

As. 110.701

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 09-070151- -00009-0000

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Señor

**DIRECTOR DIVISIÓN DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

E.

S.

D.

REF: Expediente No. **09 070151**– solicitud de patente de invención a favor de **GENENTECH, INC. And PIRAMED LIMITED** Título **COMPUESTOS DE TIENOPIRIMIDINA Y FURANOPIRIMIDINA 2-HETEROARILO MONOCÍCLICO, 4-MORFOLINO SUSTITUIDOS**

RESPUESTA A EXAMEN DE FONDO

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante me permito dar respuesta al oficio número 12508 relacionado con la patentabilidad de la presente invención y notificado por fijación en lista el 18 de Julio de 2012.

Con la intención de subsanar las objeciones emitidas por el señor Examinador, nos permitimos presentar a su consideración un nuevo pliego reivindicatorio, compuesto por veinticuatro reivindicaciones, el cual reemplaza el pliego anterior de treinta y cinco reivindicaciones, y se encuentra enteramente sustentado en la descripción.

Atendiendo a la sugerencia del señor Examinador referente al título, solicitamos el cambio del mismo por **COMPUESTOS DE TIENOPIRIMIDINA Y FURANOPIRIMIDINA 2-HETEROARILO MONOCÍCLICO, 4-MORFOLINO SUSTITUIDOS**, superando así al objeción de no patentabilidad debida a la inclusión de uso.

En lo que respecta a excepciones de patentabilidad y a reivindicaciones relativas a un uso o a un segundo uso citadas por su despacho, ponemos en manifiesto del señor Examinador que las reivindicaciones 21, 22 y 24-35 han sido eliminadas del pliego reivindicatorio con el fin de superar las objeciones pertinentes.

Con relación a la objeción que se refiere a que el capítulo reivindicatorio da lugar a un gran número de compuestos que pueden no estar reflejados en los ejemplos mostrados en la descripción, al respecto, la guía de patentabilidad establece que los ejemplos son casos particulares que sirven para ilustrar la mejor manera conocida por el solicitante para ejecutar o llevar a la práctica la invención. De esta manera, aclaramos que las reivindicaciones se encuentran enteramente sustentadas por la descripción y que los ejemplos brindados en la misma no pueden considerarse limitantes para la invención. Notamos además que la descripción contiene cerca de 350 ejemplos que sustentan debidamente la materia reivindicada.

Además, el solicitante está en su derecho de proteger el compuesto reivindicado contra la futura acción de terceros que pretendan infringir la protección solicitada, sumado lo anterior a que el solicitante ha realizado una amplia ilustración de los mismos mediante los ejemplos consignados en la descripción.

Respecto a los términos objetados "solvatos" y "metabolitos" contenidos en el pliego reivindicatorio original, consideramos que son absolutamente claros teniendo en cuenta el contexto de las reivindicaciones así como las enseñanzas de la descripción. No obstante la anterior aclaración, nos permitimos señalar que éstos términos han sido eliminados.

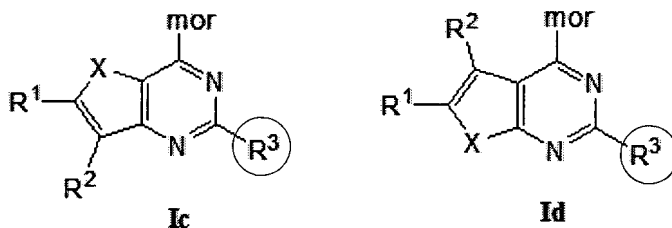
En lo que concierne a la frase "sal farmacéuticamente aceptable", consideramos que ésta se encuentra adecuadamente desarrollada en la descripción, en donde se establece que, se refiere a sales orgánicas o inorgánicas farmacéuticamente aceptables de un compuesto de la invención, es decir que, corresponden a sustancias o composiciones compatibles química y/o toxicológicamente con los otros ingredientes que constituyen una formulación, y/o el mamífero que está siendo tratado con la misma. Además se establece la forma de obtener dichas sales, sumado lo anterior a ejemplos ilustrativos de las mismas.

En conclusión, consideramos que el alcance de las nuevas reivindicaciones se encuentra debidamente sustentado en la totalidad de las enseñanzas de la descripción y más aun teniendo en cuenta que los ejemplos son plenamente ilustrativos de la invención pero de ninguna manera se deben considerar como limitativos de la materia reivindicada, por la cual solicitamos respetuosamente aceptar el nuevo pliego reivindicatorio como claro y conciso.

Nivel inventivo

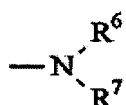
En primer lugar, deseamos resaltar que todos los compuestos revelados en las tablas 1 y 2 de la descripción son altamente activos al unirse a la fosfoinositida 3-quinasa (PI3K) tal como se puede observar en las páginas 35 y 36 de la solicitud PCT de la cual se desprende la presente solicitud. Esto indica claramente que los compuestos de la invención presentan actividad sobre el crecimiento de células tumorales al inhibir la quinasa PI3K responsable de los procesos anormales de crecimiento tumoral. Ahora bien, para demostrar el nivel inventivo de los compuestos de la invención nos enfocaremos en las diferencias con respecto al arte previo especialmente D1 que es el estado de la técnica más cercano a la invención.

La presente invención se distingue de D1 por el radical R^3 de las correspondientes formulas Ic e Id que se enseñan en la reivindicación 1:



Específicamente, el grupo R^3 en la presente invención es un grupo heteroarilo monocíclico opcionalmente sustituido por ciertos grupos: piridilo, isoxazolilo, imidazolilo, pirrolilo, tiazolilo, piridazinilo, pirimidinilo, pirazinilo, oxazolilo, furanilo, tienilo, triazoloilo, tetrazoilo.

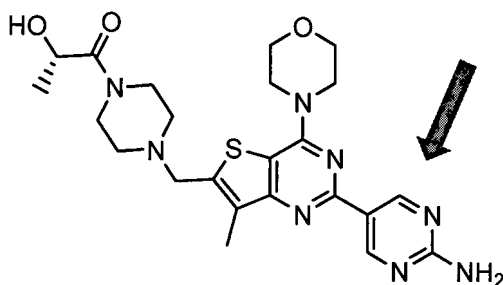
De acuerdo con lo expresado por el señor Examinador, la diferencia entre la invención definida en la reivindicación 1 y el compuesto del documento D1 consiste en que R^2 tiene otros significados distintos a morfolino. En cuanto a esta afirmación, consideramos que, de acuerdo con el Instructivo para el Examen de solicitudes de patentes de invención y modelos de utilidad, la forma correcta para el análisis de nivel inventivo corresponde a establecer las diferencias que presenta la invención frente al estado de la técnica, y no al contrario. De tal manera que, lo que diferencia a la invención de dicho estado de la técnica, además de R^3 como se mencionó con anterioridad, es que la invención limita uno de los sustituyentes del anillo de pirimidina a morfolino, mientras que en el estado de la técnica presenta la siguiente generalización para referirse a dicho sustituyente.



Como bien fue expuesto por el señor examinador, el objeto de la invención corresponde a una subclase de compuestos que difieren en los patrones de sustitución de la fórmula general de tienopirimidina y furanopirimidina 2-heteroarilo monocíclico, 4-morfolino sustituidos, pero contrario a lo que argumentado por su despacho, la selección de dicha clase de compuestos revela una mejora inesperada en la actividad anticancerígena.

Volviendo nuevamente a la diferencia entre la invención y el documento D1 (radical R³), específicamente en el grupo pirimidina, notamos que esta diferencia es la responsable del efecto inesperado en la actividad farmacológica del compuesto reivindicado contra diversos tipos de cáncer como próstata y cáncer de pulmón.

Para sustentar lo anterior, adjuntamos un artículo de los inventores publicado en el Journal of Medicinal Chemistry (Anexo 1) en donde se puede observar que el compuesto 201 (denominado GDC-0980) se compara con un inhibidor P13k del arte previo (denominado GDC-0941) y que se revela de forma general en el documento D2. Dicho compuesto GDC-0980 ahora en ensayos clínicos, muestra alta eficacia en el tratamiento de pacientes con cáncer y se encuentra representado por la siguiente fórmula química:

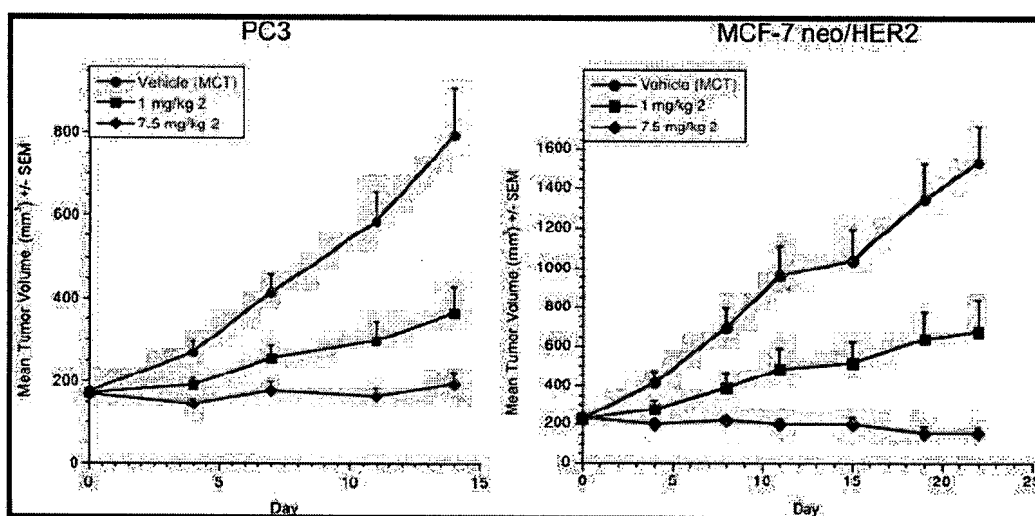


Tal como puede observar el señor examinador, esta fórmula química se encuentra dentro del alcance de la reivindicación 1 y el radical R3 corresponde efectivamente a un grupo pirimidina.

Este compuesto GDC-0980 inhibe potentemente la transducción de señales corriente abajo tanto de PI3K como de mTOR, de acuerdo a las mediciones por biomarcadores farmacodinámicos, y de esta manera actúa sobre dos vías claves para producir una inhibición lo más fuerte posible de la vía de señalización. Por lo tanto, teniendo en cuenta que tanto PI3K como mTOR son enzimas involucradas en el crecimiento celular anormal, su inhibición es importante en el control del crecimiento tumoral.

La figura 1, (incluida en el artículo 1) muestra la eficacia *in vivo* del compuesto GDC-0980 en modelos de xenoinjertos tumorales PC3 y MCF-7 neo/HER2. El compuesto fue dosificado por vía oral una vez al día durante 14 o 22 días, y el volumen del tumor fue medido en comparación con animales control tratados únicamente con el vehículo. En ambos modelos de xenoinjertos, PC-3 y MCF-7 neo/HER2, el tratamiento con 1 mg/kg de GDC-0980 resultó en una actividad antitumoral estadísticamente significativa, caracterizada como retraso en el crecimiento tumoral.

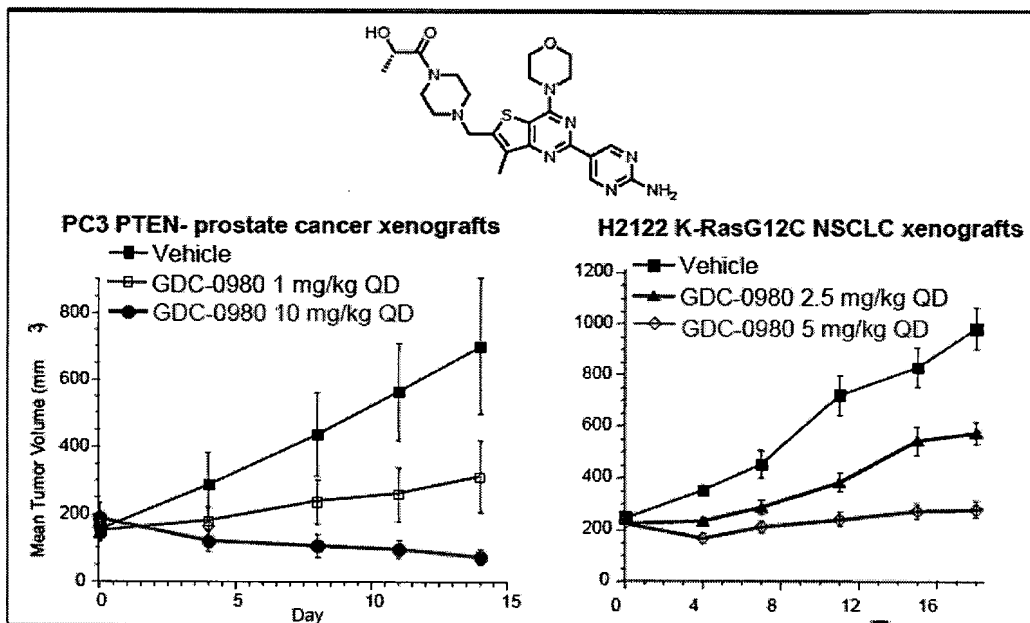
Figura 1



A partir de los datos comparativos expuestos en el artículo 1 se evidencian las contribuciones técnicas inesperadas del grupo pirimidinilo presente en R³, incluyendo una potencia incrementada hacia mTOR, menor unión a proteínas plasmáticas y mejora en de los factores farmacocinéticas observados en ratas y ratones.

Por otra parte, la figura 2 muestra que el compuesto GDC-0980 presenta una actividad significativa *in vivo* contra cáncer de próstata y cáncer de pulmón de células no-pequeñas, a partir del uso de modelos de xenoinjertos tumorales. En este caso, se observa la disminución en el volumen tumoral, comparado con el control que solo es tratado con vehículo. Vale la pena resaltar que esa figura está incluida en el anexo 2 que es una presentación realizada por el inventor Daniel Sutherlin en el Annual Meeting Education Session.

Figura 2



Finalmente, en el estudio preclínico presentado por Wallin y cols. (Anexo 3) se demostró que el compuesto GDC-0980 inhibió fuertemente la vía PI3K y disminuyó la viabilidad de células de tumor en una extensa serie de líneas celulares de cáncer. Esta propiedad adicional de inhibir mTOR (proteína blanco de rapamicina en mamíferos) sumada a la inhibición de la enzima PI3K es responsable de la elevada potencia del compuesto GDC-0980 en comparación con inhibidores convencionales clase I de PI3K.

En este orden de ideas, la naturaleza dual del compuesto GDC-0980 como bloqueador de dos puntos en la red de señalización de PI3K resulta en la fuerte caída de los marcadores proteínas-fosfato en comparación tanto con un inhibidor clase I de PI3K o un inhibidor de mTOR quinasa. Adicionalmente, el efecto dual de bloqueo del compuesto GDC-0980 también resulta en una potencia de 200 o más nmol/L en 25% de líneas celulares de cáncer, y eficacia significativa in vivo de >50% TGI en 15 de 20 modelos tumorales.

Por las razones anteriormente expuestas, se evidencia que la invención presenta efectos inesperados que permiten establecer que la solución técnica al problema propuesta no es obvia y por lo tanto se considera inventiva.

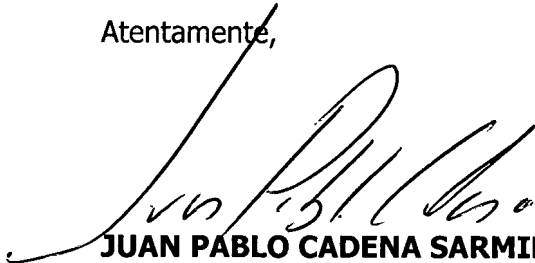
En vista de lo anteriormente expuesto, respetuosamente solicitamos el retiro de las objeciones de la presente solicitud y la concesión del privilegio solicitado, ya que en opinión del solicitante cumple con todos los requisitos establecidos en la decisión 486.

Anexos

- Nuevo pliego reivindicatorio.
- Artículo 1: Discovery of a Potent, Selective, and Orally Available Class I Phosphatidylinositol 3-Kinase (PI3K)/Mammalian Target of Rapamycin (mTOR) Kinase Inhibitor (GDC-0980) for the Treatment of Cancer. Daniel P. Sutherlin, et al.
- Presentación del inventor Daniel Sutherlin en el Annual Meeting Education Session
- Artículo 2: GDC-0980 is a Novel Class I PI3K/mTOR Kinase Inhibitor with Robust Activity in Cancer Models Driven by the PI3K Pathway. Jeffrey J. Wallin, et al.

Del Señor Director

Atentamente,



JUAN PABLO CADENA SARMIENTO

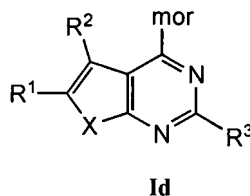
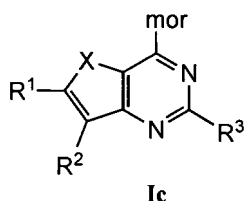
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C.C. 80.410.337 de Usaquén

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REIVINDICACIONES

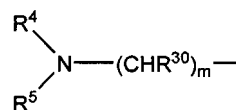
1. Un compuesto CARACTERIZADO porque se selecciona entre la Fórmula Ic y la Fórmula Id:



y estereoisómeros, isómeros geométricos, tautómeros, y sales farmacéuticamente aceptables de los mismos, donde:

X es O ó S;

R¹ es un grupo de fórmula:



R² es seleccionado entre H, F, Cl, Br, I, C₆-C₂₀arilo, C₁-C₂₀heteroarilo, C₁-C₆alquilo, C₂-C₈alqueno y C₂-C₈alquino;

R³ es un grupo heteroarilo monocíclico seleccionado entre piridilo, isoxazolilo, imidazolilo, pirrolilo, tiazolilo, piridazinilo, pirimidinilo, pirazinilo, oxazolilo, furanilo, tienilo, triazolilo, tetrazolilo, donde el grupo heteroarilo monocíclico está opcionalmente sustituido con uno o más grupos seleccionados entre F, Cl, Br, I, -CN, -NR¹⁰R¹¹, -OR¹⁰, -C(O)R¹⁰, -NR¹⁰C(O)R¹¹, -N(C(O)R¹¹)₂, -NR¹²SO₂R¹⁰, -NO₂, -SR¹⁰, -C(=O)OR¹⁰, -C(=O)NR¹⁰R¹¹, C₆-C₂₀aril, C₁-C₁₂alquilo y (C₁-C₁₂alquil)-OR¹⁰;

R⁴ y R⁵ forman, junto con el átomo de N al cual están unidos, un grupo seleccionado entre piperazina, piperidina, pirrolidina, oxazolidinona, morfina, tiomorfina, diazepano y 2,5-diazabicyclo[2.2.1]heptano, cuyo grupo está opcionalmente sustituido con uno o más grupos independientemente seleccionados entre F, Cl, Br, I, -CN, CF₃, -NO₂, oxo, -C(=Y)R¹⁰, -C(=Y)OR¹⁰, -C(=Y)NR¹⁰R¹¹, -(CR¹⁴R¹⁵)_nNR¹⁰R¹¹, -(CR¹⁴R¹⁵)_nNR¹²SO₂R¹⁰, -(CR¹⁴R¹⁵)_nOR¹⁰, -NR¹⁰R¹¹, -NR¹²C(=Y)R¹⁰, -NR¹²C(=Y)OR¹¹, -NR¹²C(=Y)NR¹⁰R¹¹, -NR¹²SO₂R¹⁰, =NR¹², OR¹⁰, -OC(=Y)R¹⁰, -OC(=Y)OR¹⁰, -OC(=Y)NR¹⁰R¹¹, -OS(O)₂(OR¹⁰), -OP(=Y)(OR¹⁰)(OR¹¹), -OP(OR¹⁰)(OR¹¹), SR¹⁰, -S(O)R¹⁰, -S(O)₂-(C₁-C₆alquil)-S(O)₂R¹⁰, -S(O)₂R¹⁰, -S(O)₂NR¹⁰R¹¹, -S(O)(OR¹⁰), -S(O)₂(OR¹⁰), -SC(=Y)R¹⁰, -SC(=Y)OR¹⁰, -SC(=Y)NR¹⁰R¹¹, C₁-C₁₂alquilo, C₂-C₈alqueno, C₂-C₈alquino, C₃-C₁₂carbociclilo, C₂-C₂₀heterociclilo, C₆-C₂₀arilo y C₁-C₂₀heteroarilo;

o uno de R^4 y R^5 es C_1 - C_6 alquilo, $-(C_1-C_6\text{alquil})_q-(C_2-C_{20})$ -heterociclilo o $-(C_1-C_6\text{alquil})_q-OR^{10}$ y el otro es un grupo piperazina, piperidina, pirrolidina, sulfonilpirano, $-(C_1-C_6\text{alquil})-(C_2-C_{20})$ heterociclilo o $-(C_1-C_6\text{alquil})-(C_1-C_{20})$ heteroarilo, donde dicho grupo piperazina, piperidina, pirrolidina, sulfonilpirano, heterociclilo o heteroarilo es no sustituido o sustituido por C_1 - C_6 alquilo, $-(C_1-C_6\text{alquil})_q-OR^{10}$ ó $-S(O)_2R^{10}$;

R^{10} , R^{11} y R^{12} son independientemente H, C_1 - C_{12} alquilo, C_2 - C_8 alquenilo, C_2 - C_8 alquinilo, C_3 - C_{12} carbociclilo, C_2 - C_{20} -heterociclilo, C_6 - C_{20} arilo o C_1 - C_{20} heteroarilo;

o R^{10} y R^{11} , junto con el nitrógeno al cual están unidos, opcionalmente forman un anillo heterocíclico C_3 - C_{20} que opcionalmente contiene uno o más átomos del anillo adicionales seleccionados entre N, O ó S, donde dicho anillo heterocíclico está opcionalmente sustituido con uno o más grupos independientemente seleccionados entre oxo, $(CH_2)_nOR^{10}$, $NR^{10}R^{11}$, CF_3 , F, Cl, Br, I, SO_2R^{10} , $C(=O)R^{10}$, $NR^{12}C(=Y)R^{11}$, $C(=Y)NR^{10}R^{11}$, C_1 - C_{12} alquilo, C_2 - C_8 alquenilo, C_2 - C_8 alquinilo, C_3 - C_{12} carbociclilo, C_2 - C_{20} heterociclilo, C_6 - C_{20} arilo y C_1 - C_{20} heteroarilo;

R^{14} y R^{15} son independientemente seleccionados entre H, C_1 - C_{12} alquilo o $-(CH_2)_n$ -arilo;

o R^{14} y R^{15} , junto con los átomos con los cuales están unidos, forman un anillo carbocíclico C_3 - C_{12} saturado o parcialmente insaturado;

