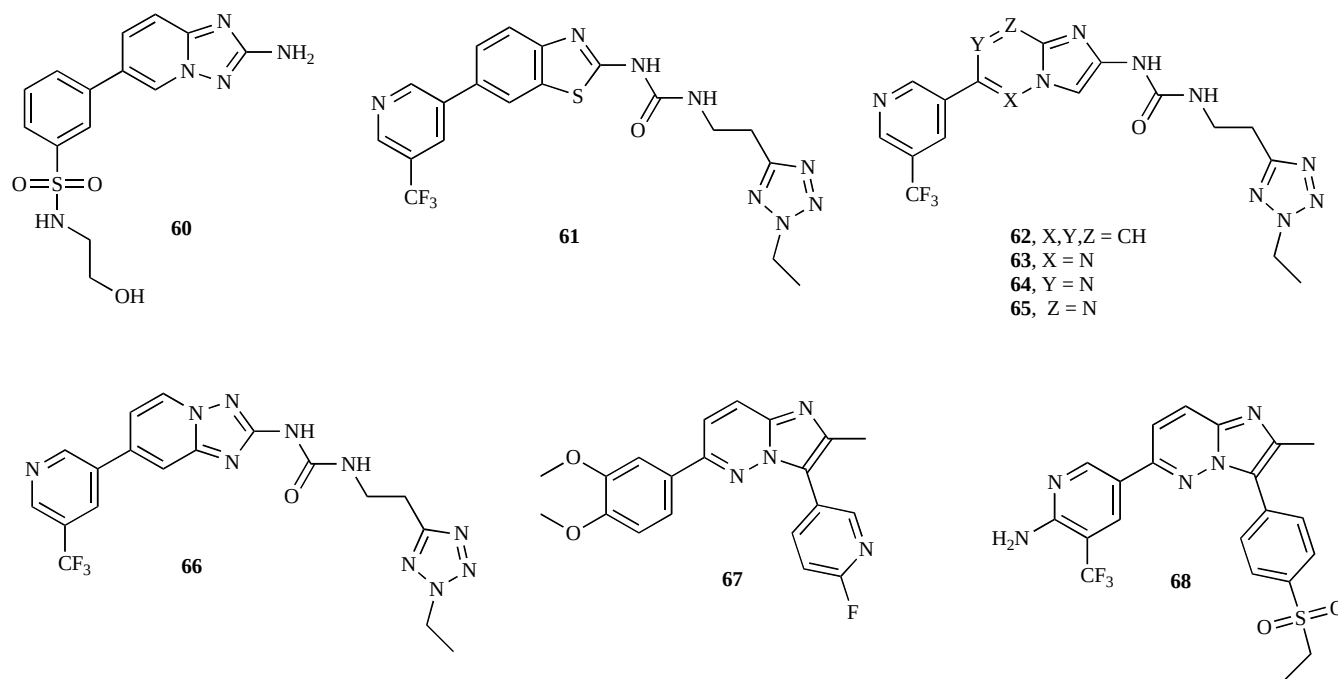


Fig. (11). 6, 5-Fused heterocycles as PI3K γ inhibitors.

nM; β = 1682 nM; δ = 1353 nM) and **68** (IC₅₀: PI3K γ = 1223 nM; α = 63 nM; β = 240 nM; δ = 103 nM) are representative structures from two additional Novartis patent applications [138,139].

7. CONCLUDING REMARKS

The clear clinical and commercial success of imatinib (Gleevec) has helped to validate the development of protein kinase inhibitors as anti-cancer agents. However, due to the conserved topology of the ATP-binding site across the human kinome, the design of small-molecule inhibitors that exhibit a sufficient level of cross-kinase selectivity to enable oral treatment of chronic inflammatory diseases remains an elusive goal. A variety of genetic and pharmacologic tools have defined clear roles for PI3K δ and PI3K γ in leukocyte trafficking and immune responses, and the last several years have witnessed a flurry of activity within the pharmaceutical industry to identify selective inhibitors of these lipid kinases. Although most of the known PI3K inhibitors were originally identified from natural product leads or high-throughput screening campaigns, recent x-ray cocrystal structures of several structurally diverse compounds bound to PI3K γ may greatly facilitate the rational design of future isoform-selective PI3K inhibitors. However, despite the tremendous effort already expended by the pharmaceutical industry, there are no reports of PI3K γ -specific compounds progressing to the clinic, and limited examples with PI3K δ -selective or PI3K δ/γ dual inhibitors. This may reflect the inherent difficulty in identifying the optimal balance of PI3K-isoform selectivity (γ and/or δ vs. α and β) for achieving *in vivo* efficacy with minimal side effects. The therapeutic window is further diminished by the need to attenuate the activity of these ATP-competitive inhibitors against off-target protein kinases. As a result, the choice of which disease indication to pursue in a clinical setting may

vary greatly based on the overall potency, selectivity, and pharmacokinetic profile of the individual PI3K inhibitor. It is anticipated that these factors will be delineated over time as the many ongoing PI3K programs begin to mature.

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