R^{30} es H o C_1 - C_6 alquilo;

mor es un grupo morfolina opcionalmente sustituido con uno o más grupos seleccionados entre F, Cl, Br, I, $-(C_1-C_6\text{alquil})_2NR^{10}R^{11}$, $-(CR^{14}R^{15})_nNR^{10}R^{11}$, $C(R^{14}R^{15})_nNR^{12}C(=Y)R^{10}$, $-(CR^{14}R^{15})_nNR^{12}S(O)_2R^{10}$, $-CH(OR^{10})R^{10}$, $-(CR^{14}R^{15})_nOR^{10}$, $-(CR^{14}R^{15})_nS(O)_2R^{10}$, $-(CR^{14}R^{15})_nS(O)_2NR^{10}R^{11}$, $-C(=Y)R^{10}$, $-C(=Y)OR^{10}$, $-C(=Y)NR^{10}R^{11}$, $-C(=Y)NR^{12}OR^{10}$, $-C(=O)NR^{12}S(O)_2R^{10}$, $-C(=O)NR^{12}(CR^{14}R^{15})_mNR^{10}R^{11}$, $-NO_2$, $-NR^{10}R^{11}$, $-NR^{12}C(=Y)R^{11}$, $-NR^{12}C(=Y)OR^{11}$, $-NR^{12}C(=Y)NR^{10}R^{11}$, $-NR^{12}S(O)_2R^{10}$, $-NR^{12}SO_2NR^{10}R^{11}$, $-S(O)_2R^{10}$, $-S(O)_2NR^{10}R^{11}$, $-SC(=Y)R^{10}$, $-SC(=Y)OR^{10}$, C_1 - C_{12} alquilo, C_2 - C_8 alquenilo, C_2 - C_8 alquinilo, C_3 - C_{12} carbociclilo, C_2 - C_{20} heterociclilo, C_6 - C_{20} arilo o C_1 - C_{20} heteroarilo;

donde dichos alquilo, alquenilo, alquinilo, carbociclilo, heterociclilo, arilo y heteroarilo están opcionalmente sustituidos con uno o más grupos independientemente seleccionados entre F, Cl, Br, I, $-CN$, CF_3 , $-NO_2$, oxo, $-C(=Y)R^{10}$, $-C(=Y)OR^{10}$, $-C(=Y)NR^{10}R^{11}$, $-(CR^{14}R^{15})_nNR^{10}R^{11}$, $-(CR^{14}R^{15})_nNR^{12}SO_2R^{10}$, $-(CR^{14}R^{15})_nOR^{10}$, $-NR^{10}R^{11}$, $-NR^{12}C(=Y)R^{10}$, $-NR^{12}C(=Y)OR^{11}$, $-NR^{12}C(=Y)NR^{10}R^{11}$, $-NR^{12}SO_2R^{10}$, $=NR^{12}$, OR^{10} , $OC(=Y)R^{10}$, $OC(=Y)OR^{10}$, $OC(=Y)NR^{10}R^{11}$, $-OS(O)_2(OR^{10})$, $-OP(=Y)(OR^{10})(OR^{11})$, $OP(OR^{10})(OR^{11})$, SR^{10} , $-S(O)R^{10}$, $-S(O)_2R^{10}$, $-S(O)_2NR^{10}R^{11}$, $-S(O)(OR^{10})$, $-S(O)_2(OR^{10})$, $-SC(=Y)R^{10}$, $-SC(=Y)OR^{10}$, $-SC(=Y)NR^{10}R^{11}$, C_1 - C_{12} alquilo opcionalmente sustituido, C_2 - C_8 alquenilo opcionalmente sustituido, C_2 - C_8 alquinilo opcionalmente sustituido, C_3 -

C₁₂carbociclilo opcionalmente sustituido, C₂-C₂₀heterociclilo opcionalmente sustituido, C₆-C₂₀arilo opcionalmente sustituido y C₁-C₂₀-heteroarilo opcionalmente sustituido;

Y es O, S ó NR¹²;

cada q es independientemente 0 ó 1;

m es 0 ó 1; y

n es 1, 2, 3, 4, 5 ó 6;

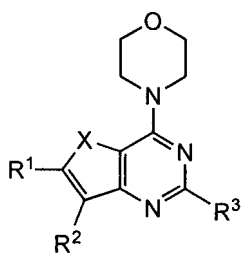
con las salvedades de que:

(i) cuando en la Fórmula (Ic) mor es morfolino no sustituido, X es S, R¹ es un grupo (4-metilsulfonilpiperazin-1-il)metilo y R² es H, entonces R³ no es un grupo seleccionado entre imidazolilo que es no sustituido o sustituido con un grupo CH₃, pirimidinilo que es no sustituido y piridinilo que es no sustituido o sustituido con un grupo F;

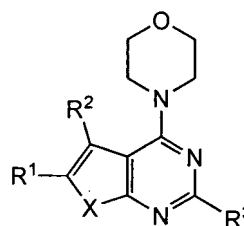
(ii) cuando en la Fórmula (Ic) mor es morfolino no sustituido, X es S, R¹ es un grupo (4-metilpiperazin-1-il)metilo y R² es H, entonces R³ no es piridinilo que está sustituido por un grupo OH; y

(iii) cuando en la Fórmula (Id) mor es morfolino no sustituido, X es S, R¹ es un grupo (4-metilsulfonilpiperazin-1-il)metilo y R² es H, entonces R³ no es un grupo seleccionado entre piridinilo que es no sustituido y pirimidinilo que es no sustituido o sustituido por un grupo -OCH₃ ó -N(CH₃)₂.

2. El compuesto de la reivindicación 1, CARACTERIZADO porque se selecciona entre la Fórmula Ia y la Fórmula Ib:

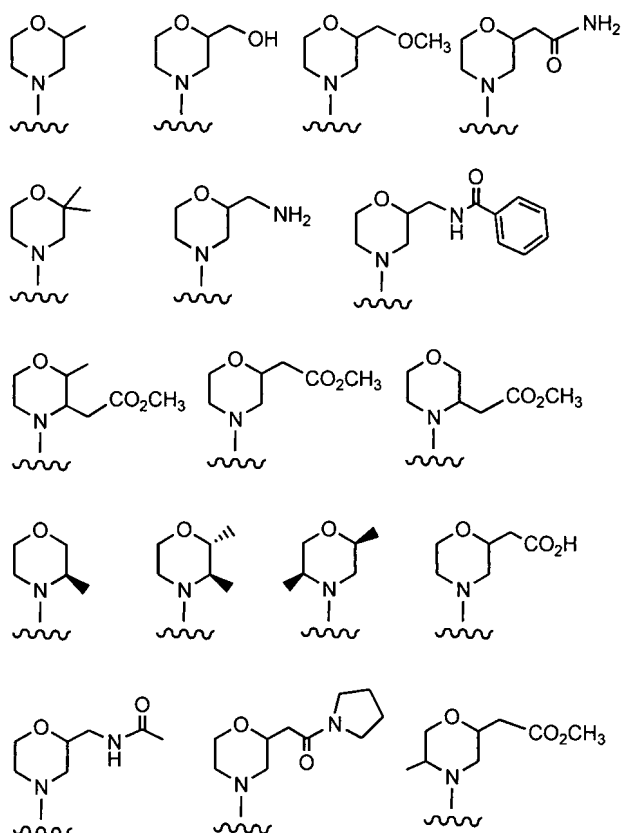


Ia



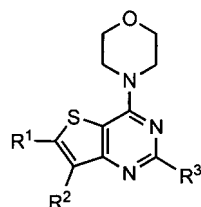
Ib

3. El compuesto de la reivindicación 1, CARACTERIZADO porque mor es seleccionado entre las estructuras:

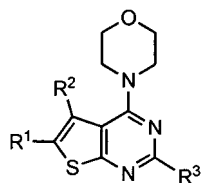


donde la línea ondulada indica la unión a la posición 4 del anillo pirimidina.

4. El compuesto de la reivindicación 2, CARACTERIZADO porque tiene la Fórmula Ia, donde X es S y tiene la fórmula:



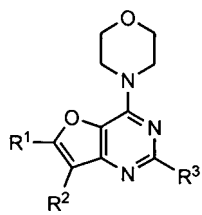
5. El compuesto de la reivindicación 2, CARACTERIZADO porque tiene la Fórmula Ib, donde X es S y tiene la fórmula:



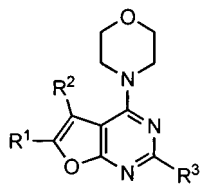
6. El compuesto de la reivindicación 2, CARACTERIZADO porque tiene la Fórmula Ia,

12

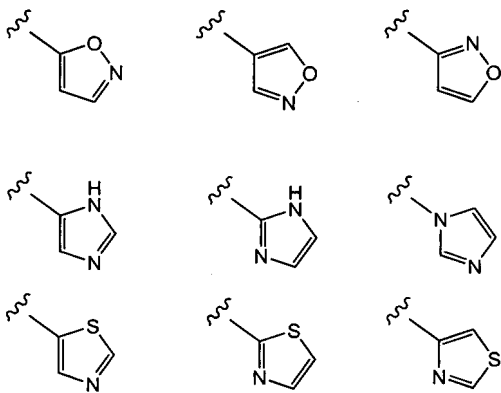
donde X es O y tiene la fórmula:



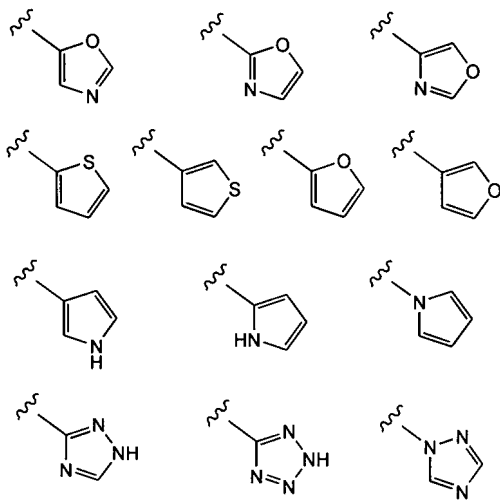
7. El compuesto de la reivindicación 2, CARACTERIZADO porque tiene la Fórmula Ib, donde X es O y tiene la fórmula:



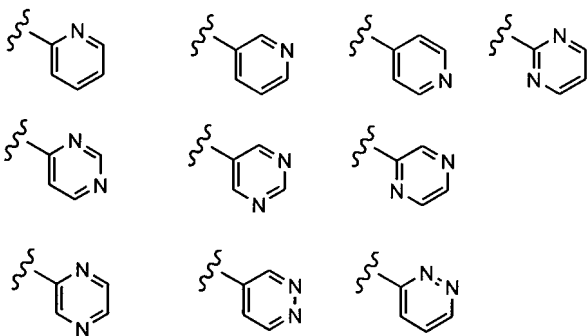
8. El compuesto de la reivindicación 1, CARACTERIZADO porque R² es seleccionado entre H, F, Cl, Br, I y C₁-C₆ alquilo.
9. El compuesto de la reivindicación 1, CARACTERIZADO porque R³ es seleccionado entre las estructuras:



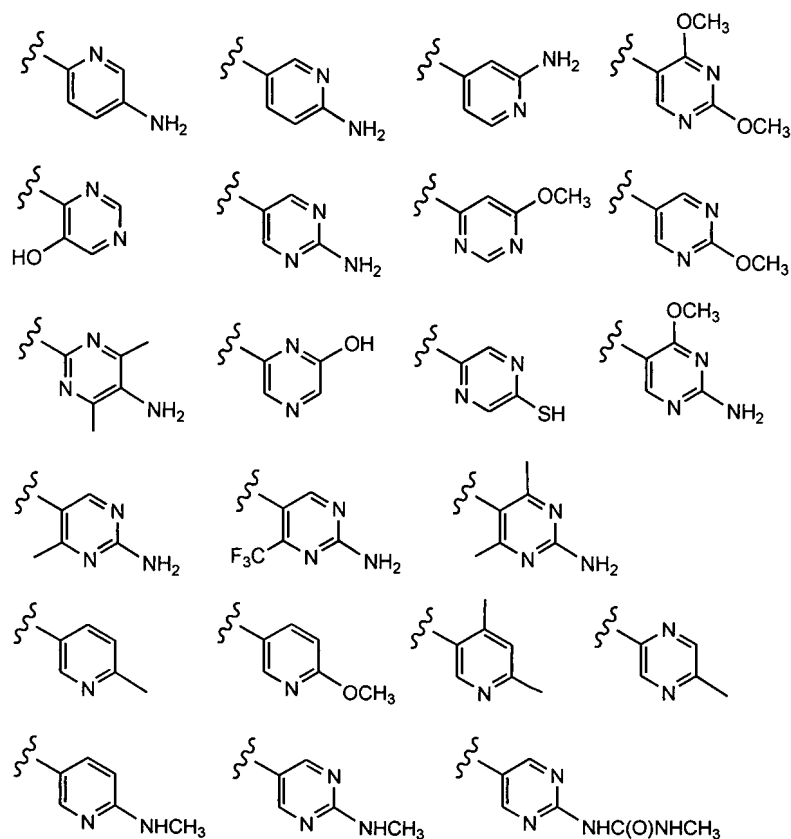
13



10. El compuesto de la reivindicación 1, CARACTERIZADO porque R³ es seleccionado entre las estructuras:



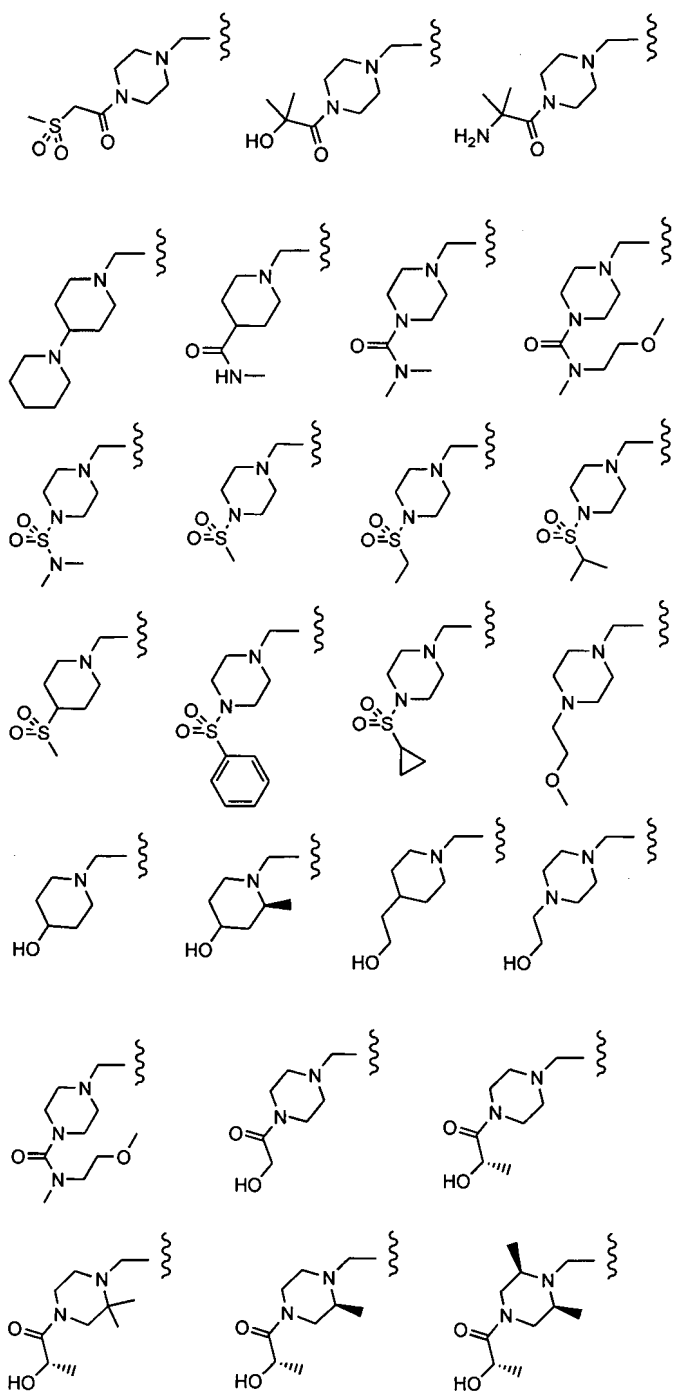
11. El compuesto de la reivindicación 1, CARACTERIZADO porque R³ es seleccionado entre las estructuras:

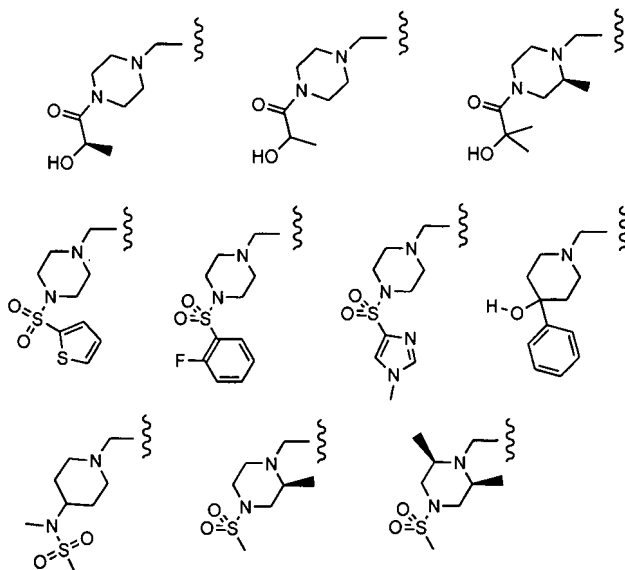


12. El compuesto de la reivindicación 1, CARACTERIZADO porque el grupo heteroarilo monocíclico esta sustituido con uno o más grupos seleccionados entre F, -NH₂, -NHCH₃, -N(CH₃)₂, -OH, -OCH₃, -C(O)CH₃, -NHC(O)CH₃, -N(C(O)CH₃)₂, -NHC(O)NH₂, -CO₂H, -CHO, -CH₂OH, -C(=O)NHCH₃, -C(=O)NH₂ y -CH₃.

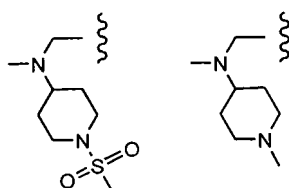
13. El compuesto de la reivindicación 1, CARACTERIZADO porque R⁴ y R⁵ forman, junto con el átomo de N al cual están unidos, un grupo seleccionado entre piperazina, piperidina, pirrolidina, oxazolidinona, diazepano y 2,5-diazabicyclo[2.2.1]-heptano, cuyo grupo es no sustituido o sustituido por -[(C₁-C₆alquil)-NR]_q-S(O)₂-(C₁-C₆alquil)_q-Z ó -C(O)-(C₁-C₆alquil)_q-S(O)₂Z donde Z es R¹⁰ ó -NR¹¹R¹², o por C₁-C₆alquilo no sustituido, hidroxil-C₁-C₆alquilo, oxo (=O), -(C₁-C₆alquil)_q-OR, -C(O)-CR₂-NR₂, -CR₂-C(O)-NR₂, -C(O)-(NR)_q-(C₁-C₆alquil)_q-OR, -C(O)-ciclilo, -C(O)R, -C(O)OR, -C(O)-Tet ó -NR¹³R¹⁴.

14. El compuesto de la reivindicación 1, CARACTERIZADO porque R¹ es seleccionado entre los grupos:





15. El compuesto de la reivindicación 1, CARACTERIZADO porque R^1 es seleccionado entre los grupos:



16. El compuesto de la reivindicación 1, CARACTERIZADO porque es seleccionado entre:

1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-(metilsulfonil)etanona;

1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxi-2-metilpropan-1-ona;

2-amino-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-metilpropan-1-ona;

(S)-2-amino-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno-[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-(dimetilamino)etanona;

2-amino-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)etanona;

5-(4-morfolino-6-((4-(piperidin-1-il)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-

il)piridin-2-amina;

1-((2-(2,4-dimetoxipirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metilpiperidina-4-carboxamida;

4-((2-(2,4-dimetoxipirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperazina-1-carboxamida;

N-(2-metoxietil)-4-((2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)-N-metilpiperazina-1-carboxamida;

5-(4-morfolino-6-((4-N-dimetilaminosulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(4-morfolino-6-((4-N-dimetilaminosulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-N-dimetilamino-sulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidina;

5-(4-morfolino-6-((4-(piperidin-1-il)piperidin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)pirimidin-2-amina;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metilpiperidina-4-carboxamida;

5-(4-morfolino-6-((4-N-isopropilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

2-(2,4-dimetoxipirimidin-5-il)-7-metil-6-((4-(metilsulfonil)-piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidina;

5-(7-metil-6-((4-(metilsulfonil)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(7-metil-6-((4-(metilsulfonil)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-fenilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(4-morfolino-6-((4-N-ciclopropilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-ciclopropilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

1-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metilpiperidina-4-carboxamida;

1-((2-(2,4-dimetoxipirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperidina-4-carboxamida;

4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperazina-1-carboxamida;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperidina-4-carboxamida;

4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)-N,N-dimetilpiperazina-1-carboxamida;

5-(6-((4-(metilsulfonyl)piperidin-1-il)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)piperidin-2-amina;

5-(6-((4-(metilsulfonyl)piperidin-1-il)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)piperidin-2-amina;

2-(2,4-dimetoxipirimidin-5-il)-6-((4-(metilsulfonyl)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidina;

2-(6-metilpiridin-3-il)-4-morfolino-6-((4-(piperidin-1-il)-piperidin-1-il)metil)tieno[3,2-d]pirimidina;

1-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperidina-4-carboxamida;

2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-(piperidin-1-il)piperidin-1-il)metil)tieno[3,2-d]pirimidina;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno-[2,3-d]pirimidin-2-il)tiazol-2-amina;

5-(6-((4-(1-metilpiperidin-4-il)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piperidin-2-amina;

5-(6-((4-(2-metoxietil)piperazin-1-il)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)piperidin-2-amina;

5-(4-morfolino-6-((4-(pirimidin-2-il)piperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)piperidin-2-amina;

6-((4-metilpiperazin-1-il)metil)-4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidina;

4-morfolino-2-(piridin-3-il)-6-((4-(pirrolidin-1-il)piperidin-1-il)metil)tieno[3,2-d]pirimidina;

6-((3-(metilsulfonyl)pirrolidin-1-il)metil)-4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidina;

1-((4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperidin-4-ol;

2-(1-((4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperidin-4-il)etanol;

5-(6-((4-metilpiperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piperidin-2-amina;

5-(4-morfolino-6-((4-(pirrolidin-1-il)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(6-((3-(metilsulfonil)pirrolidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperidin-4-ol;
 (R)-1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperidin-3-ol;
 2-(1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperidin-4-il)etanol;
 (R)-1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)pirrolidin-3-ol;
 2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-il)metil)tieno[2,3-d]pirimidina;
 4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-2-(tiazol-5-il)tieno[3,2-d]pirimidina;
 5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)tiazol-2-amina;
 N-(2-metoxietil)-N-metil-4-((2-(6-metilpiridin-3-il)-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazina-1-carboxamida;
 4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-(2-metoxietil)-N-metilpiperazina-1-carboxamida;
 4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-(2-metoxietil)-N-metilpiperazina-1-carboxamida;
 2-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)etanol;
 2-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)etanol;
 5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-amina;
 5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;
 2-(4-metoxipiridin-3-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-il)metil)tieno[3,2-d]pirimidina;
 2-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-N,N-dimetilacetamida;

6-((4-metilpiperazin-1-il)metil)-4-morfolino-2-(pirimidin-5-il)tieno[3,2-d]pirimidina;
 5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-furo[2,3-d]pirimidin-2-il)pirimidin-2-amina;
 4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperazina-1-carboxamida;
 2-(6-metilpiridin-3-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-il)metil)tieno[2,3-d]pirimidina;
 N,1-dimetil-N-((4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidin-6-il)metil)piperidin-4-amina;
 6-((4-metilpiperazin-1-il)metil)-4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidina;
 5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridina-3-carbonitrilo;
 2-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-N,2-dimetilpropanamida;
 2-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-N,N-dimetilacetamida;
 2-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-N,2-dimetilpropanamida;
 N,N-dimetil-2-(4-((2-(6-metilpiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)acetamida;
 4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N,N-dimetilpiperazina-1-carboxamida;
 2-(2-isopropil-1H-imidazol-1-il)-4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidina;
 N,2-dimetil-2-(4-((2-(6-metilpiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propanamida;
 1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxietanona;
 1-(4-((2-(6-aminopiridin-3-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxietanona;
 1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxietanona;
 1-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxietanona;
 (S)-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

(S)-1-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

N-(5-(6-((4-(2-hidroxiacetil)piperazin-1-il)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)piridin-2-il)acetamida;

1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiestanona;

1-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiestanona;

5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirazin-2-amina;

2-(5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-ilamino)etanol;

5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)piridin-2-ol;

5-(6-((1-metilpiperidin-4-il-N-metilamino)metil)-7-metil-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(7-metil-4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

7-metil-4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-2-(pirimidin-5-il)tieno[3,2-d]pirimidina;

(S)-1-((S)-4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-3-metilpiperazin-1-il)-2-hidroxiopropan-1-ona;

2-(6-metilpiridin-3-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-il)metil)tieno[3,2-d]pirimidina;

N-metil-5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

N-metil-N-(5-(4-morfolino-6-((piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-il)acetamida;

5-(6-((1-metilpiperidin-4-ilamino)-N-metilaminometil)-7-metil-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;

N,1-dimetil-N-((7-metil-4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperidin-4-amina;

N-(5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-furo[3,2-d]pirimidin-2-il)piridin-2-il)formamida;

N-(5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-il)formamida;

(S)-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno-[3,2-d]pirimidin-6-

il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;
 (S)-1-(4-((2-(6-aminopiridin-3-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;
 (S)-2-hidroxi-1-(4-((2-(2-metoxipirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((2-(6-metoxipiridin-3-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((7-metil-4-morfolino-2-(pirimidin-5-il)-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((7-metil-4-morfolino-2-(piridin-3-il)tieno-[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;
 (S)-1-(4-((2-(6-aminopiridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;
 (S)-2-hidroxi-1-(4-((2-(2-metoxipirimidin-5-il)-4-morfolinotieno-[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((2-(6-metoxipiridin-3-il)-4-morfolinotieno-[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((4-morfolino-2-(pirimidin-5-il)tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 (S)-2-hidroxi-1-(4-((4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;
 N,1-dimetil-N-((4-morfolino-2-(piridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperidin-4-amina;
 N,1-dimetil-N-((4-morfolino-2-(6-aminopiridin-3-il)tieno[3,2-d]pirimidin-6-il)metil)piperidin-4-amina;
 N-(5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-il)acetamida;
 5-(6-((4-N-metilsulfonilpiperazin-1-il)metil)-7-metil-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;
 7-metil-4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-2-(piridin-3-il)tieno[3,2-d]pirimidina;
 N-((2-(2-aminopiridin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metil(N-1-metilsulfonil)piperidin-4-amina;
 N-((2-(2,4-dimetoxipirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-

metil(N-1-metilsulfonyl)piperidin-4-amina;

N-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metil(N-1-metilsulfonyl)piperidin-4-amina;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)furo-[3,2-d]pirimidin-2-il)pirimidin-2-amina;

N-(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-furo[3,2-d]pirimidin-2-il)piridin-2-il)acetamida;

4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-2-(piridin-3-il)furo[3,2-d]pirimidina;

2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno[3,2-d]pirimidina;

(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)furan-2-il)metanol;

2-(6-metoxipiridin-3-il)-4-morfolino-6-((piperazin-1-il)metil)-tieno[3,2-d]pirimidina;

4-morfolino-6-((piperazin-1-il)metil)-2-(4-N-metilpiridin-4-il)tieno[3,2-d]pirimidina;

(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)tiofen-2-il)metanol;

2-(5-metoxipiridin-3-il)-4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno[2,3-d]pirimidina;

2-(furan-3-il)-4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno[3,2-d]pirimidina;

(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-tieno[2,3-d]pirimidin-2-il)piridin-3-il)metanol;

N-(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridin-2-il)acetamida;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-tieno[2,3-d]pirimidin-2-il)piridin-2-amina;

(5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridin-3-il)metanol;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)-furo[3,2-d]pirimidin-2-il)piridin-2-amina;

2-(2-metoxipirimidin-5-il)-4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)furo[3,2-d]pirimidina;

5-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)furo-[3,2-d]pirimidin-2-

il)piridina-3-carbaldehído;

2-(5-metoxipiridin-3-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-

il)metil)furo[3,2-d]pirimidina;

5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-

il)piridin-2-amina;

5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-

il)piridina-3-carbaldehído;

2-(5-metoxipiridin-3-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-

il)metil)tieno[3,2-d]pirimidina;

N,N-dimetil-5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-

d]pirimidin-2-il)pirimidin-2-amina;

4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-2-(pirimidin-5-il)furo[3,2-

d]pirimidina;

2-(2-metoxipirimidin-5-il)-4-morfolino-6-((4-N-metilsulfonil-piperazin-1-

il)metil)tieno[3,2-d]pirimidina;

1-(5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)furo-[3,2-d]pirimidin-2-

il)piridin-2-il)urea;

5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-

il)piridin-2-metilsulfonilamina;

6-metil-5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-

d]pirimidin-2-il)piridin-2-amina;

5-(4-morfolino-6-((4-N-isobutilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-

il)piridin-2-amina;

2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-N-fenilsulfonil-piperazin-1-

il)metil)tieno[3,2-d]pirimidina;

2-(2,4-dimetoxipirimidin-5-il)-4-morfolino-6-((4-N-ciclopropil-sulfonilpiperazin-1-

il)metil)tieno[3,2-d]pirimidina;

5-(6-(((S)-3-metil-(4-N-metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-

d]pirimidin-2-il)piridin-2-amina;

5-(6-(((S)-3-metil-(4-N-metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-

d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((2R,6S)-2,6-dimetil-(4-N-metilsulfonil)piperazin-1-il)metil)-4-

morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(6-(((2R,6S)-2,6-dimetil-(4-N-metilsulfonil)piperazin-1-il)-metil)-4-

morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(4-morfolino-6-((1-*O,S*-tiomorfolin-4-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-amina;
 N,N-dimetil-1-((4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidin-6-il)metil)piperidina-4-carboxamida;
 N,N-dimetil-4-((4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidin-6-il)metil)piperazina-1-carboxamida;
 N-metil-1-((4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidin-6-il)metil)piperidina-4-carboxamida;
 N-(2-metoxietil)-N-metil-1-((4-morfolino-2-(pirimidin-5-il)tieno[2,3-d]pirimidin-6-il)metil)piperidin-4-amina;
 5-(7-metil-4-morfolino-6-((4-N-ciclopropilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(7-metil-4-morfolino-6-((4-N-fenilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(7-metil-4-morfolino-6-((4-N-isopropilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 N,1-dimetil-N-((4-morfolino-2-(pirimidin-5-il)tieno[3,2-d]-2-aminopirimidin-6-il)metil)piperidin-4-amina;
 5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridina-2-carboxamida;
 5-(4-morfolino-6-((1-*O,S*-tiomorfolin-4-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(6-(((R)-3-metil-(4-N-metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;
 5-(6-(((R)-3-metil-(4-N-metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 4-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)piridina-2,6-diamina;
 5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)piridina-2-carbonitrilo;
 5-(4-morfolino-6-((4-N-(tiofen-2-il)sulfonil)piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(4-morfolino-6-((4-N-2-fluorofenilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
 5-(6-((4-(metilsulfonil)piperidin-1-il)metil)-4-morfolino-tieno[2,3-d]pirimidin-2-

il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-3-fluorofenilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-(1-metilimidazol-4-il)piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-4-fluorofenilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-(dimetilaminosulfonil)piperidin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-(dimetilaminosulfonil)piperidin-1-il)metil)tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-fenilpiperidin-4-ol;

5-(6-((4-(N-(2-metoxietil)-N-metilamino)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-etilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(N-metil,N-metilsulfonilamino)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;

4-metoxi-5-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(N-metil,N-metilsulfonilamino)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-isobutirilsulfonilpiperazin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

6-(4-morfolino-6-((4-N-metilsulfonilpiperazin-1-il)metil)tieno-[2,3-d]pirimidin-2-il)piridin-2-amina;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)pirrolidin-3-ol;

5-(4-morfolino-6-(1-(4-N-metilsulfonilpiperazin-1-il)etil)-tieno[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(4-morfolino-6-(1-(4-N-metilsulfonilpiperazin-1-il)etil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

(R)-1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperidin-3-ol;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperidin-4-

ol;

5-(6-((3-(metilsulfonyl)pirrolidin-1-il)metil)-4-morfolino-tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

2-(2-metilpirimidin-5-il)-morfolino-6-(4-N-metilsulfonyl-(piperazin-1-il)metil)tieno[2,3-d]pirimidina;

2-(2-metilaminopirimidin-5-il)-morfolino-6-(4-N-metilsulfonyl-(piperazin-1-il)metil)tieno[2,3-d]pirimidina;

2-(2-metilpirimidin-5-il)-4-morfolino-6-(4-N-metilsulfonyl-(piperazin-1-il)metil)tieno[3,2-d]pirimidina;

5-(4-morfolino-6-(4-N-(tiofen-2-il)sulfonyl(piperazin-1-il)metil)tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-N-ciclopropilsulfonylpiperazin-1-il)metil)-tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

2-(1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperidin-4-ilsulfonyl)-N,N-dimetil-acetamida;

5-(4-morfolino-6-((4-(tiazol-2-ilsulfonyl)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

4-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)piridina-2,6-diamina;

5-(6-((4-((metilsulfonyl)metilsulfonyl)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

2-(2-metilaminopirimidin-5-il)-morfolino-6-(4-N-metilsulfonyl-(piperazin-1-il)metil)tieno[3,2-d]pirimidina;

4-(4-morfolino-6-((4-N-metilsulfonylpiperazin-1-il)metil)tieno-[3,2-d]pirimidin-2-il)piridin-2-amina;

N-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-N-metil(N-1-isopropil)piperidin-4-amina;

5-(7-metil-6-(((2R,6S)-2,6-dimetil-(4-N-metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-(4-clorofenil)piperidin-4-ol; y

2-(2-(metiltio)pirimidin-5-il)-4-morfolino-6-((4-N-metilsulfonyl-piperazin-1-il)metil)tieno[3,2-d]pirimidina.

17. El compuesto de la reivindicación 1, CARACTERIZADO porque es seleccionado entre:

5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[2,3-d]pirimidin-2-il)-4-(trifluorometil)pirimidin-2-amina;

5-(6-((metil(piperidin-4-ilmetil)amino)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(1-metilpiperidin-4-il)amino)metil)-4-morfolinotieno-[2,3-d]pirimidin-2-il)-4-(trifluorometil)pirimidin-2-amina;

4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazina-1-carboxilato de ter-butilo;

5-(7-metil-4-morfolino-6-(piperazin-1-ilmetil)tieno[3,2-d]pirimidin-2-il)-4-(trifluorometil)pirimidin-2-amina;

(S)-1-(4-((2-(2-amino-4-metilpirimidin-5-il)-4-morfolinotieno-[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;

(S)-1-(4-((2-(2-amino-4-(trifluorometil)pirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona;

1-(4-((2-(2-amino-4-metilpirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxi-2-metilpropan-1-ona;

5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)-4-(trifluorometil)pirimidin-2-amina;

5-(6-((4-((2-metil-1H-imidazol-1-il)metil)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-(morfolinometil)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-(piperidin-1-ilmetil)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-(pirrolidin-1-ilmetil)piperidin-1-il)metil)-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-((dimetilamino)metil)piperidin-1-il)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((1-metilpiperidin-4-ilamino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-isopropilpiperidin-4-il)(2-metoxietil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil((1-metilpiperidin-4-il)metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(4-morfolino-6-((4-morfolinopiperidin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(dimetilamino)piperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(1-metilpirrolidin-3-il)amino)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-ciclopropilpiperidin-4-il)(metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-aminopiperidin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((3-(dimetilamino)pirrolidin-1-il)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(piperidin-4-il)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-(2-metoxietil)piperidin-4-il)(metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(1-propilpiperidin-4-il)amino)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-ciclohexilpiperidin-4-il)(metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-isobutilpiperidin-4-il)(metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((1-etilpiperidin-4-il)(metil)amino)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

4-metil-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

(S)-1-(4-((2-(2-amino-4-metilpirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-(metilsulfonil)etanona;

(1-aminociclopropil)(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-metanona;

2-amino-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-metilpropan-1-ona;

(R)-2-amino-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

(S)-2-amino-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-(dimetilamino)etanona;

2-amino-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)etanona;

1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxi-2-metilpropan-1-ona;

5-(7-metil-4-morfolino-6-(piperazin-1-ilmetil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[2,3-d]pirimidin-2-il)piridin-3-amina;

5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[2,3-d]pirimidin-2-il)-3-nitropiridin-2-amina;

N,3-dimetil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

(5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[2,3-d]pirimidin-2-il)piridin-2-il)metanol;

4-(2-(4-metilpiridin-3-il)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[2,3-d]pirimidin-4-il)morfolina;

4-(2-(5-metilpiridin-3-il)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[2,3-d]pirimidin-4-il)morfolina;

N-etil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

5-(6-((4-((2-metoxietil)(metil)amino)piperidin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

5-(6-((4-((2-metoxietil)(metil)amino)piperidin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)-N-metilpiridin-2-amina;

5-(6-((4-((2-metoxietil)(metil)amino)piperidin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)-N-metilpirimidin-2-amina;

N-metil-5-(6-((metil(1-metilpiperidin-4-il)amino)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

4-metil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

4-(2-(5,6-dimetilpiridin-3-il)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[2,3-d]pirimidin-4-il)morfolina;

3,4-dimetil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

(S)-2-hidroxi-1-(4-((2-(6-(metilamino)piridin-3-il)-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

(S)-2-hidroxi-1-(4-((2-(6-(metilamino)piridin-3-il)-4-morfolino-tieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

(S)-2-hidroxi-1-(4-((7-metil-2-(6-(metilamino)piridin-3-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

3-cloro-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

3-cloro-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)piridin-2-amina;

3-metil-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

4-metil-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

1-((2-(2-(metilamino)pirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)pirrolidin-3-ol;

1-((2-(2-(metilamino)pirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperidin-4-ol;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-metilpiperidin-4-ol;

N-(5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolino-tieno[2,3-d]pirimidin-2-il)piridin-2-il)acetamida;

N-metil-5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)piridin-2-amina;

2-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)etanol;

N,N-dimetil-2-(5-(6-((4-(metilsulfonil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-il)etanamina;

4-(6-((4-(metilsulfonil)piperazin-1-il)metil)-2-(6-fenilpiridin-3-il)tieno[2,3-d]pirimidin-4-il)morfolina;

(S)-2-hidroxi-1-(4-((2-(2-(metilamino)pirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

(S)-2-hidroxi-1-(4-((7-metil-2-(2-(metilamino)pirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)propan-1-ona;

5-(6-(1,4'-bipiperidin-1'-ilmetil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

N-isopropil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

N-etil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(1-metilpiperidin-4-il)amino)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

N-isopropil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

N-etil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(bencil(metil)amino)piperidin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-((2-metoxietil)(metil)amino)piperidin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

(R)-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[2,3-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

(R)-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

(R)-1-(4-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

N-(5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)tiazol-2-il)acetamida;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-(piperidin-2-il)piperidin-4-ol;

4-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-2-(5-fenilpiperidin-3-il)tieno[2,3-d]pirimidin-4-il)morfolina;

(S)-5-(6-((4-(2-hidroxiopropanoil)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)picolinonitrilo;

(S)-5-(6-((4-(2-hidroxiopropanoil)piperazin-1-il)metil)-7-metil-4-morfolinotieno[3,2-d]pirimidin-2-il)picolinonitrilo;

(S)-1-(4-((2-(2,4-dimetoxipirimidin-5-il)-7-metil-4-morfolino-tieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

(S)-1-(4-((2-(2-(dimetilamino)pirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxiopropan-1-ona;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-(tiazol-2-il)piperidin-4-ol;

4-(2-(2-metilpirimidin-5-il)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[2,3-d]pirimidin-4-il)morfolina;

N-metil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

4-(2-(2-metilpirimidin-5-il)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[3,2-d]pirimidin-4-il)morfolina;

5-(4-morfolino-6-((4-(tiofen-2-ilsulfonyl)piperazin-1-il)metil)-tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(ciclopropilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

2-(1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]-pirimidin-6-il)metil)piperidin-4-ilsulfonyl)-N,N-dimetil-acetamida;

5-(4-morfolino-6-((4-(tiazol-2-ilsulfonyl)piperidin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((4-(metilsulfonylmetilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

N-metil-5-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

4-(6-((4-(metilsulfonyl)piperazin-1-il)metil)-4-morfolinotieno-[3,2-d]pirimidin-2-il)piridin-2-amina;

5-(6-(((1-isopropilpiperidin-4-il)(metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-(((2R,6S)-2,6-dimetil-4-(metilsulfonyl)piperazin-1-il)-metil)-7-metil-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

1-((2-(2-aminopirimidin-5-il)-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)-4-(4-clorofenil)piperidin-4-ol;

(S)-3-metil-4-(6-((4-metilpiperazin-1-il)metil)-2-(pirimidin-5-il)tieno[3,2-d]pirimidin-4-il)morfolina;

(S)-5-(6-((metil(1-metilpiperidin-4-il)amino)metil)-4-(3-metil-morfolino)tieno[2,3-d]pirimidin-2-il)pirimidin-2-amina;

(S)-5-(4-(3-metilmorfolino)-6-((4-(metilsulfonyl)piperazin-1-il)metil)tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

(S)-1-(4-((2-(2-aminopirimidin-5-il)-4-(3-metilmorfolino)tieno-[3,2-d]pirimidin-6-il)metil)-1,4-diazepan-1-il)etanona;

5-(6-((metil(piridin-4-ilmetil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;

5-(6-((metil(piridin-3-ilmetil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
5-(6-((metil(piridin-2-ilmetil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina;
5-(6-((metil((4-metiltiazol-2-il)metil)amino)metil)-4-morfolino-tieno[3,2-d]pirimidin-2-il)pirimidin-2-amina; y
5-(6-((metil((1-metil-1H-imidazol-4-il)metil)amino)metil)-4-morfolinotieno[3,2-d]pirimidin-2-il)pirimidin-2-amina.

18. Una composición farmacéutica CARACTERIZADA porque comprende un compuesto de la reivindicación 1 y un vehículo farmacéuticamente aceptable.
19. La composición de acuerdo con la reivindicación 18, CARACTERIZADA porque además comprende un agente terapéutico adicional seleccionado entre un agente quimioterapéutico, un agente anti-inflamatorio, un agente inmunomodulador, un factor neurotrópico, un agente para tratar una enfermedad cardiovascular, un agente para tratar una enfermedad hepática, un agente antiviral, un agente para tratar trastornos de la sangre, un agente para tratar diabetes y un agente para tratar trastornos de inmunodeficiencia.
20. Una composición CARACTERIZADA porque comprende un compuesto de la reivindicación 1 en una cantidad para inhibir en forma detectable la actividad de PI3 quinasa, y un portador, adyuvante o vehículo farmacéuticamente aceptable.
21. Un proceso para preparar una composición farmacéutica, CARACTERIZADO porque comprende combinar un compuesto de la reivindicación 1 con un vehículo farmacéuticamente aceptable.
22. El compuesto (S)-1-(4-((2-(2-aminopirimidin-5-il)-7-metil-4-morfolinotieno[3,2-d]pirimidin-6-il)metil)piperazin-1-il)-2-hidroxipropan-1-ona.

25

ANEXO 1

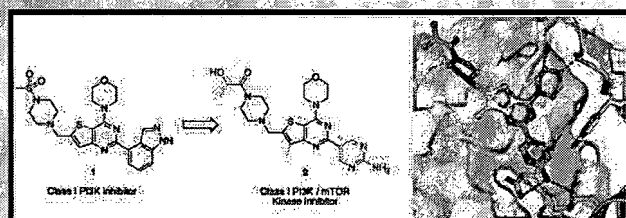
Discovery of a Potent, Selective, and Orally Available Class I Phosphatidylinositol 3-Kinase (PI3K)/Mammalian Target of Rapamycin (mTOR) Kinase Inhibitor (GDC-0980) for the Treatment of Cancer

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ABSTRACT: The discovery of **2** (GDC-0980), a class I PI3K and mTOR kinase inhibitor for oncology indications, is described. mTOR inhibition was added to the class I PI3K inhibitor **1** (GDC-0941) scaffold primarily through the substitution of the indazole in **1** for a 2-aminopyrimidine. This substitution also increased the microsomal stability and the free fraction of compounds as evidenced through a pairwise comparison of molecules that were otherwise identical. Highlighted in detail are analogues of an advanced compound **4** that were designed to improve solubility, resulting in **2**. This compound, is potent across PI3K class I isoforms with IC₅₀s of 5, 27, 7, and 14 nM for PI3K α , β , δ , and γ , respectively, inhibits mTOR with a K_i of 17 nM yet is highly selective versus a large panel of kinases including others in the PIKK family. On the basis of the cell potency, low clearance in mouse, and high free fraction, **2** demonstrated significant efficacy in mouse xenografts when dosed as low as 1 mg/kg orally and is currently in phase I clinical trials for cancer.



INTRODUCTION

Both PI3K and mTOR have been identified as promising kinase targets for the treatment of cancer. These enzymes participate in related, but not redundant, signaling networks to transmit cellular growth and survival signals, which are hallmarks of tumor growth.^{1–5} An interest in targeting two important points along this critical signaling pathway, along with the fact that PI3Ks and mTOR have a high degree of active site similarity, has spurred the development of PI3K/mTOR inhibitors for oncology indications.^{6–8}

PI3K α , a member of the class I PI3Ks (α , β , δ , γ), has been linked to cancer through the identification of activating mutations in the kinase domain,^{9–12} loss of function mutations in the PI3K negative regulator PTEN,^{13,14} through its key role in transmitting signals to or from known oncogenes (such as EGFR, HER2, RAS, and AKT)^{1,2} and as a recognized mechanism for resistance to known therapies.¹⁵ Functionally, this lipid kinase is responsible for the phosphorylation of phosphatidylinositol (4,5) bis-phosphate to phosphatidylinositol (3,4,5) tris-phosphate, which then recruits additional kinases to the membrane, activating the oncogene AKT. AKT, in turn is then responsible for a broad number of downstream signaling events that includes mTOR activation. Several class I PI3K inhibitors, including **1**

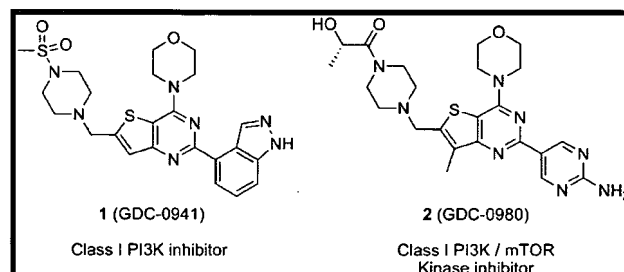


Figure 1. Structures of morpholino thienopyrimidine inhibitors of class I PI3Ks, **1**, and class I PI3Ks and mTOR Kinase, **2**.

(GDC-0941, Figure 1),^{16,17} have entered clinical trials. Additionally, isoform selective compounds have also entered clinical development for various oncology indications.¹⁸

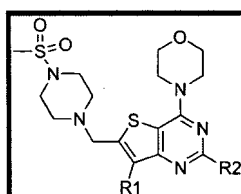
The kinase mTOR, a member of the PIKK (phosphatidylinositol like kinase) family, is activated downstream of AKT and leads to increased protein synthesis and growth.⁵ Rapamycin analogues such as temsirolimus, which inhibits mTOR when complexed in

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Table 1. Relevant Potency and PK Data for Previously Described Molecules 1, 3, and 4^a



Comp	R1	R2	IC ₅₀ or K _{i app} *(nM)				rat Cl _p (ml/min/kg)	dog Cl _p (ml/min/kg)	PPB human	thermodynamic solubility pH6.5 (μg/mL)
			p110α	mTOR*	PC3 cell	MCF7 -neo/HER2 cell				
1	H		3	580	280	290	60	12	93%	16
3	H		2	29	310	130	24	22	51%	24
4	CH ₃		4	21	220	143	13	5	77%	9

^a Male Sprague–Dawley rats and male beagle dogs were dosed intravenously at 1 mg/kg for rat and dog (1 rat, dimesylate salt in 5% DMSO, 5% cremaphore (5 mg/kg); dog, dimesylate salt in 30% SBE-β-CD. 3 rat, TFA salt in 5% DMSO, 5% cremaphore; dog, HCl salt in 20% HP-β-CD. 4 rat, TFA salt in 5% DMSO, 5% cremaphore; dog, HCl salt in 10% HP-β-CD). *K_{i app} is reported for mTOR, all others are IC₅₀s.^{16,31,32}

part to rictor (mTORC1 complex), have been approved for the treatment of advanced renal cell carcinoma and mantle cell lymphoma, validating this target in humans.¹⁹ One potential limitation of exclusive mTORC1 inhibition by rapamycin analogues is that mTOR kinase also participates in the mTORC2 protein complex that can activate the oncogene AKT, through phosphorylation of AKT Ser473, and promote cell survival through other signaling mechanisms.²⁰ Experimentally, it has been shown that inhibition of mTORC1 by rapamycin analogues fails to repress a negative feedback loop that results in the phosphorylation and activation of AKT.^{21–24} To avoid this undesired feedback mechanism and potential to reactivate the pathway and cause resistance, ATP competitive mTOR kinase inhibitors that can inhibit both mTORC1 and mTORC2 have been pursued as alternatives to the rapamycin analogues.²⁵

Given the quantity of evidence implicating both PI3K and mTOR in cancer, we and others have developed compounds that inhibit both kinases. Several of these inhibitors have begun early clinical development.^{26–29} The discovery of 2 (GDC-0980), also in the clinic, is described herein. By inhibiting two nodes in this critical pathway, this compound may provide a therapeutic advantage relative to inhibitors that target PI3K or mTOR alone.

RESULTS AND DISCUSSION

The class I PI3K inhibitor 1 served as a useful template to explore the potential addition of mTOR potency, as this compound possessed good potency, was selective against a large panel of kinases, and was efficacious in mice xenograft studies when dosed orally.¹⁶ At the outset of our work, it was known that the tool compound PI-103, also possessing a morpholino pyrimidine core, did inhibit mTOR and PI3Ks, and it was conceivable that mTOR potency could be acquired through modification of 1.³⁰ Secondary to this effort, we aimed to improve clearance of

the scaffold while also maintaining or improving potency and solubility.

Recent communications from our lab have described the discovery of the 2-aminopyrimidyl-thienopyrimidine 3 and the 7-methyl analogue 4 (Table 1).^{31–33} These compounds are potent inhibitors of mTOR and the class I PI3Ks and confirm the potential to inhibit both kinases in this structural class. In addition to biochemical assays, these compounds are also potent in cell-based proliferation assays where human tumor cell growth is driven by loss of the negative regulator PTEN (PC3 line) or activating mutations in PI3Kα (MCF7-neo/HER2 line). Proliferation IC₅₀s correlated well with cellular pAKT IC₅₀s measured in PC3 cells, a downstream readout of pathway inhibition (correlation coefficients of 0.78 and 0.74 for the PC3 and MCF7-neo/HER2 lines, respectively, when looking across >200 compounds from multiple scaffolds). The replacement of the indazole with a 2-aminopyrimidine, found in 1 and 3, respectively, was a key factor in increasing potency for mTOR by 10-fold on average across a number of diverse morpholino thienopyrimidines. When this change was applied to the lead compound 1 to generate 3, the result was a 20-fold improvement in mTOR inhibition. Proliferation IC₅₀s for potent PI3Kα inhibitors were not dramatically affected by additional mTOR inhibition in these two specific cell lines. This observation was not surprising in that these two lines used in the discovery phase of the program depended exclusively on PI3Kα activity for growth.

In addition to the increased mTOR inhibition brought about through the aminopyrimidine substitution, the cLogP of the scaffold was lowered by 1.9 units via this change and consequently increased the free fraction and the in vitro stability of the molecules across species (measured logDs for 1 and 3 were 2.4 and 1.3, respectively). To systematically observe these effects, we examined matched pairs of compounds where the core and pendant R groups are held constant and the data is compared

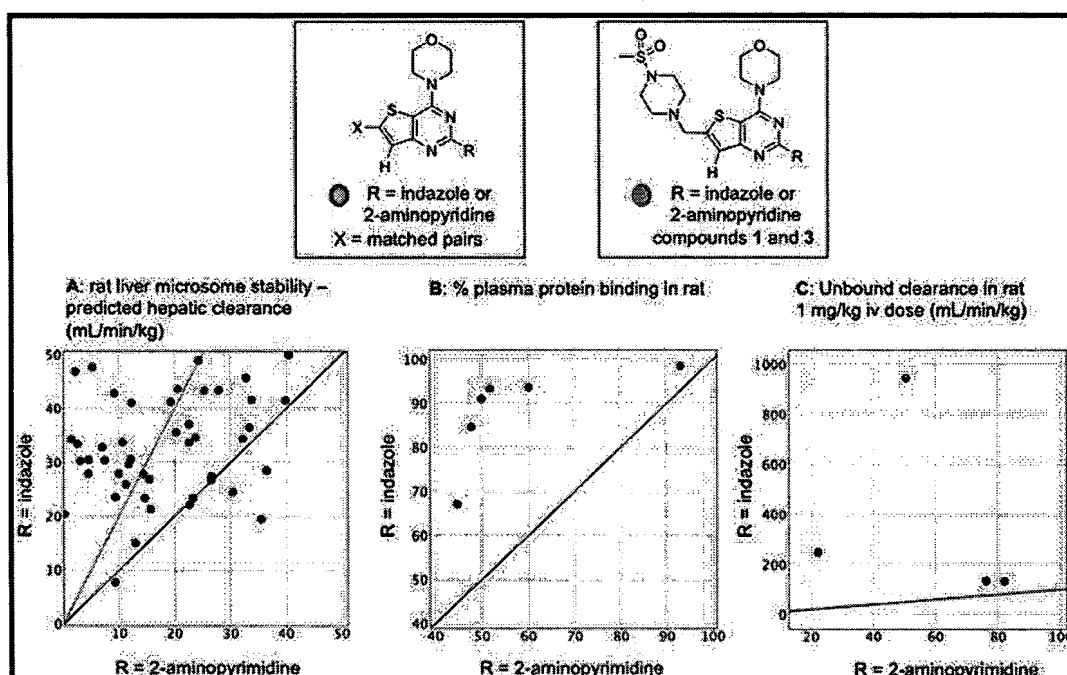
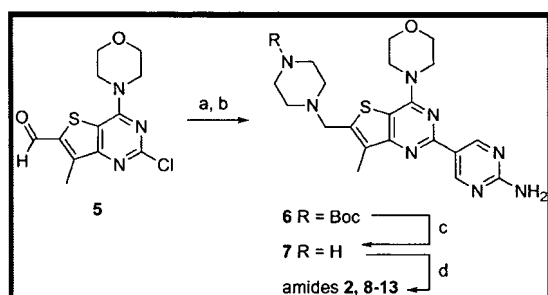


Figure 2. Comparisons of rat liver microsome stability, plasma protein binding, and unbound clearance in rat for matched pairs of compounds having either an indazole or a 2-aminopyrimidine. Points on each graph represents two compounds where R = indazole (data on the y axis) or R = 2-aminopyrimidine (x axis). Blue points have undisclosed X groups, and the red point indicates the data for compounds 1 and 3 specifically. The black lines represent the line of unity, and the blue line in (A) represents a 2-fold change from the line of unity. Hepatic clearance in (A) was predicted from liver microsome incubations using the “in vitro $t_{1/2}$ method”.³⁵

Scheme 1. Synthesis of Compounds Found in Table 2^a



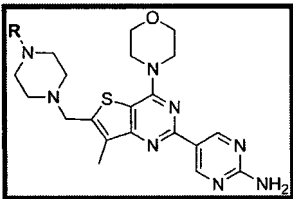
^a Reagents and conditions: (a) Boc-piperazine, 1,2-DCE, $\text{HC}(\text{OCH}_3)_3$, $\text{Na}(\text{OAc})_3\text{BH}$, 96%; (b) 2-aminopyrimidine-5-boronic acid pinacol ester, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, 1 M Na_2CO_3 , CH_3CN , microwave, 140 °C, 15 min, 82%; (c) 4N HCl in dioxane, DCM, 25 °C, 3 h, 100%; (d) RCOOH , DIPEA, HATU, DMF, 25 °C, 30 min, 42–56%.

exclusively between the indazole and the aminopyrimidine (Figure 2A–C). Figure 2A shows that when 46 indazole/pyrimidine matched pairs are compared, 21 of the pyrimidines were >2 fold more stable than the corresponding indazole-containing analogue in rat liver microsomes. The remaining 30 compounds are within an accepted 2-fold margin of error for this assay. Additionally, for the comparisons that can be made, the PPB in rat is also lowered significantly for all compounds (Figure 2B). These findings were not restricted to rat and could be seen across species, including human (data not shown). The increased microsomal stability correlated well with in vivo data and contributed to equivalent or significantly lower unbound clearances for all molecules tested in rat (Figure 2C).

Where 3 displayed good clearance in rats, methyl substitution of the core found in 4 was required to give low clearances in dogs as well. While this change improved dog clearances, compounds such as 4 suffered from relatively low solubility and was considered to increase the compounds potential risk of solubility limited exposure and PK variability in humans. Thus, the focus of further work was to determine whether analogues could be generated with better solubility while maintaining the biological and pharmacokinetic properties of 4. With this goal in mind, we considered solubility improvements to be best accessed through modification of the solvent exposed sulfonamide found on the piperazine ring. This was thought to be possible due to the added potency afforded by the 2-aminopyrimidine that allowed greater flexibility in the SAR surrounding the solvent exposed region of the core.³¹

Chemistry. Piperazine amides 2 and 8–13 (Scheme 1 and Table 2) were pursued to maintain the neutral charge of the distal nitrogen of the piperazine ring to provide a balance of polarity without added molecular weight and to utilize readily available amino acid starting materials. We explored both amino and hydroxy acids to improve solubility and with and without substitution at the α carbon to modulate metabolic stabilities. Synthetically, the amides were installed in the last step due to instability of this group to the high temperature Suzuki reaction used to install the aminopyrimidine. Unsubstituted piperazine 7 was generated from the previously described aldehyde 5 after reductive amination with Boc-piperazine, Suzuki coupling with 2-aminopyrimidine-4-boronic acid, and Boc deprotection of 6. The final products were made with Boc protected amino acids which were deprotected following coupling or with the unprotected hydroxy acids to give 2 and 8–13.

Table 2. Biochemical and Cell-Based Potency, Predicted and in Vivo Hepatic Clearances, and Thermodynamic Solubility for Compounds 2 and 8–13^a



Compound	R	IC ₅₀ or K _{i app} [*] (nM)					Clearance (ml/min/kg)			thermodynamic solubility pH 6.5 (mg/mL)	
		p110α	mTOR [*]	p110β/δ/γ	PC3	MCF7	human pred. Cl _h	rat pred. Cl _h	Rat Cl _p	F%	
8		0.7	12	11/4.3/17	228	518	10.8	16.1	140	0	0.69
9		2.1	6.3	29/2.4/37	556	472	7.1	6.7	-	-	-
10		1.0	14	44/1.5/20	367	289	8.6	19.2	84	6	1.0
11		2.8*	-	19/5.6/10	457	288	4.1	3.6	-	-	-
2		4.8	17	27/6.7/14	307	255	3.1	4.4	15	100	0.084
12		8.7	-	145/11/33	295	356	6.5	11	17	77	-
13		1.7	14	45/5.0/25	116	161	7.2	13.7	24	71	0.6

^a Male Sprague–Dawley rats were dosed with TFA salts of each compound intravenously at 1 mg/kg as a solution in 5% DMSO/5% cremophor and dosed orally in 80% PEG400 (or 0.5% methylcellulose)/0.2% Tween-80 for compound 2). *K_{i app} is reported for mTOR and PI3Kα for compound 11, all others are IC₅₀s. Hepatic clearance was predicted from liver microsome incubations using the “in vitro t_{1/2} method”.³⁵

A subset of structurally related analogues are shown in Table 2. The amine-based glycine, alanine, and dimethyl glycine derivatives of 4 all had respectable potencies in biochemical and cell-based assays along with the desired significant improvements in thermodynamic solubility at pH 6.5. However, in vivo clearance for the two analogues tested, 8 and 10, were at or above liver blood flow³⁴ and the compounds had little or no oral bioavailability. The alcohol-based analogues had much better PK properties as exemplified by 2, 12, and 13, which had increased solubility, low to moderate clearance, and good oral bioavailability. Compound 11 was not advanced further as the cellular potency in the PC3 line was above 400 nM. Lactic amide 12 had biochemical potencies and human microsomal stabilities that were less preferred relative to it is enantiomer 2, even though rodent PK and cellular potencies were roughly similar. Lactic amide 2 was chosen for further study primarily due to its low predicted clearance in human as measured in vitro with human liver microsomes, its low in vivo clearance in rat, and maximized oral bioavailability relative to tertiary alcohol 13. In addition to measuring cellular proliferation in the PC3 line, direct inhibition of PI3K could be monitored in these same cells by monitoring

the phosphorylation of Ser473 on AKT. In this assay, 2 had an IC₅₀ of 36 nM. Additionally, to confirm the binding mode of 2, we obtained a cocrystal structure of this compound bound to PI3Kγ (Figure 3). The structure bound in accordance with a similar previously disclosed amino-pyridine.³¹

Kinase Selectivity. Previous PI3K inhibitors from this and other chemical classes have maintained a high degree of selectivity over off-target kinases even while maintaining dual inhibition for mTOR and the PI3K isoforms. To confirm this, 2 was submitted for screening in a panel of kinases provided by Invitrogen’s SelectScreen service. Of the 240 total kinases in the SelectScreen panel, only 5 kinases other than the PI3K isoforms and mTOR consistently displayed greater than 60% inhibition when treated with 1 μM of 2: Fgr, Mlk1, PAK4, Syk, and Yes1. Measurement of IC₅₀s for the most potently inhibited kinases in the panel revealed significantly weaker values for the off-target kinases compared to PI3K/mTOR; IC₅₀s for Fgr, Mlk1, and Syk were 697, 232, and 134 nM, respectively. Compound 2 was also remarkably selective for several other members of the closely related PIKK family kinases: C2alpha IC₅₀ = 1300 nM; C2beta IC₅₀ = 794 nM; VPS34 IC₅₀ = 2000 nM;

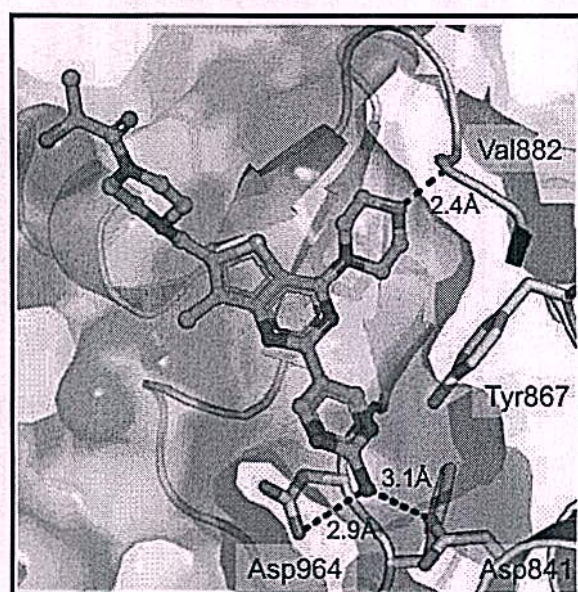


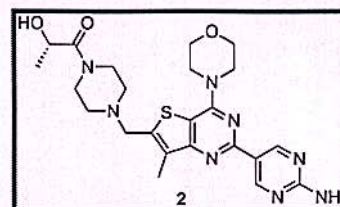
Figure 3. Crystal structure (2.8 Å) of 2 bound to PI3K γ . The backbone atoms of PI3K γ are shown in a cartoon representation with carbon atoms colored gray, 2 is shown in ball and stick representation with carbon atoms colored cyan. Critical contacts include the morpholine oxygen with the hinge residue Val882 and key contacts from the aminopyrimidine NH₂ to the carbonyl oxygens of Asp841 and Asp964. Crystals were grown at 18 °C from 20% PEG 3350, 0.2 M NH₄SO₄ by hanging drop vapor diffusion.¹⁶

PI4K α >10 μ M; PI4K β >10 μ M; DNA-PK K_{app} = 623 nM, respectively. Thus, the biochemical data illustrate that 2 had the desired selectivity profile, with potent inhibition of all class I PI3K isoforms and mTOR and selectivity over the other profiled PIKK family enzymes and a large, diverse panel of kinases.

Pharmacokinetics. On the basis of this promising profile, 2 was further characterized through PK studies conducted in several preclinical species. PK parameters obtained in mice after intravenous administration at 1 mg/kg and when dosed orally at 5 and 50 mg/kg are highlighted in Table 3 and are relevant to xenograft studies discussed herein. Clearance and PPB were low, and 2 showed dose-proportional exposure from 5 mg/kg dosed in PEG to 50 mg/kg dosed in suspension in MCT, a finding attributed partially to the compound's good solubility. PK parameters for 2 were also favorable in the other species tested, including dog (data not shown, manuscript in preparation). Plasma protein binding was similar across species.

In Vivo Efficacy. We evaluated the efficacy of compound 2 in vivo using xenografts derived from the same two tumor cell lines that were used to screen for cellular potency: PC-3 and MCF-7 neo/HER2 (Figure 4). The compound was dosed orally as a crystalline suspension in 0.5% methylcellulose/0.2% Tween-80 (MCT) once a day for either 14 or 22 days, and the tumor volume was measured relative to control animals treated with MCT vehicle only. In both PC-3 and MCF-7 neo/HER2 xenograft models, treatment with 1 mg/kg of compound 2 resulted in statistically significant antitumor activity, characterized as tumor growth delay [p < .001 vs vehicle treated group based on Dunnett's t test on day 14 (for PC3) and day 22 (for MCF-7 neo/HER2)]. At the maximum tolerated dose of 7.5 mg/kg, compound 2, tumor stasis or regressions were observed in the PC-3 and MCF-7 neo/HER2 xenograft models, respectively [p < .0001 vs

Table 3. Pharmacokinetic and Plasma Protein Binding Data for Compound 2 in Mouse^a



predicted Cl_h (mL/min/kg)	IV (1 mg/kg)		PO				F	PPB
	Cl_p (mL/min/kg)	V_{ss} (L/kg)	dose (mg/kg)	C_{max} (μ M)	AUC (μ M·h)	%		
33	9.2	1.7	5	2.0	10	56	71	
			50	24	121	66		

^a Female nu/nu mice were dosed with the HCl salt, either intravenously as a solution in 5% DMSO/5% cremophor, orally at 5 mg/kg in 80% PEG400, and orally at 50 mg/kg as a crystalline suspension in 0.5% methylcellulose/0.2% Tween-80. Hepatic clearance was predicted from mouse liver microsome incubations using the "in vitro $t_{1/2}$ method".³⁵

vehicle treated group based on Dunnett's t test on day 14 (for PC3) and day 22 (for MCF-7 neo/HER2)]. The significant antitumor activity of compound 2 at low doses is likely due to high cellular potency, low plasma clearance, and relatively high free fraction of the drug in vivo.

CONCLUSION

A potent and selective inhibitor of class I PI3 kinases and mTOR kinase was developed using lead compound 1 as a structural template. The replacement of an indazole with a 2-aminopyrimidine was responsible for increased potency for mTOR, lower PPB, and improved predicted and measured total and unbound clearances in rats and mice. A methyl group was added to the thienopyrimidine core to lower the in vivo clearances in rats and dogs further. Finally, a lactic amide was used to replace a sulfonamide in order to increase the thermodynamic solubility at a neutral pH, resulting in the discovery of 2. On the basis of the potency for PI3K and mTOR, selectivity against other kinases, pharmacokinetics, solubility, and efficacy in PI3K driven animal models of cancer, this compound was advanced into development where it is currently in phase I clinical trials.

EXPERIMENTAL SECTION

Chemistry. All solvents and reagents were used as obtained. ¹H NMR spectra were recorded with a Bruker Avance DPX400 spectrometer or a Varian Inova 400 NMR spectrometer and referenced to tetramethyl silane. Chemical shifts are expressed as δ units using tetramethylsilane as the external standard (in NMR description, s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra were measured with a Finnigan SSQ710C spectrometer using an ESI source coupled to a Waters 600MS HPLC system operating in reverse mode with an X-bridge Phenyl column of dimensions 150 mm by 4.6 mm, with 5 μ m sized particles. All compounds were purified to $\geq$ 95% purity using reversed phase HPLC.

2-Chloro-7-methyl-4-morpholinothieno[3,2-d]pyrimidine-6-carbaldehyde (5). To 4-(2-chloro-7-methylthieno[3,2-d]pyrimidin-4-yl)morpholine (3.18 g, 11.8 mmol) in THF (65 mL) at -78 °C

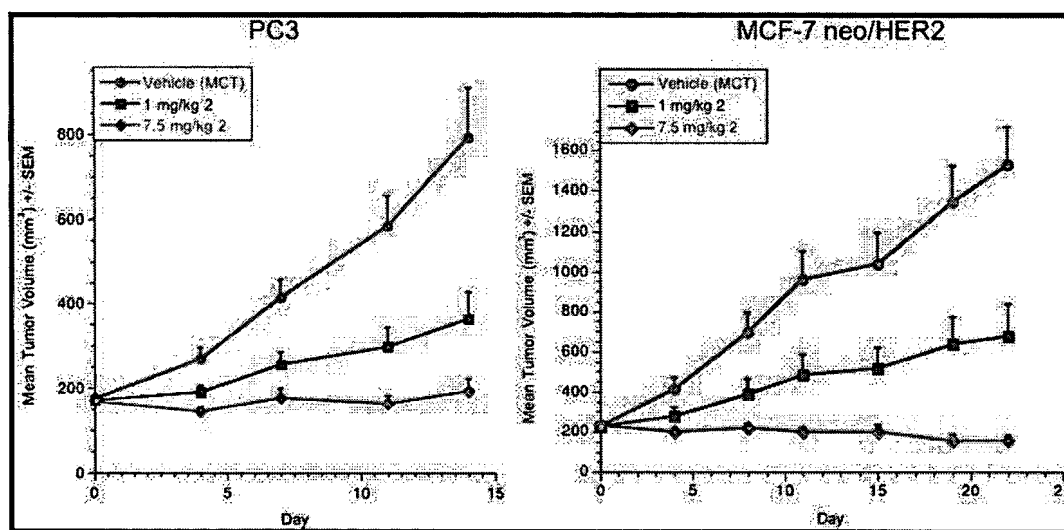


Figure 4. In vivo efficacy for 2 in PC3 and MCF-7 neo/HER2 xenograft tumor models when dosed orally at 1 and 7.5 mg/kg on a once daily schedule.

was added a 2.5 M solution of *n*-butyllithium in hexane (10.3 mL). The resulting solution was warmed to -40°C and stirred 2 h. DMF (4.2 mL, 54.2 mmol) was added, and the reaction mixture stirred 2 h at -40°C . The reaction was quenched by pouring slowly onto 0.25 M HCl with ice. After 1 h, the solid was filtered and lyophilized to yield 3.44 g of (5). ^1H NMR (400 MHz, CDCl_3) δ 10.32 (s, 1H), 4.02 (m, 4H), 3.79 (m, 4H), 2.68 (s, 3H). MS: (ESI+) 298.1.

tert-Butyl 4-((2-chloro-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazine-1-carboxylate. 2-Chloro-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidine-6-carbaldehyde (0.58 g) and *tert*-butyl piperazine-1-carboxylate (0.52 g) were dissolved in 1,2-dichloroethane (10 mL). Acetic acid (1.22 mL) was added and the reaction stirred 15 min before the addition of sodium triacetoxyborohydride (0.55 g). The resulting solution was stirred 24 h at room temperature. The reaction mixture was quenched with saturated aqueous NaHCO_3 , extracted with CH_2Cl_2 , dried over Na_2SO_4 , filtered, and concentrated in vacuo. The crude material was purified by flash chromatography (EtOAc/hexanes) to provide 880 mg of *tert*-butyl 4-((2-chloro-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazine-1-carboxylate. ^1H NMR (400 MHz, DMSO) δ 3.88 (m, 4H), 3.82 (s, 2H), 3.74 (m, 4H), 3.34 (m, 4H), 2.43 (m, 4H), 2.23 (s, 3H), 1.39 (s, 9H). MS: (ESI+) 468.3.

tert-Butyl 4-((2-(2-Aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazine-1-carboxylate (6). *tert*-Butyl 4-((2-chloro-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazine-1-carboxylate (2.74 g, 5.8 mmol) was combined with bis(triphenylphosphine)palladium(II) chloride (0.21 g) and 2-aminopyrimidine-5-boronic acid pinacol ester (1.68 g) in 12 mL of acetonitrile and 12 mL of 1 M sodium carbonate in water. The reaction mixture was heated in a 50 mL microwave vessel and microwaved at 300 W to 140°C for 12 min. After cooling to room temperature, the resultant gray solid was washed with water and acetonitrile before solubilizing in a mixture of DMF, methanol, and DCM and stirred with charcoal overnight prior to filtering. This filtrate was treated with florisil for 2 h, filtered, and evaporated to give 6 as an off-white solid (2.52 g). ^1H NMR (400 MHz, DMSO) δ 9.15 (s, 2H), 7.06 (s, 2H), 3.97–3.91 (m, 4H), 3.83 (s, 2H), 3.80–3.74 (m, 4H), 3.34 (brs, 4H), 2.48–2.41 (m, 4H), 2.35 (s, 3H), 1.40 (s, 9H). MS: (ESI+) 527.2.

(7-Methyl-4-morpholino-6-(piperazin-1-ylmethyl)thieno[3,2-*d*]pyrimidin-2-yl)pyrimidin-2-amine (7). *tert*-Butyl 4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazine-1-carboxylate 6 (9.0 g) was dissolved in 43 mL

of 4 M HCl in 1,4-dioxane and stirred for 3 h. The solution was evaporated and dried overnight under vacuum to afford 7 in 100% yield. NMR (400 MHz, DMSO) δ 9.15 (s, 2H), 7.06 (s, 2H), 4.05–3.86 (m, 4H), 3.85–3.69 (m, 6H), 2.70 (brs, 4H), 2.42 (brs, 4H), 2.34 (s, 3H). MS: (ESI+) 427.2.

2-Amino-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazin-1-yl)ethanone (8). *N,N*-Diisopropylethylamine (150 μL , 0.87 mmol) was added to a solution of 5-(7-methyl-4-morpholino-6-(piperazin-1-ylmethyl)thieno[3,2-*d*]pyrimidin-2-yl)pyrimidin-2-amine 7 (74 mg, 0.17 mmol) in DMF (1 mL). HATU (97 mg, 0.26 mmol) and Boc-glycine (60 mg, 0.3 mmol) was added to the reaction mixture, which was stirred for 30 min at room temperature. The reaction mixture was diluted with ethyl acetate (10 mL) and extracted with a saturated sodium bicarbonate solution (10 mL). The organic layer was dried with magnesium sulfate, filtered, and then concentrated to afford the penultimate product. This intermediate was then subjected to a Boc-deprotection with TFA before final reverse-phase HPLC purification to give 75.3 mg (56%) of 8 ($2\times$ TFA salt) as a white solid. ^1H NMR (400 MHz, DMSO) δ 9.18 (s, 2H), 8.03 t, $J = 5.2$ Hz, 3H), 7.15 (brs, 2H), 4.00–3.94 (m, 4H), 3.89 (q, $J = 5.2$ Hz, $J = 10.8$ Hz, 2H), 3.82–3.76 (m, 4H), 3.51 (br m, 4H), 2.89 (br m, 4H), 2.42 (s, 3H). MS: (ESI+) 484.1.

(5)-2-Amino-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazin-1-yl)propan-1-one (9). Compound 9 was prepared from intermediate 7 and Boc-*L*-alanine by the procedure for compound 8 in 42% yield. ^1H NMR (400 MHz, DMSO) δ 9.18 (s, 2H), 8.09 (d, $J = 3.8$ Hz, 3H), 7.10 (br s, 2H), 4.43–4.32 (m, 1H), 4.25 (br s, 2H), 4.01–3.93 (m, 4H), 3.83–3.75 (m, 5H), 3.62 (br m, 4H), 2.89 (br m, 4H), 2.42 (s, 3H), 1.31 (d, $J = 6.8$ Hz, 3H). MS: (ESI+) 498.1.

2-Amino-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-methylpropan-1-one (10). Compound 10 was prepared from intermediate 7 and *N*-Boc-aminoisobutyric acid by the procedure for compound 8 in 56% yield. ^1H NMR (400 MHz, DMSO) δ 9.18 (s, 2H), 8.12 (s, 3H), 7.09 (br s, 2H), 4.32 (br s, 2H), 4.03–3.91 (m, 4H), 3.90–3.53 (m, 4H), 2.93 (br m, 4H), 2.43 (s, 3H), 1.55 (s, 6H). MS: (ESI+) 512.1.

1-(4-((2-(2-Aminopyrimidin-5-yl)-7-methyl-4-morpholinethieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxyethanone (11). Compound 11 was prepared from intermediate 7 and 2-hydroxyacetic acid by the procedure for compound 8 in 42% yield

but did not necessitate final Boc-deprotection prior to reverse phase HPLC purification. ¹H NMR (400 MHz, DMSO) δ 9.15 (s, 2H), 7.04 (s, 2H), 4.51 (t, J = 5.5 Hz, 1H), 4.07 (d, J = 5.5 Hz, 2H), 3.97–3.91 (m, 4H), 3.85 (s, 2H), 3.81–3.75 (m, 4H), 3.54–3.45 (m, 2H), 3.42–3.32 (m, 2H), 2.35 (s, 3H). MS: (ESI+) 485.3.

(S)-1-(4-((2-(2-Aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one (2). Compound 2 was prepared from intermediate 7 and L-lactic acid by the procedure for compound 11 in 87% yield. Chiral HPLC analysis for 2 was performed with a Chiral Technologies Chiralcel OJ-RH (4.6 mm $\times$ 150 mm, 5 μ m) column and found to be >99% enantiomerically pure (isocratic elution with 0.1% DEA in methanol (v/v), run time = 20 min, flow rate of 1.0 mL/min, detection = 270 nm, injection volume = 5 μ L, 2 t_R = 5.8 min, 12 t_R = 7.6 min). ¹H NMR (400 MHz, DMSO- d_6) δ 1.18 (d, J = 6.53 Hz, 3H), 2.34 (s, 3H), 2.38–2.60 (br, 4H), 3.36–3.64 (br, 4H), 3.67–3.78 (m, 4H), 3.84 (s, 2H), 3.89–4.00 (m, 4H), 4.35–4.48 (m, 1H), 4.84 (d, J = 6.98 Hz, 1H), 7.05 (s, 2H), 9.15 (s, 2H). MS: (ESI+) 499.1.

(R)-1-(4-((2-(2-Aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one (12). Compound 12 was prepared from intermediate 7 and L-lactic acid by the procedure for compound 11 in 61% yield. ¹H NMR (400 MHz, DMSO) δ 9.15 (s, 2H), 7.02 (s, 2H), 4.81 (d, J = 6.9 Hz, 1H), 4.41 (dq, J = 6.6 Hz, 1H), 3.97–3.91 (m, 4H), 3.84 (s, 2H), 3.81–3.74 (m, 4H), 3.55–3.41 (br m, 4H), 2.55–2.41 (br m, 4H), 2.35 (s, 3H), 2.07 (s, 2H), 1.18 (d, J = 6.5 Hz, 3H). MS: (ESI+) 499.1.

1-(4-((2-(2-Aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxy-2-methylpropan-1-one (13). Compound 13 was prepared from intermediate 7 and 2-hydroxy-2-methylpropanoic acid by the procedure for compound 11 in 54% yield. ¹H NMR (400 MHz, DMSO) δ 9.16 (s, 2H), 7.04 (s, 2H), 5.35 (s, 1H), 3.98–3.91 (m, 5H), 3.85–3.81 (m, 2H), 3.81–3.75 (m, 5H), 2.51–2.47 (m, 4H), 2.35 (s, 3H), 1.31 (s, 6H). MS: (ESI+) 513.3.

Characterization of Biochemical and Cellular Activity in Vitro. Enzymatic activity of the class I PI3K isoforms and human recombinant mTOR were measured using a fluorescence polarization assay as described previously.³¹ VPS34 and C2b were assayed using a scintillation proximity assay as described previously.¹⁶ DNA-PK was assayed using a capillary electrophoresis assay that monitors production of phosphorylated peptide as well as loss of unphosphorylated substrate. In a total volume of 5 μ L, DNA-PK (2 units/ μ L; Promega) was incubated with 1.5 μ M peptide substrate (S-FAM-AEPPLSQEAFADLWKK-NH₂) in the presence of 10 mM Hepes (pH 7.2), 10 mM MgCl₂, 10 μ M ATP, 10 μ g/mL calf thymus DNA, 0.015% (v/v) Brij-35, 1 mM dithiothreitol, and 2% dimethylsulfoxide.³⁶ After incubation for 60 min at 25 °C the reaction was terminated with a final concentration of 25 mM Hepes (pH 7.2), 0.0037% Brij-35, 37.5 mM EDTA. Dose–response curves were fit to an equation for tight-binding inhibition using the measured K_m for ATP for DNA-PK of 9.3 μ M.

Kinase selectivity was measured using the SelectScreen service at Invitrogen (Life Technologies), profiling 145 or 240 kinases for % inhibition at 1 μ M compound using the Z-lyte assay format or the Adapta assay format as specified by Lifetechnologies.

IC₅₀s were calculated from the fit of the dose–response curves to a four-parameter equation and were measured at the experimental K_m for ATP for the kinase of interest. Alternatively, apparent K_s were calculated by fitting the data to an equation for tight-binding inhibition as previously described.³¹

Antiproliferative cellular assays were conducted as described³¹ using PC3 and MCF7.1 human tumor cell lines provided by the ATCC or Genentech Research laboratories, respectively. MCF7.1 is an in vivo selected line developed at Genentech and originally derived from the parental human MCF7 breast cancer cell line (ATCC, Manassas, VA).

All IC₅₀ or K_{iapp} values for both biochemical and cellular assays represent geometric means of multiple replicates or of duplicates. Biochemical assays typically showed <30% difference between duplicates or replicates and cellular assays were typically within 2-fold agreement between replicates.

In Vivo Xenograft Studies. Human prostate cancer PC3 cells obtained from the National Cancer Institute (Frederick, MD) were resuspended in Hank's Balanced Salt Solution and 3×10^6 cells implanted subcutaneously into the right hind flank of athymic nu/nu (nude) mice. Tumors were monitored until they reached a mean tumor volume of 150–200 mm³ prior to the initiation of dosing. MCF7.1 cells resuspended in a 1:1 mixture of Hank's Buffered Salt Solution and Matrigel Basement Membrane Matrix (no. 356237, BD Biosciences; Santa Cruz, CA) were 5×10^6 subcutaneously implanted into the right hind flank of athymic nu/nu (nude) mice. Prior to cell inoculation, 17 β -estradiol (0.36 mg/pellet, 60-day release, no. SE-121) obtained from Innovative Research of America (Sarasota, FL) were implanted into the dorsal shoulder blade area of each nude mouse. After implantation of cells, tumors were monitored until they reached a mean tumor volume of 250–350 mm³ prior to initiating dosing. Compound 2 was dissolved in 0.5% methylcellulose with 0.2% Tween-80 (MCT). Female nude (nu/nu) mice that were 6–8 weeks old and weighed 20–30 g were obtained from Charles River Laboratories (Hollister, CA). Tumor bearing mice were dosed daily for 14–21 days depending on the xenograft model with 100 μ L of vehicle (MCT) or test agent orally.

Tumor volume was measured in two dimensions (length and width) using Ultra Cal-IV calipers (model 54–10–111; Fred V. Fowler Company; Newton, MA) and was analyzed using Excel version 11.2 (Microsoft Corporation; Redmond, WA). The tumor volume (mm³) = (longer measurement $\times$ shorter measurement²) $\times$ 0.5. Animal body weights were measured using an Adventurer Pro AV812 scale (Ohaus Corporation; Pine Brook, NJ). Percent weight change = [1 – (new weight/initial weight)] $\times$ 100. Tumor sizes were recorded twice weekly over the course of the study (14–21 days). Mouse body weights were also recorded twice weekly, and the mice were observed daily. Mice with tumor volumes $\geq$ 2000 mm³ or with losses in body weight $\geq$ 20% from their initial body weight were promptly euthanized per IACUC guidelines. Mean tumor volume and SEM values (n = 10) were calculated using JMP statistical software, version 5.1.2 at end of treatment. % Tumor inhibition = 100(mean volume of tumors in vehicle treated animals – mean volume of tumors in test article treated animals given the test article)/mean volume of tumors in vehicle treated animals. Data were analyzed and p -values were determined using the Dunnett t test with JMP statistical software, version 5.1.2 (SAS Institute; Cary, NC).

Accession Codes

The coordinates and structure factors for compound 2 has been deposited (PDB code 3TL5).

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for coordinating and dosing animals for the reported in vivo efficacy studies.

ABBREVIATIONS USED

AKT, protein kinase B; DCE, dichloroethane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EGFR, epidermal growth factor receptor 1; HATU, (2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); HER2, epidermal growth factor receptor 2; PEG, polyethylene glycol; PI3K, phosphoinositide 3-kinase; PPB, plasma protein binding; PTEN, phosphatase and tensin homologue; mTOR, mammalian target of rapamycin; TFA, trifluoroacetic acid; THF, tetrahydrofuran

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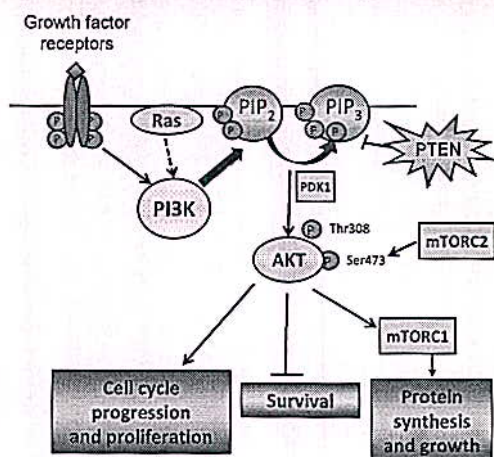
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ANEXO 2

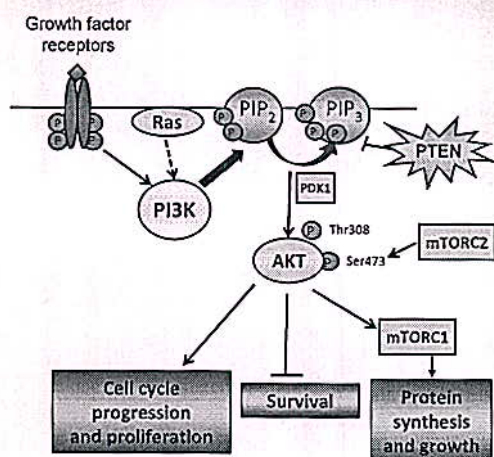
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General overview of the PI3K signaling pathway



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PI3K signaling in cancer



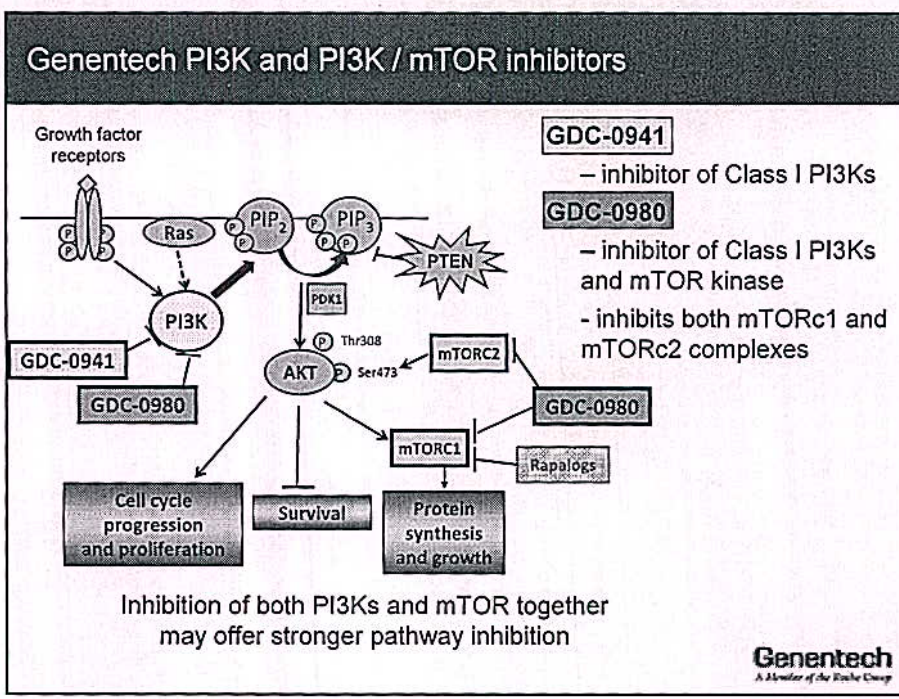
PI3K

1. PI3Kα mutations found in 20-40% breast, endometrial, colon, bladder cancers
2. Negatively regulated by tumor suppressor PTEN – loss of function in prostate, melanoma, glioblastoma...
3. Downstream of known oncogenes (ex. EGFR, HER2, RAS)
4. Upstream of known oncogenes (ex. AKT, TSC)

mTOR

Multiple mTORC1 inhibitors (rapalogs) approved for the treatment of cancer

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GDC-0941: A Class I PI3K inhibitor

PI3K α = 3 nM
 PI3K β = 33 nM
 PI3K δ = 3 nM
 PI3K γ = 75 nM
 mTOR = 580 nM

PC3 (cell) = 280 nM
 MCF7-neo/HER2 (cell) = 320 nM

Preclinical data driving goals for next generation compounds

- Potent inhibitor of Class I PI3Ks
- Selective over >250 protein kinases
- Efficacious in PI3K driven xenograft models
 - *maximum efficacy at 150 mg/kg QD (a high dose)*
- Moderate-high clearance in rodents, dogs, monkey, and in human microsomes
 - *in vivo clearance in rat and dog is 60 and 12 mL/min/kg respectively*
- a significant degree of uncertainty in human PK and dose prediction

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Opportunities for further medicinal chemistry	
Goals	Key Data
Biological differentiation - Kinase / Isoform Selectivity Lower predicted human dose - Improved Potency - Lower <i>in vivo</i> clearance - Increased free fraction - Maximize oral bioavailability	<i>In vitro</i> potency PI3K α, β, γ, δ, & mTOR IC₅₀ (nM) Cellular potency PC3 (PTEN neg) IC₅₀ (uM) MDA-361 (p110α mut) IC₅₀ (uM) <i>in vitro</i> pharmacology Liver microsome stability (ml/min/kg) Liver hepatocyte stability (ml/min/kg) Plasma protein binding (% bound) Thermodynamic Solubility (ug/ml) <i>in vivo</i> pharmacology Clearance (CL, CLu (ml/min/kg)) Bioavailability (F, %)

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A Starting point: understanding key structural features of GDC-0941

Piperazine sulfonamide:
 Provides solubility and some potency
 Exposed to solvent
 Design compounds that maintain potency but...
 - are metabolised differently
 - and retain or improve solubility

--- H-bonds

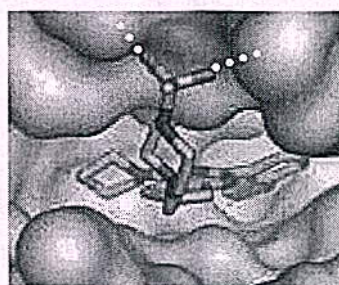
Core

Morpholine:
 Critical for binding to the hinge region

Indazole:
 Back pocket binder important for binding affinity
 optimized to maintain potency and impart bioavailability

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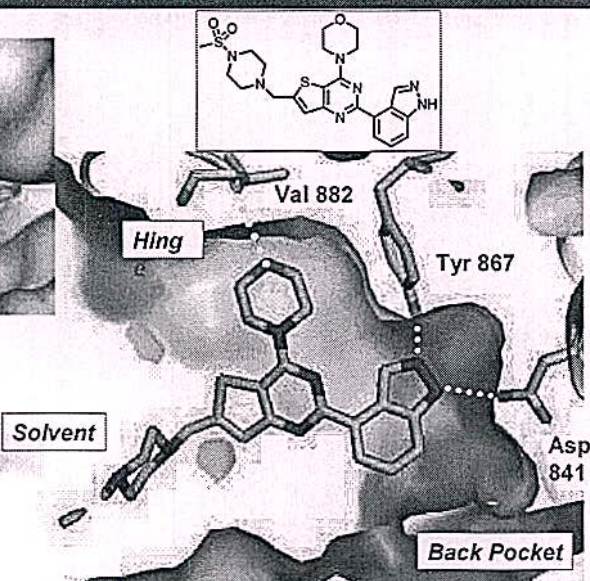
A Starting point: understanding key structural features of GDC-0941 bound to PI3K γ



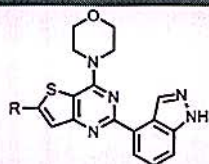
PI3K α = 3 nM
 PI3K β = 33 nM
 PI3K δ = 3 nM
 PI3K γ = 75 nM
 mTOR = 580 nM

PC3 (cell) = 280 nM
 MCF7-neo/HER2 (cell) = 320 nM

Chris Wiesmann, Mark Ultsch



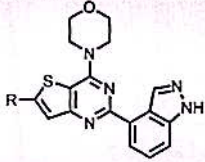
Truncation of piperazine reduces clearance



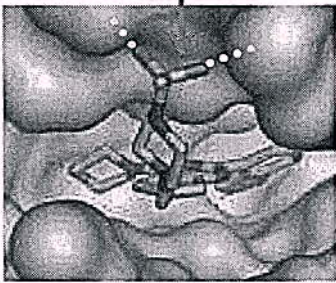
R	Clearance in rat (ml/min/kg)	F%
	60	32
	42	8
	22	35
	16	29

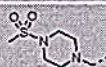
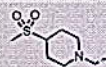
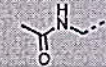
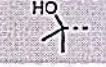
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Truncation of piperazine reduces clearance



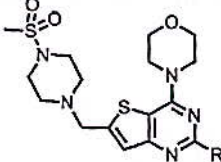
Loss of key binding contacts results in a loss of potency



R	Clearance in rat (ml/min/kg)	F%	PI3Kα (nM)
	60	32	3
	42	8	22
	22	35	22
	16	29	22

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Discovery of new aminopiperidine back pocket binders

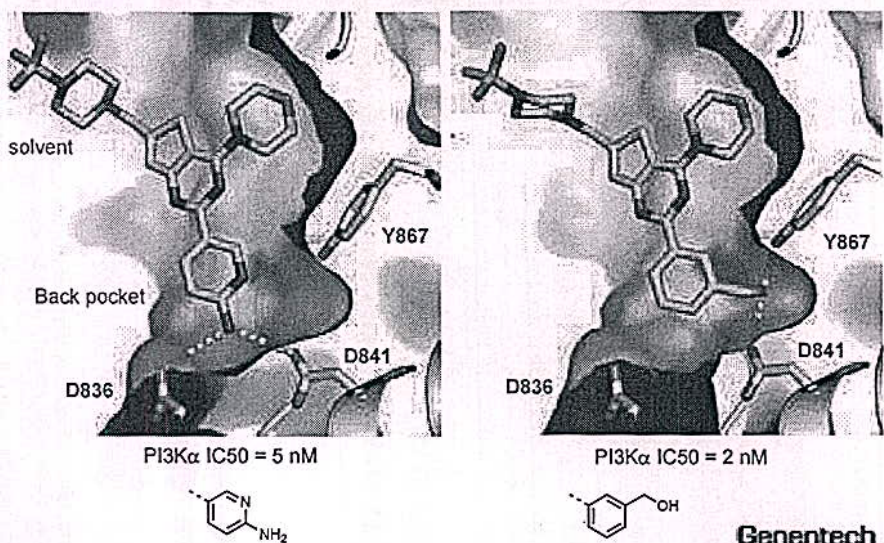


R	PI3Kα (nM)	PC3 Cell uM
	3	0.28
	430	3.7
	240	1.2

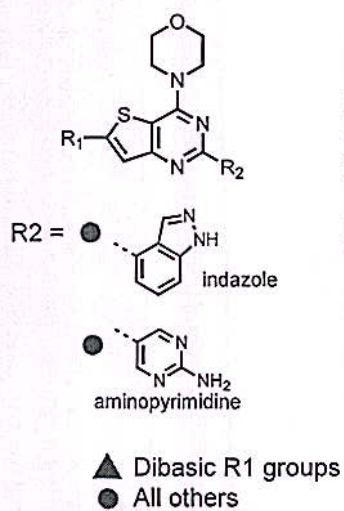
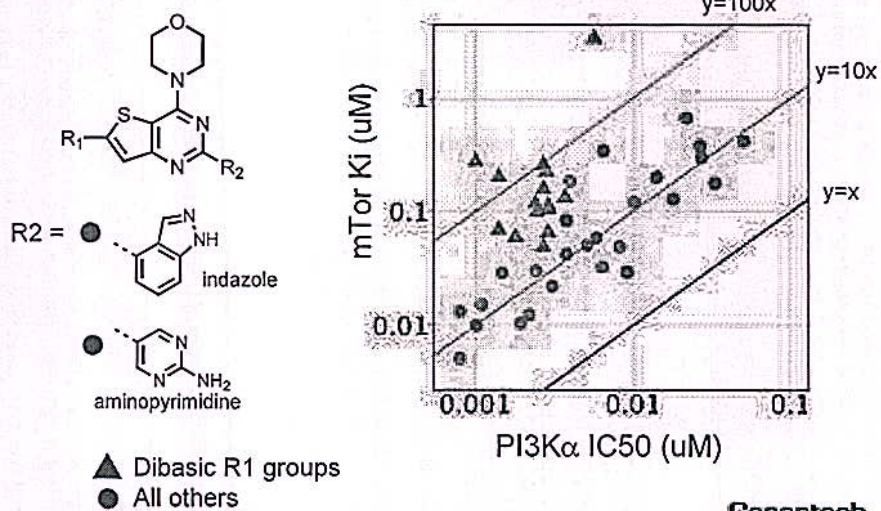
R	PI3Kα (nM)	PC3 Cell uM
	21	1.8
	5.5	0.27
	2	0.3

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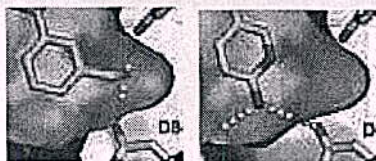
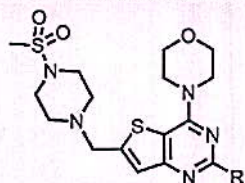
Structures in PI3Kγ explain observed SAR



Aminopyrimidine adds potency for PI3K & mTOR overall but additional features influence selectivity



Change to the aminopyrimidine in the lead series adds potency for mTOR and maintains PI3K α



R	PI3K α (nM)	mTOR (nM)	Fold selectivity
	3	580	>100
	20	1000	50
	2	170	85

R	PI3K α (nM)	mTOR (nM)	Fold selectivity
	5	45	9
	2	29	15

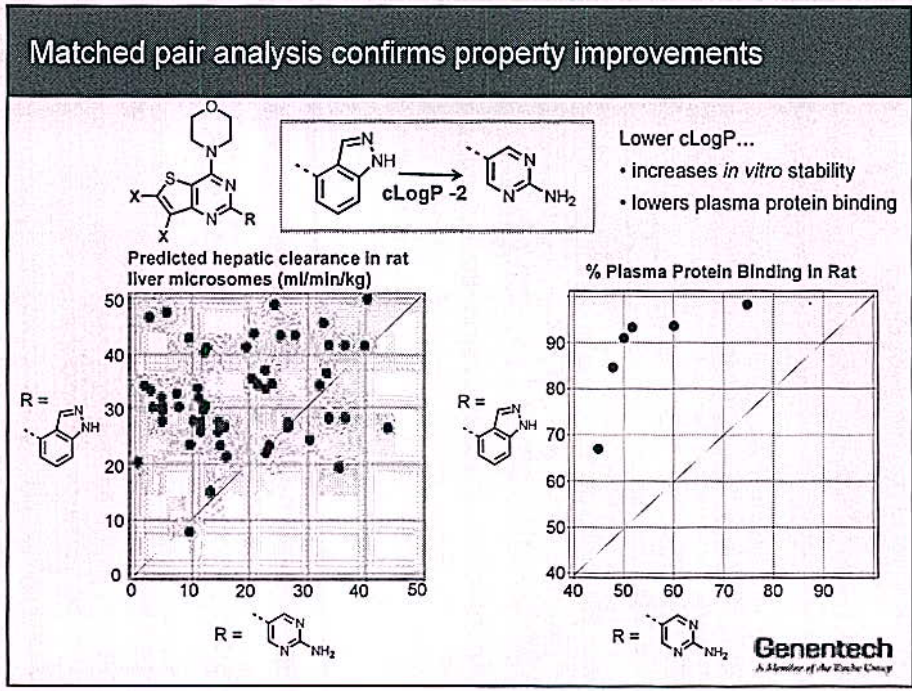
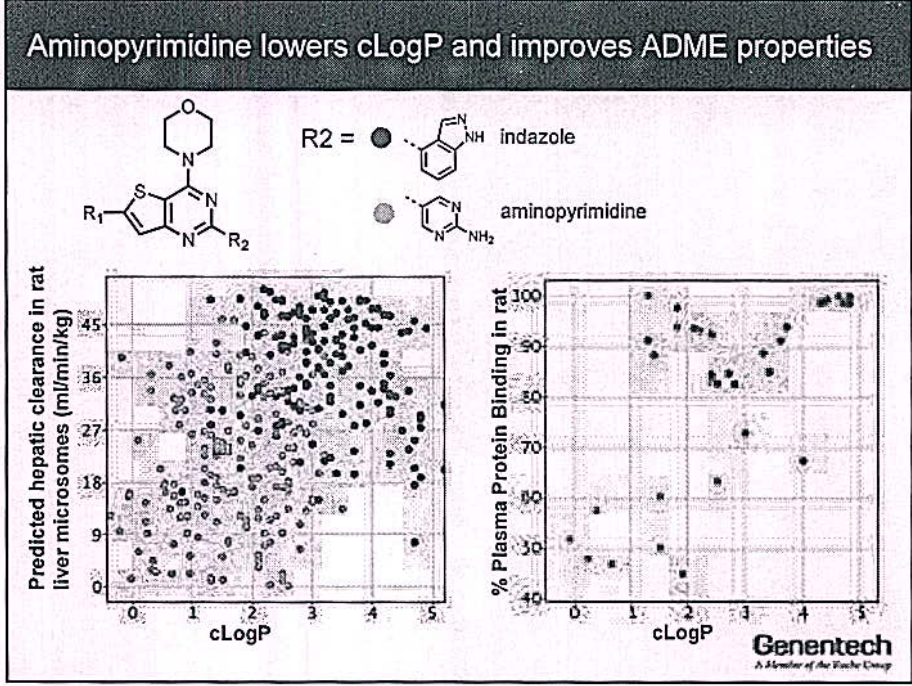
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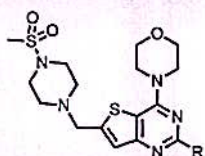
PI3K γ / mTOR sequence alignment & homology

[illegible]

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Property Improvements lower unbound clearance in rat

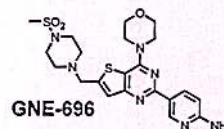
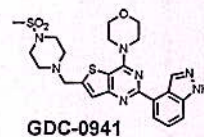
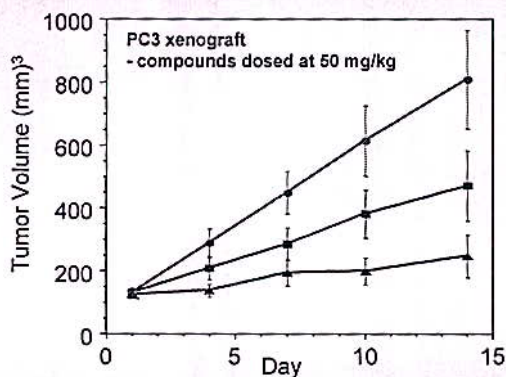


Better stability and larger free fraction lowers unbound clearance in rat

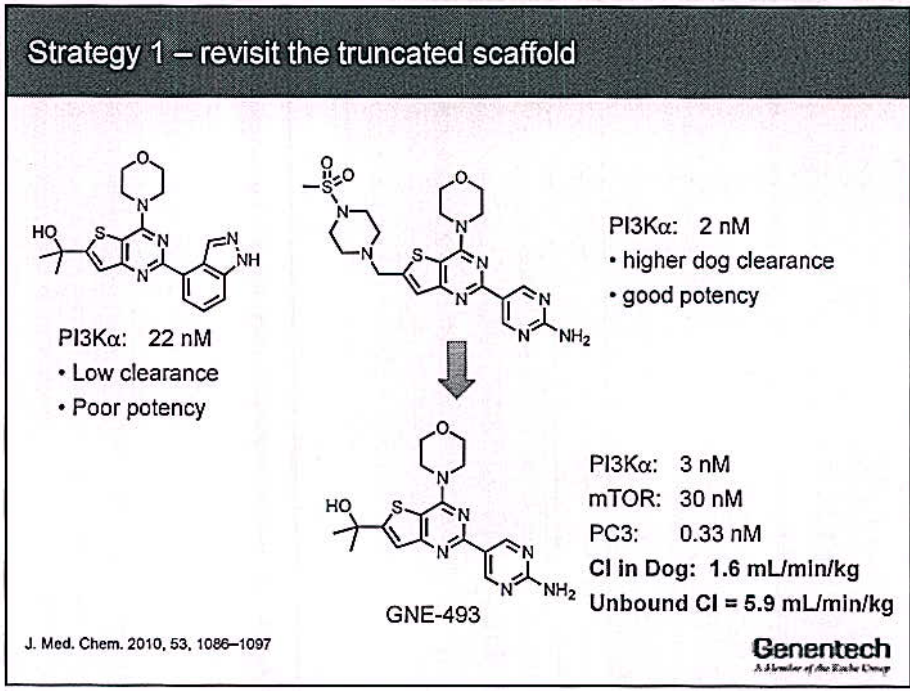
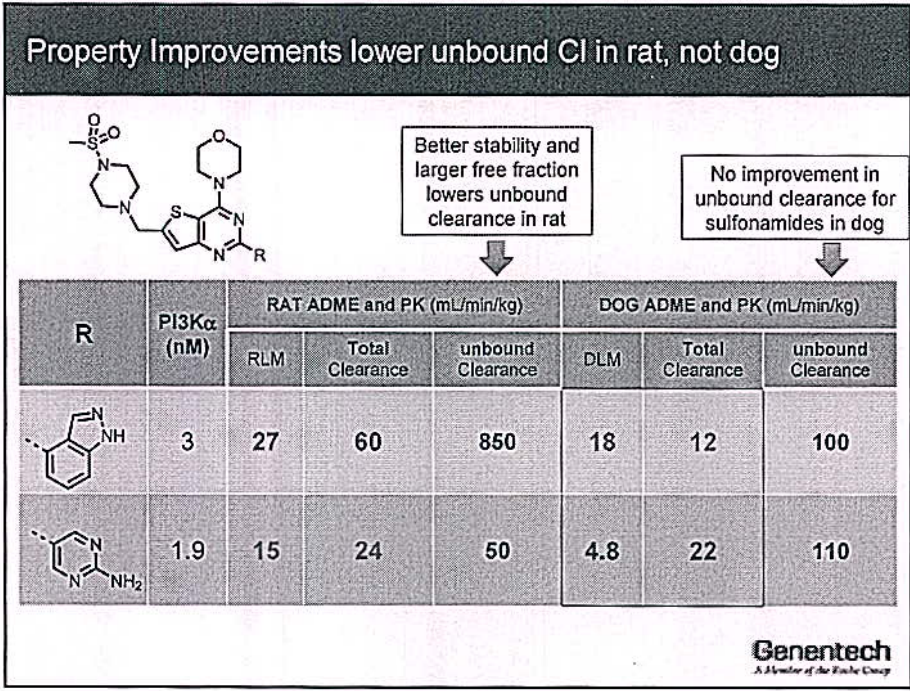
R	PI3K α (nM)	RAT ADME and PK (Cl in mL/min/kg)			
		RLM	Total Clearance	PPB % bound	unbound Clearance
	3	27	60	93	850
	1.9	15	24	49	50

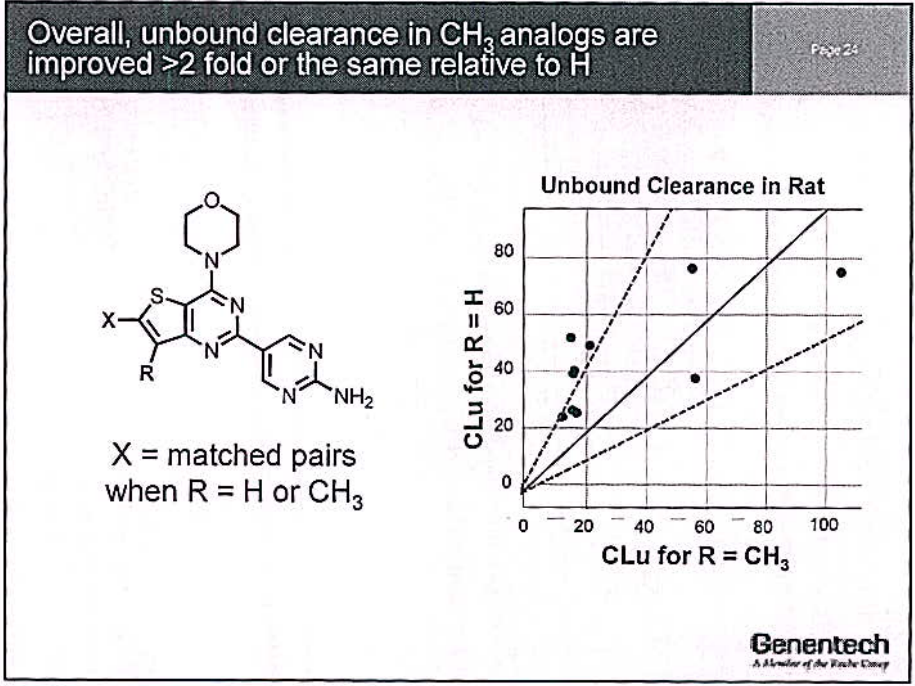
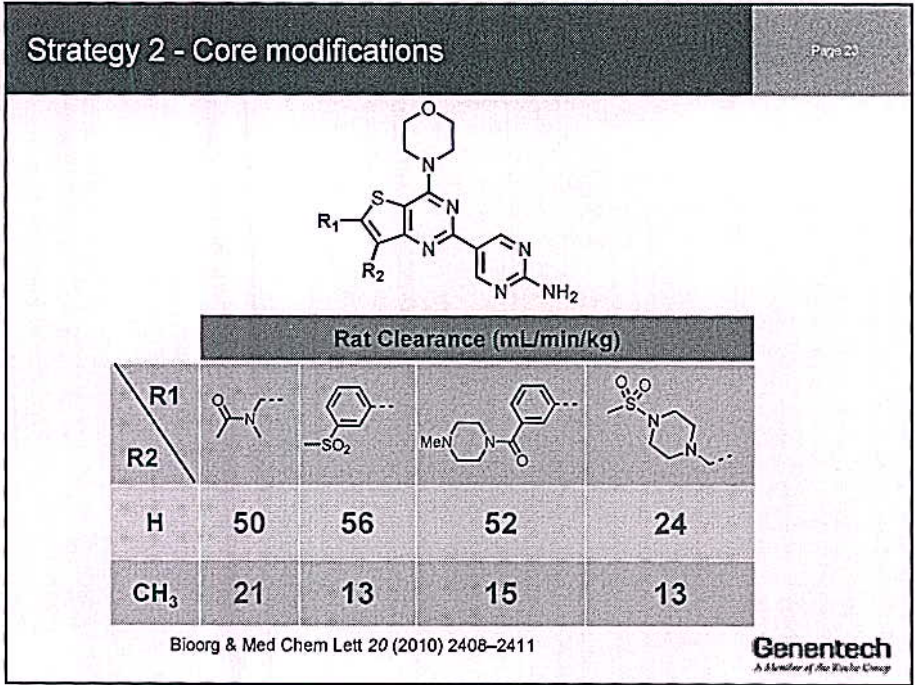
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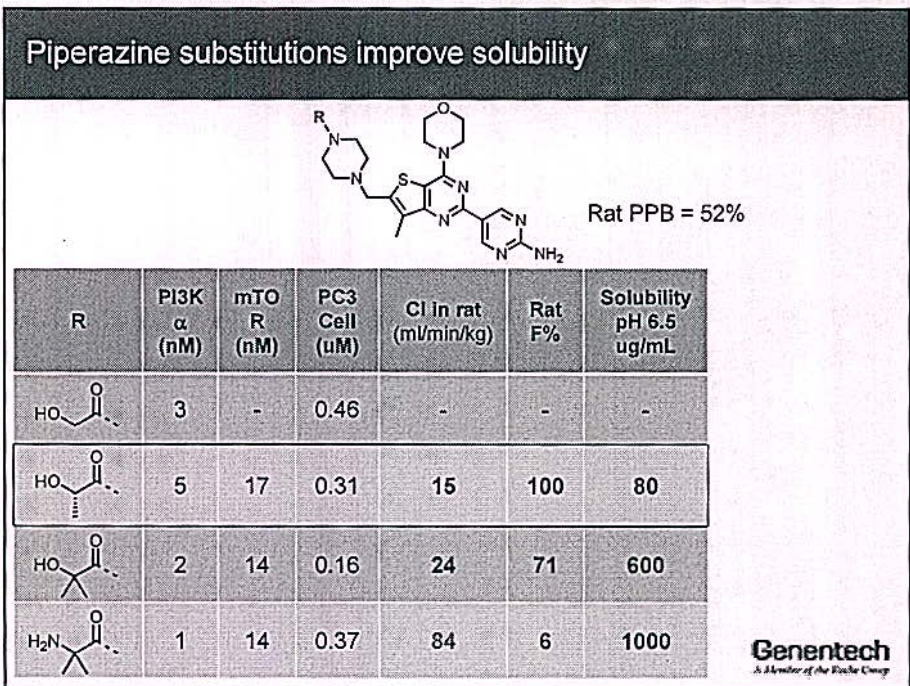
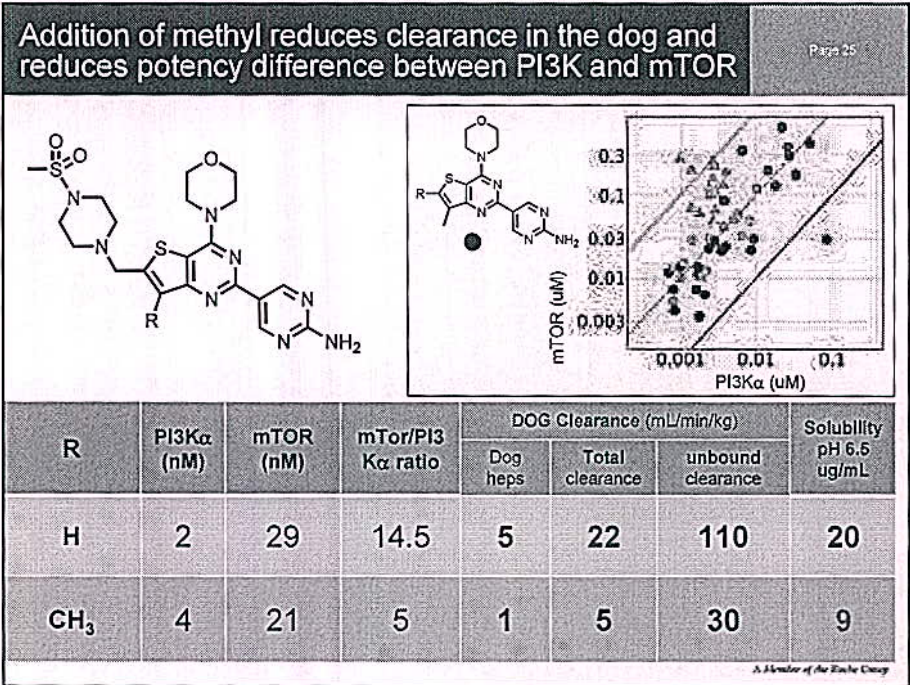
Increased free fraction & stability improves *in vivo* potency



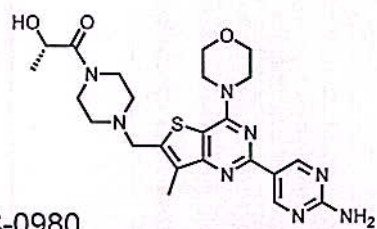
R	PI3K α (nM)	mTOR (nM)	PC3 (μ M)	Mouse Clearance mL/min/kg	PPB mouse	Free AUC at 50 mg/kg μ M·h
GDC-0941	3	580	0.28	60	95.6	1
GNE-696	5	45	0.27	24	81	8







**Dog PK remains low in more soluble molecule.
GDC-0980 selected as clinical candidate**

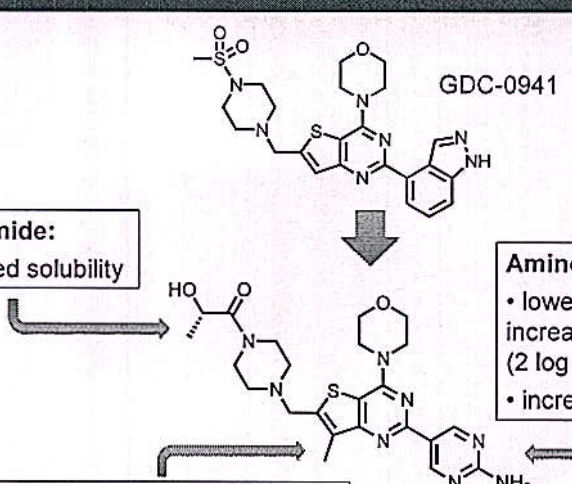


GDC-0980

PI3Kα (nM)	mTOR (nM)	PC3 Cell (μM)	Clearance in dog (ml/min/kg)			F%	Solubility pH 6.5 μg/mL
			in vitro	unbound clearance	unbound clearance		
5	17	0.31	4.4	6	12	100	80

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Key structural changes on route to GDC-0980



Lactic amide:

- increased solubility

Aminopyrimidine:

- lower clearance and increased free fraction (2 log drop in cLogP)
- increased potency

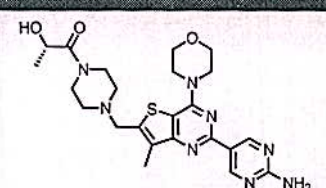
Methylthienopyrimidine core:

- lower clearance
- lower mTOR/PI3K ratio

GDC-0980

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GDC-0980 *in vitro* profile is excellent



- potent inhibitor of cell proliferation in PI3K driven lines
 - PC3: 310 nM
 - MCF7-neo/HER2: 470 nM
- selective for >145 kinases
- no significant responses in an *in vitro* pharmacology receptor screening assay (n=68 @10 uM)
- *in vitro* hERG assay clean (IC50>100 uM)
- negative for genotoxicity
- stable in human microsomes and hepatocytes
- <50% inhibition of CYP450s at 40 uM
- high free fraction across species (40-72%, 58% in human)

PI3K α : 5 nM

PI3K β : 27 nM

PI3K δ : 7 nM

PI3K γ : 14 nM

mTOR: 17 nM

DNAPK: 423 nM

VPS34: 1989 nM

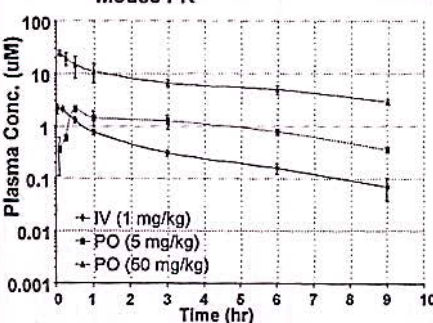
PI3KC2a: 1300 nM

PI3KC2b: 794 nM

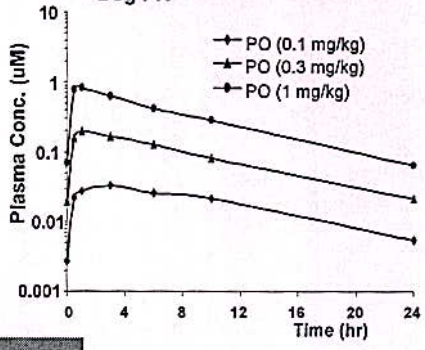
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Good solubility contributes to predictable PK for GDC-0980

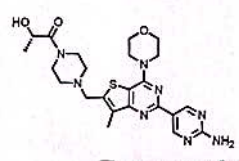
Mouse PK



Dog PK

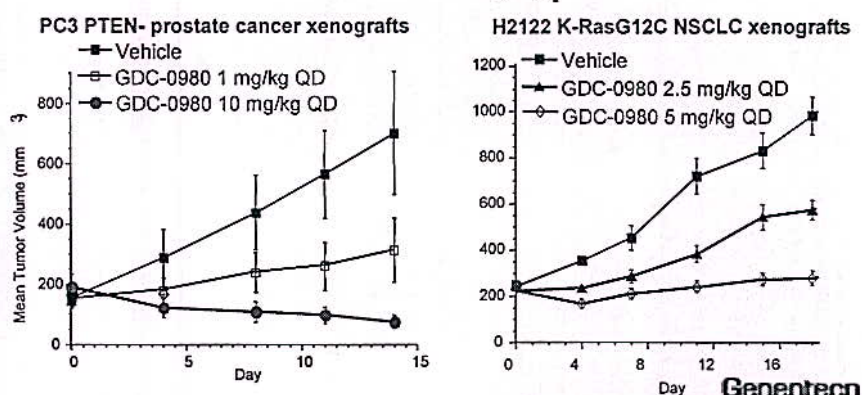
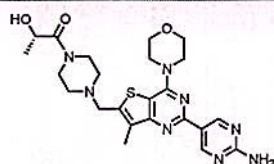


Species	Cl (mL/min/kg)	F%	% PPB
Mouse	9	100	71
Rat	15	100	59
Dog	6	100	48
Monkey	19	23	52

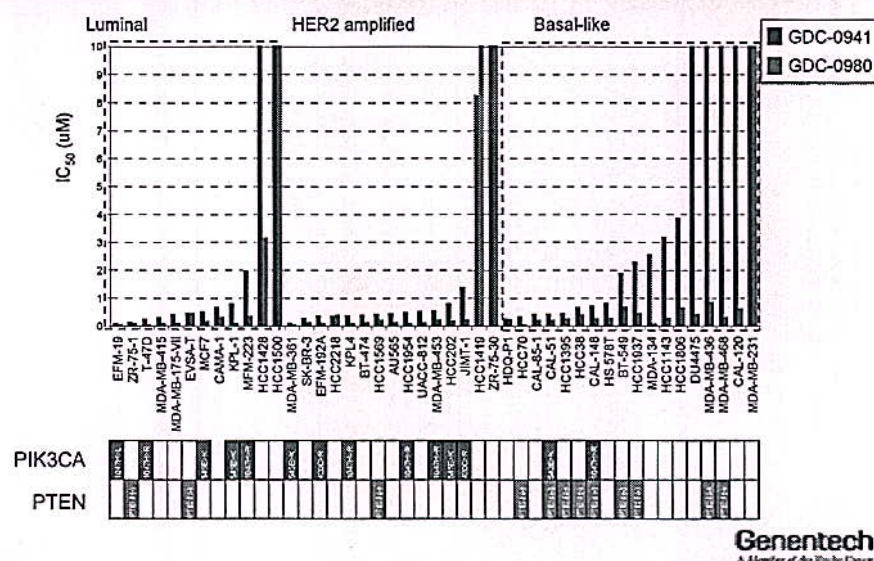


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GDC-0980 – low dose efficacy driven by good potency, a low clearance and a high free fraction



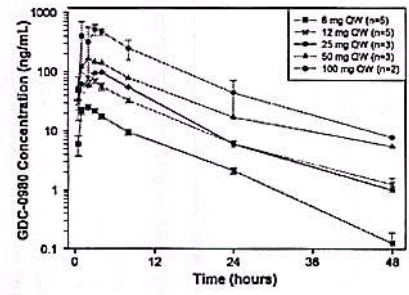
GDC-0941 and GDC-0980 Have Excellent Activity in Breast Cancer Cell Lines



GDC-0980 Phase I trials Ongoing: Favorable PK properties contributes to predictable PK in humans

- GDC-0980 Phase Ia daily and weekly dosing study started in 2009
- GDC-0980 is rapidly absorbed with a terminal half-life range of 6–18 hours and displays dose proportional increases in AUC and Cmax after a single oral dose (Figure A).

Figure A. Day 1 GDC-0980 Concentration-Time Profile (Mean Plot)



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GDC-0980 Phase I trials Ongoing: Pharmacokinetics and Pharmacodynamics in humans

- GDC-0980 Phase Ia daily and weekly dosing study started 2009
- GDC-0980 is rapidly absorbed with a terminal half-life range of 6–18 hours and displays dose proportional increases in AUC and Cmax after a single oral dose (Figure A).
- Decreased levels of the surrogate tissue marker pAKT in Platelet Rich Plasma were inversely correlated with plasma GDC-0980 concentrations (Figure B).
- Pre- and post-dose tumor biopsies demonstrate pS6 decrease, in addition to FDG-PET uptake decreases

Figure A. Day 1 GDC-0980 Concentration-Time Profile (Mean Plot)

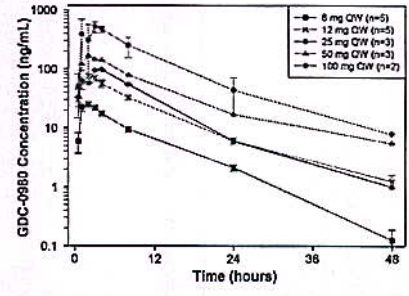
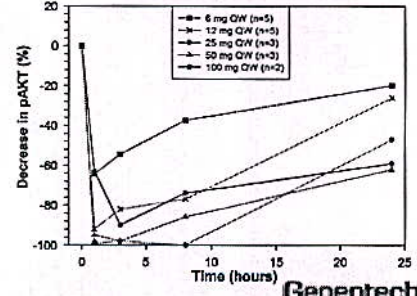


Figure B. Percent Change in pAKT from Baseline by Dose



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Wagner et al Abstract #389, 22nd EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics, 2010

Summary / Conclusion

Medicinal Chemistry en route to GDC-0980:

- Aminopyrimidine significantly changed overall properties relative to an indole
 - potency, stability, free fraction (contributes to efficacy at a low dose)
 - mTOR potency
- methyl thienopyrimidine lowered unbound clearance for analogs with piperazine sulfonamide
- lactic amides maintained favorable properties and added solubility

GDC-0980:

- GDC-0980 has an excellent *in vitro* and *in vivo* preclinical profile
- PK characteristics in human are consistent with preclinical results
- GDC-0980 demonstrated significant modulation of PD biomarkers in preclinical and clinical studies

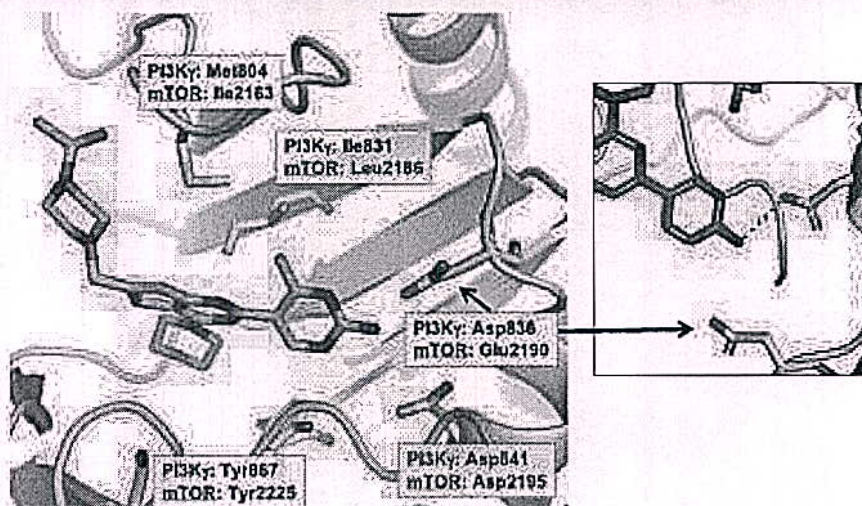
GDC-0980 is currently being studied in ongoing phase I and Ib trials in combination with additional therapies. Phase II trials are anticipated to start this year.

GDC-0980 presented on April 4th: Abs. # 2787

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Differences in the N-terminal lobe help to explain SAR

Page 34

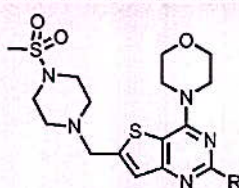


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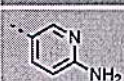
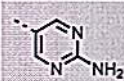
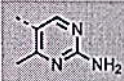
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Cell based potency for PI3K/mTOR inhibitors

Page 37



PC3: PTEN null (PI3K negative regulator)
MCF7.1: PI3K α activating mutation

R	PI3K α (nM)	mTOR (nM)	Fold selectivity	PC3 (μ M)	MCF7.1 (μ M)
	5	45	9	0.27	0.19
	2	29	15	0.31	0.13
	1	880	880	0.23	0.29

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ANEXO 3

Molecular Cancer Therapeutics



GDC-0980 Is a Novel Class I PI3K/mTOR Kinase Inhibitor with Robust Activity in Cancer Models Driven by the PI3K Pathway

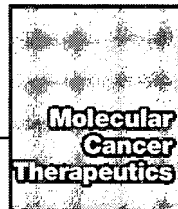
Jeffrey J. Wallin, Kyle A. Edgar, Jane Guan, et al.

Mol Cancer Ther 2011;10:2426-2436. Published OnlineFirst October 13, 2011.

Updated Version	Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-11-0446
Supplementary Material	Access the most recent supplemental material at: http://mct.aacrjournals.org/content/suppl/2011/10/07/1535-7163.MCT-11-0446.DC2.html

Cited Articles	This article cites 27 articles, 12 of which you can access for free at: http://mct.aacrjournals.org/content/10/12/2426.full.html#ref-list-1
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GDC-0980 Is a Novel Class I PI3K/mTOR Kinase Inhibitor with Robust Activity in Cancer Models Driven by the PI3K Pathway

Jeffrey J. Wallin¹, Kyle A. Edgar¹, Jane Guan¹, Megan Berry¹, Wei Wei Prior¹, Leslie Lee¹, John D. Lesnick², Cristina Lewis², Jim Nonomiya², Jodie Pang³, Laurent Salphati³, Alan G. Olivero⁴, Daniel P. Sutherlin⁴, Carol O'Brien⁵, Jill M. Spoerke⁵, Sonal Patel⁶, Letitia Lensun⁶, Robert Kassees¹, Leanne Ross¹, Mark R. Lackner⁵, Deepak Sampath¹, Marcia Belvin¹, and Lori S. Friedman¹

Abstract

Alterations of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway occur broadly in cancer via multiple mechanisms including mutation of the *PIK3CA* gene, loss or mutation of phosphatase and tensin homolog (PTEN), and deregulation of mammalian target of rapamycin (mTOR) complexes. The dysregulation of this pathway has been implicated in tumor initiation, cell growth and survival, invasion and angiogenesis, thus, PI3K and mTOR are promising therapeutic targets for cancer. We discovered GDC-0980, a selective, potent, orally bioavailable inhibitor of Class I PI3 kinase and mTOR kinase (TORC1/2) with excellent pharmacokinetic and pharmaceutical properties. GDC-0980 potently inhibits signal transduction downstream of both PI3K and mTOR, as measured by pharmacodynamic (PD) biomarkers, thereby acting upon two key pathway nodes to produce the strongest attainable inhibition of signaling in the pathway. Correspondingly, GDC-0980 was potent across a broad panel of cancer cell lines, with the greatest potency in breast, prostate, and lung cancers and less activity in melanoma and pancreatic cancers, consistent with KRAS and BRAF acting as resistance markers. Treatment of cancer cell lines with GDC-0980 resulted in G1 cell-cycle arrest, and in contrast to mTOR inhibitors, GDC-0980 induced apoptosis in certain cancer cell lines, including those with direct pathway activation via PI3K and PTEN. Low doses of GDC-0980 potently inhibited tumor growth in xenograft models including those with activated PI3K, loss of LKB1 or PTEN, and elicited an exposure-related decrease in PD biomarkers. These preclinical data show that GDC-0980 is a potent and effective dual PI3K/mTOR inhibitor with promise for the clinic. *Mol Cancer Ther*; 10(12); 2426–36. ©2011 AACR.

Introduction

Upregulation of the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway is a common feature in most cancers (reviewed in ref. 1). Genetic deviations in the pathway have been detected in many human cancers (2) and act primarily to stimulate cell proliferation, migration, and survival. Activation of the pathway occurs following activating point mutations or amplifications of the *PIK3CA* gene encoding the p110 α PI3K isoform (3, 4). Genetic deletion or loss of function mutations within the tumor suppressor PTEN, a phosphatase with opposing

function to PI3K, also increases PI3K pathway signaling (5). These aberrations lead to increased downstream signaling through kinases such as Akt and mTOR, and increased activity of the PI3K pathway has been proposed as a hallmark of resistance to cancer treatment (6–10).

Oncogenes such as PI3K, AKT, epidermal growth factor receptor (EGFR), and human epithelial growth factor receptor 2 (HER2) stimulate proliferation, growth and survival by activating mTOR kinase (reviewed in ref. 11). The mTOR kinase activity has been attributed to 2 protein complexes, mTORC1 and mTORC2 (12). Rapamycin specifically disrupts the mTORC1 complex and rapalogs have activity in renal cancers though patients all progress eventually, which may be due, in part, to feedback to IRS-1 and activation of AKT (reviewed in ref. 13). Therefore, inhibiting both mTOR complexes with an mTOR kinase inhibitor is predicted to counter feedback by blocking the PDK2 function of mTORC2. In addition, inhibiting PI3K in the same molecule creates a blockade higher in the pathway where IRS1 signals to PI3K.

Therapeutic targeting of the PI3K pathway with small molecule inhibitors may have clinical benefit, either as single agents in PI3K-addicted cancers or used more broadly in combination with other conventional or

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Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

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targeted therapies. Several inhibitors targeting the PI3K pathway have now entered clinical trials (14–16). Here, we describe preclinical data for the novel PI3K/mTOR inhibitor GDC-0980. We show that GDC-0980 potently inhibits pathway signaling and viability in the majority of solid tumor cancer cell lines investigated. Furthermore, we show that GDC-0980 is efficacious in the majority of xenograft models investigated. The pharmacokinetic properties of GDC-0980 allow for intermittent *in vivo* dosing without sacrificing antitumor efficacy.

Materials and Methods

Cell culture

Cell lines were obtained from the American Type Culture Collection or from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). Cell lines were tested and authenticated using gene expression and single nucleotide polymorphism genotyping arrays, as previously described (17, 18). Lines were cultured in DMEM or RPMI supplemented with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C under 5% CO₂. MCF7-neo/HER2 is an *in vivo* selected tumor cell line developed at Genentech and derived from the parental MCF7 human breast cancer cell line.

Materials

GDC-0941 and GDC-0980 were generated at Genentech, Inc. mTOR1/2 inhibitor is from patent WO 2008/023159 A1. Antibodies used include phospho-Akt^{Thr308}, phospho-AKT^{Ser473}, AKT, phospho-PRAS40^{Thr246}, phospho-S6^{Ser235/236}, phospho-S6^{Ser240/242}, S6, phospho-ERK^{Thr202/Tyr204}, ERK, cleaved PARP, and cyclin D1 obtained from Cell Signaling and a β-actin antibody was obtained from Sigma.

Mutation detection

Screening for mutation was done by sequencing of exons and adjacent intronic junctions (Polymorphic DNA Technologies, Inc.). Primers for PCR and sequencing were designed using the primer3 program (Integrated DNA Technologies, Inc.). Nested PCR products were treated with ExoSAP from USB. PCR products were sequenced using BigDye Terminator Mix using an ABI3730xl (Applied Biosystems). Trace files were analyzed using the software program Agent (Paracel), Sequencher (Genecodes) and Mutation Surveyor 3.0 (SoftGenetics).

Cell viability assays

Three hundred and eighty-four-well plates were seeded with 2,000 cells/well in a volume of 54 µL per well followed by incubation at 37°C under 5% CO₂ overnight (~16 hours). Compounds were diluted in dimethyl sulfoxide to generate the desired stock concentrations then added in a volume of 6 µL per well. All treatments were tested in quadruplicate. After 4 days incubation, relative numbers of viable cells were estimated using CellTiter-Glo (Promega) and total luminescence was measured on a

Wallac Multilabel Reader (PerkinElmer). The concentration of drug resulting in 50% inhibition of cell viability (IC₅₀) or 50% maximal effective concentration (EC₅₀) was determined using Prism software (GraphPad). For cell lines that failed to achieve an IC₅₀ the highest concentration tested (20 µmol/L) is listed.

Protein assays

Ten centimeter square dishes were seeded with 2 million cells in a volume of 10 mL followed by incubation at 37°C under 5% CO₂ overnight (~16 hours). Cells were treated with the indicated concentration of GDC-0941, GDC-0980, or mTOR1/2 inhibitor for the time indicated. Following treatment, cells were washed with cold PBS and lysed in 1X Cell Extraction Buffer from Biosource supplemented with protease inhibitors (Roche), 1 mmol/L PMSF, and Phosphatase Inhibitor Cocktails 1 and 2 from Sigma. Protein concentration was determined using the Pierce BCA Protein Assay Kit. For immunoblots, equal protein amounts were separated by electrophoresis through NuPage Bis-Tris 10% gradient gels (Invitrogen); proteins were transferred onto polyvinylidene difluoride membranes using the Criterion system and protocol from Bio-Rad.

Levels of phospho-Akt^{Ser473} and total Akt were assessed in xenograft tumors using biomarker kits from Meso Scale Discovery.

Xenograft studies

In vivo efficacy of compounds was evaluated in the following cancer cell line tumor xenograft models: breast cancer cell lines MX-1, MCF7, MDA-MB-231, Cal-51, MCF7-neo/HER2, KPL4, the Fo5 HER2+ mouse transplant model, and the MAXF1162 primary human transplant model (Oncotest); non-small cell lung cancer (NSCLC) lines—NCI-H1299, NCI-H2122, A549 LXFL529 (Oncotest); pancreatic cancer cell lines—KP4 and Mia-PaCa-2; colon cancer cell lines—HCT-116, Colo205, LoVo, and DLD-1; prostate cancer lines—LuCap35V and PC3; melanoma line A375. Cells or tumor fragments were implanted subcutaneously into the flank of each mouse. Before cell inoculation with MCF7 or MCF7-neo/HER2 cells, 17β-estradiol pellets (0.36 mg/pellet, 60-day release, No. SE-121; Innovative Research of America) were implanted into the dorsal shoulder. After implantation of cells into mice, tumors were monitored until they reached mean tumor volumes of 180 to 350 mm³ and were distributed into groups of no less than 8 animals/group ensuring each group had equivalent mean tumor volumes before initiating dosing. Female nude (*nu/nu*, athymic nude or NMRI nude) mice that were 6 to 8 weeks old and weighed 20 to 30 g were obtained from Charles River Laboratories, Harlan Laboratories, or Taconic. GDC-0980 was formulated at various concentrations in 0.5% methylcellulose with 0.2% Tween-80 to achieve the indicated dosages and was administered daily, every 4 days, or weekly with 100 µL via oral gavage. Docetaxel (Sanofi Aventis) was dosed at 5 or 10 mg/kg,

intraperitoneally, every week. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula $(L \times W \times W)/2$. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that $\%TGI = 100 \times 1 - (AUC_{\text{treatment}}/\text{day})/(AUC_{\text{vehicle}}/\text{day})$. Curve fitting was applied to $\log_2$ transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1 to 97 in R v2.12.0.

Tumor sizes and body weights were recorded twice weekly over the course of the study. Mice with tumor volumes 2,000 or more mm^3 or with losses in body weight 20% or more from their body weight at the start of treatment were euthanized per IACUC guidelines.

For pharmacodynamic (PD) marker analysis, PC3 or MCF7-neo/HER2 xenograft tumors were excised from animals and immediately snap frozen in liquid nitrogen. Frozen tumors were weighed and processed using a pestle (Scieneware) in Lysis Buffer (see above). Levels of phospho-Akt^{Ser473} and total Akt were assessed in xenograft tumors using biomarker kits from Meso Scale Discovery.

Statistics

Significant differences (*P* values) comparing lines with and without evaluated genetic abnormalities was determined by 2-tailed Mann-Whitney test calculated using the JMP statistical software, version 5.1.2 (JMP Software).

Results

GDC-0980 reduces viability in cancer cell lines by cell-cycle inhibition and induction of apoptosis

GDC-0980 is a potent small-molecule inhibitor of class I PI3K isoforms and mTOR kinase and displays excellent selectivity against a large panel of other kinases, including closely related family members DNA-PK, VPS34, c2alpha, and c2beta (Fig. 1; ref. 19). We assessed a panel of breast, non-small cell lung, colon, pancreatic, melanoma, and prostate cancer cell lines in cell viability experiments to profile the activity of GDC-0980 *in vitro* (Fig. 1). GDC-0980 was most effective in prostate ($IC_{50} < 200$ nmol/L 50%, < 500 nmol/L 100%), breast ($IC_{50} < 200$ nmol/L 37%, < 500 nmol/L 78%) and NSCLC lines ($IC_{50} < 200$ nmol/L 29%, < 500 nmol/L 88%), and was less effective in pancreatic ($IC_{50} < 200$ nmol/L 13%, < 500 nmol/L 67%) and melanoma cell lines ($IC_{50} < 200$ nmol/L 0%, < 500 nmol/L 33%). Taken together, GDC-0980 had broad cellular activity with potency below 500 nmol/L in 124 out of the 167 cell lines tested.

GDC-0941 is a potent inhibitor of class I PI3K isoforms, but not mTOR and is also currently being evaluated in clinical trials (14, 20). Overall, GDC-0980 was more effective than GDC-0941 in cell viability experiments (Fig. 1). The increased potency of GDC-0980 was evident across all evaluated tumor tissue types ($P < 0.001$) and all genotypes except those that are HER2 amplified (Supplementary Fig. S1).

Cell viability IC_{50} values and half-maximal EC_{50} values for rapamycin are different due to the shallow dose-titration curves (Supplementary Fig. S2). Consistent with previous reports (21), we found that direct inhibition of the mTOR kinase and PI3K was more effective than rapamycin treatment alone.

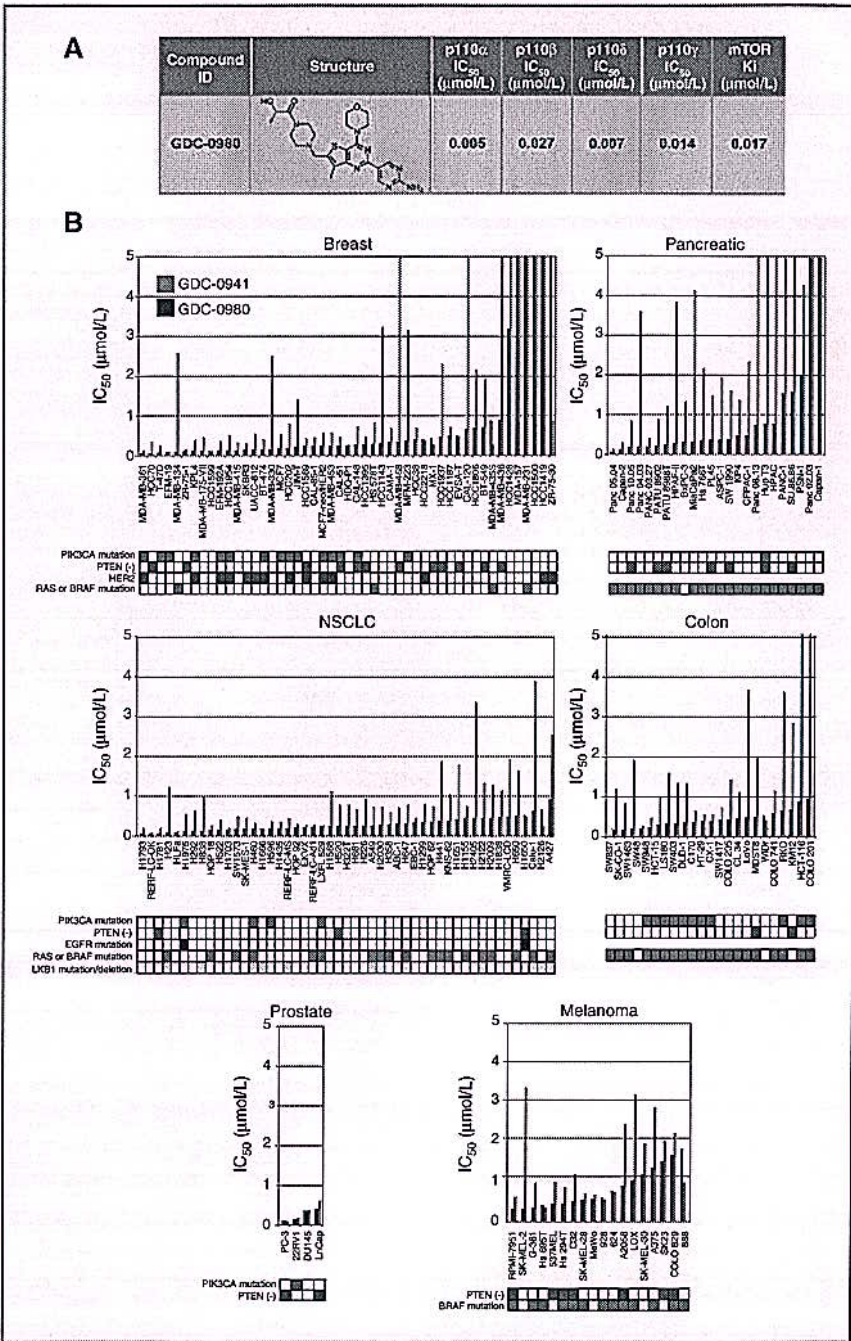
To investigate potential molecular predictors of response to GDC-0980, we examined whether key alterations in the PI3K and MAPK pathways such as HER2 amplification, *PIK3CA* mutations, loss of PTEN protein, *LKB1* inactivating mutations, deletions or loss, *EGFR* activating mutations, *RAS* hotspot mutations, or *BRAF* hotspot mutations were associated with increased or decreased sensitivity to GDC-0980 (Supplementary Tables S2–8). Across all tumor types hotspot mutations within *BRAF* or *RAS* proteins were negative predictors of GDC-0980 potency ($P = 0.01$), while no significant positive predictors of potency were identified. Across all lines a trend was observed for PTEN loss [PTEN (–)] association with GDC-0980 potency, but this was not statistically significant ($P = 0.07$). Sensitivity of breast tumor cell lines to GDC-0980 was similar to what has been previously described for the class I PI3K inhibitor GDC-0941 (22). Breast tumor cell lines harboring mutations in the *PIK3CA* gene exhibit increased sensitivity to GDC-0980 ($P = 0.01$). In NSCLC lines, mutations in *BRAF* or *RAS* proteins approached significance ($P = 0.07$) as negative predictors of GDC-0980 sensitivity.

We next examined signaling components of the PI3K signaling network (Fig. 2A) in cell lines representing each of these 6 tumor tissue types. In each cell line treated with GDC-0980 for 4 hours, phosphorylation of all downstream markers was reduced (Fig. 2B). These included signaling markers downstream of PI3K, such as phospho-Akt (Thr308), which is phosphorylated by PDK1, and biomarkers downstream of mTOR such as phospho-Akt (Ser473) and phospho-S6 (Fig. 2A). We also assessed phospho-ERK (Thr202/Tyr204) in GDC-0980 treated samples and observed a slight reduction of this marker in 2 of 6 cell lines.

To determine the downstream molecular consequences of PI3K pathway inhibition by GDC-0980 on cancer cells, we assessed markers of cell cycle, cyclin D1, and apoptosis, cleaved-poly (ADP-ribose) polymerase (cleaved-PARP), 24 hours after GDC-0980 treatment (Fig. 2C). Cyclin D1 is expressed in proliferating cells and helps control progression of cells through the cell cycle (23). A dose-dependent reduction in cyclin D1 levels in response to GDC-0980 was observed in most cancer lines, with the exception of the pancreatic cell line KP4 and the melanoma line A375, which were both less responsive to GDC-0980 in the viability assay (Fig. 1). PARP is one of the main cleavage targets of caspase-3 and cleaved PARP serves as a marker for apoptotic cells (24). Cleaved PARP was detected following GDC-0980 treatment in both the KPL4 (HER2+, PI3K^{H1047R}) and LoVo (KRas^{G13D}) cell lines, indicating that an apoptotic response had been triggered in these cells as early as 24 hours.

70

Figure 1. Potency of GDC-0980 PI3K/mTOR inhibitor in biochemical and cell-based assays. A, GDC-0980 IC₅₀ for PI3K α , β , δ , and γ isoforms, and Ki for mTOR kinase. B, GDC-0980 and GDC-0941 (class I PI3K inhibitor) IC₅₀ values were determined in a 96-hour viability assay in breast, NSCLC, pancreatic, colon, melanoma, and prostate cancer lines. Cell lines were categorized into the tumor tissue types from which they were derived. Any detected mutation for PIK3CA, RAS, BRAF, EGFR, or LKB1 is indicated by a colored square. PTEN (–) indicates a nondetectable signal for this protein by Western blot.



Inhibition of mTOR does not augment apoptotic responses

We used inhibitors with different target specificities to investigate the signaling and apoptotic consequences of solely blocking PI3K, solely blocking mTOR, or dual

blockade of both. In these studies, GDC-0980 was compared with GDC-0941 PI3K inhibitor and an mTOR kinase inhibitor. To evaluate the effects of inhibition of mTOR kinase, we used mTOR kinase inhibitor, an ATP-competitive selective small molecule inhibitor of TORC1 and

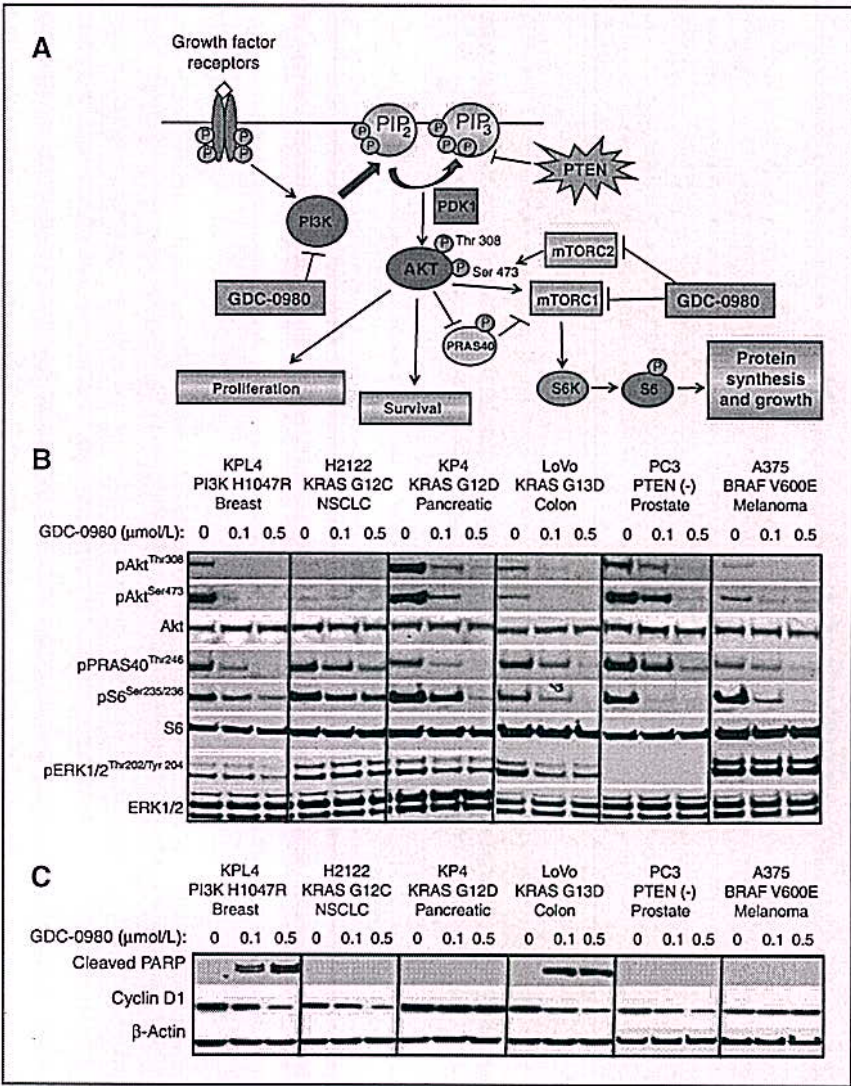


Figure 2. Cell pathway and mechanistic effects of GDC-0980 treatment in tumor cell lines. A, GDC-0980 decreases PI3K pathway signaling in all tumor cell lines. Immunoblots from 4-hour-treated samples showing protein concentrations for phospho-Akt^{Thr308} (pAkt^{Thr308}), phospho-Akt^{Ser473} (pAkt^{Ser473}), Akt, phospho-PRAS40^{Thr246} (pPRAS40^{Thr246}), phospho-S6^{Ser235/236} (pS6^{Ser235/236}), and S6. B, disparate apoptosis and cell-cycle effects with GDC-0980 treatment. Protein concentrations of apoptosis marker cleaved PARP and cell-cycle marker cyclin D1 after 24 hours. C, PI3K pathway signaling and GDC-0980 treatment.

TORC2 (25). The potency (Ki) against mTOR kinase for GDC-0980, GDC-0941, and mTOR1/2 inhibitor is 17 nmol/L, 580 nmol/L, and 5 nmol/L, respectively. For these studies, single agent treatments with the 3 inhibitors were used in the KPL4 PI3K mutant breast cancer cell line, at concentrations ranging from 78 nmol/L to 5 μmol/L (Fig. 3). At 4 hours of treatment, all 3 inhibitors had reduced phospho-Akt (Ser473) levels. The phospho-S6 (Ser235/236) reduction was strongest for GDC-0980. GDC-0941 resulted in a reduction of both phospho-S6 sites at 4 hours, but the pS6 decrease was not as significant as phospho-Akt. By 24 hours phospho-S6 (Ser240/242) levels were strongly decreased with GDC-0941 treatments. GDC-0980 was most effective against all 4 phosphorylation markers of pathway activity, indicating a more complete blockade was

quickly achieved and sustained with the PI3K/mTOR inhibitor.

To investigate the consequences of inhibiting the pathway at different nodes, we looked at the marker of apoptosis cleaved-PARP at 4 and 24 hours posttreatment in the KPL4 (PI3K^{H1047R}) cell line and at 24 hours in EVSA-T [PTEN (-)] and HDQ-P1 cell lines. We detected a difference between mTOR inhibitor and PI3K inhibitors in their ability to induce apoptosis (Fig. 3). An increase in cleaved PARP was minimally detected with mTOR inhibition at any concentration, but an increase of cleaved-PARP was observed in a dose-dependent manner with GDC-0941 or GDC-0980 treatments in all 3 lines, regardless of genotype. Thus mTOR kinase inhibition alone was not enough to induce apoptosis, while PI3K inhibition was sufficient.

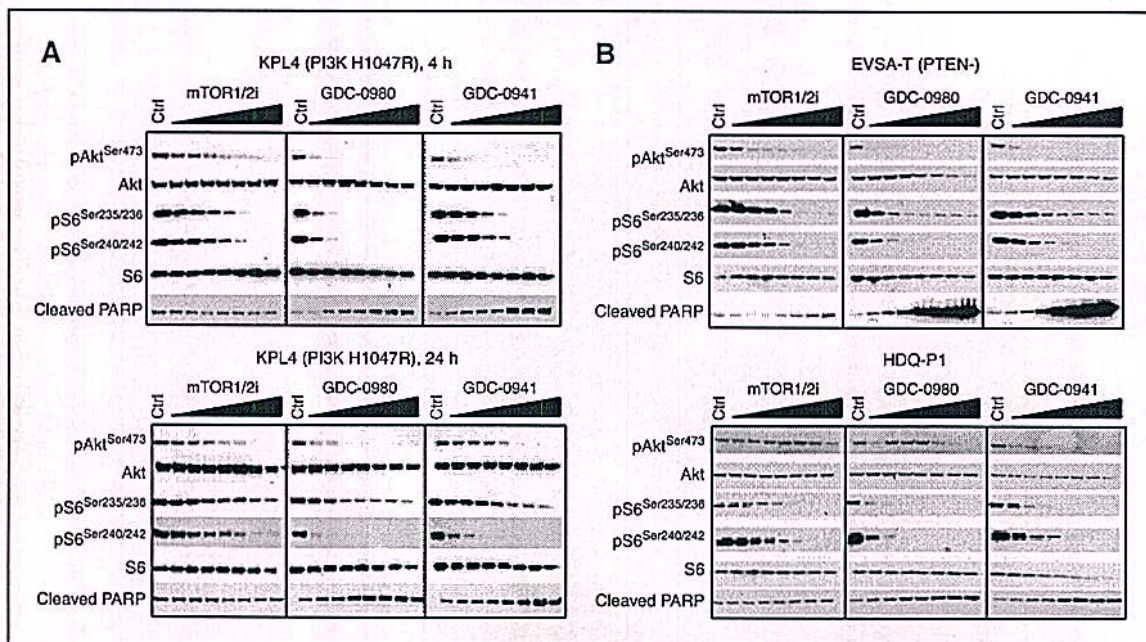


Figure 3. Pharmacodynamic and apoptotic responses to PI3K pathway node targeted small molecule inhibitors. A, immunoblots from 4-hour and 24-hour-treated KPL4 cell samples for PI3K pathway proteins phospho-Akt (pAkt^{Ser473}), Akt, phospho-S6 (phospho-S6^{Ser235/236} and phospho-S6^{Ser240/242}), S6, and the apoptotic marker cleaved PARP. Small molecule inhibitors started at 5 μ M and were dose-titrated with 2-fold dilutions. B, immunoblots from 24-hour-treated EVSA-T and HDQ-P1 cell samples. Treatments are identical to A.

Oral dosing of GDC-0980 results in significant antitumor responses in xenograft models on daily or intermittent schedules

Pharmacokinetic (PK) studies indicated that GDC-0980 has excellent PK properties in mouse, with high oral bioavailability, low to moderate clearance and low plasma protein binding, consistent with its solubility and pharmaceutical properties (Supplementary Table S1, Supplementary Fig. S4, and ref. 19). The *in vivo* antitumor activity of GDC-0980 was investigated in 20 xenograft tumor models representative of 6 different human tumor types (Fig. 4A). For these studies, mice-bearing established tumors (7–10 days postimplantation, tumor volume 150–250 mm³) were dosed orally with GDC-0980 at 5 mg/kg GDC-0980 daily for 21 days. The 5 mg/kg dose was approximately 70% of the maximum tolerated dose based on body weight loss. A low dose of GDC-0980 was sufficient to generate significant efficacy because of the high exposure achieved following oral dosing and the high-free fraction. The data in Fig. 4A is represented as percent tumor growth inhibition (%TGI) at the end of the dosing period, with 100% TGI indicating tumor stasis. Overall, dosing GDC-0980 at 5 mg/kg daily resulted in greater than 50% TGI in 15 of the 20 xenograft models. The weakest *in vivo* effects were observed in the melanoma A375 (BRAF^{V600E}) model, triple negative breast cancer MX-1 [PTEN (–)] model, and 2 pancreatic KRAS mutant xenograft models, KP4 (KRAS^{G12C}) and MiaPaCa2 (KRAS^{G12C}). Consistent with *in vitro* data in other cell

lines, we detected cleaved-PARP in 2 MCF7-neo/HER2 xenograft tumors 4 hours after the last dose (Fig. 2B).

The possibility of changing dose and schedule may enhance the ability to balance tolerability and activity in the clinic. In the PC3 xenograft model dose-dependent tumor growth inhibition resulted from GDC-0980 administered orally either daily, every 4 days, or on a once weekly schedule (Fig. 4C). Tumor growth delay was observed on each schedule, and was dose dependent.

The PTEN null PC3 prostate cancer xenograft was used to investigate the relationship of PK, PD, and efficacy of GDC-0980 (Fig. 5). In mice that were administered GDC-0980 daily, tumor growth delay occurred at 2.5 mg/kg, tumor stasis at 5 mg/kg, and tumor regression at 10 mg/kg (Fig. 5A). Single dose GDC-0980 PK/PD relationships were evaluated in animals with established PC3 tumors. In these studies, GDC-0980 was dosed at 2.5, 5, or 10 mg/kg and plasma concentrations of GDC-0980 and tumor levels of pAkt were evaluated more than 48 hours. As expected, an increase in unbound GDC-0980 was detected in the plasma, and drug concentration decreased over time (Fig. 5B). Phosphorylated Akt at the Ser473 site was evaluated in tumors and normalized with total Akt protein (pAkt/tAkt) at the same time points. All 3 doses of GDC-0980 reduced pAkt/tAkt levels significantly for the first 8 hours, and an unbound GDC-0980 concentration of at least 0.2 μ M/L was required to decrease this marker by at least 75%. By 48 hours unbound GDC-0980 was not detected in any of the dose groups. Relationships between

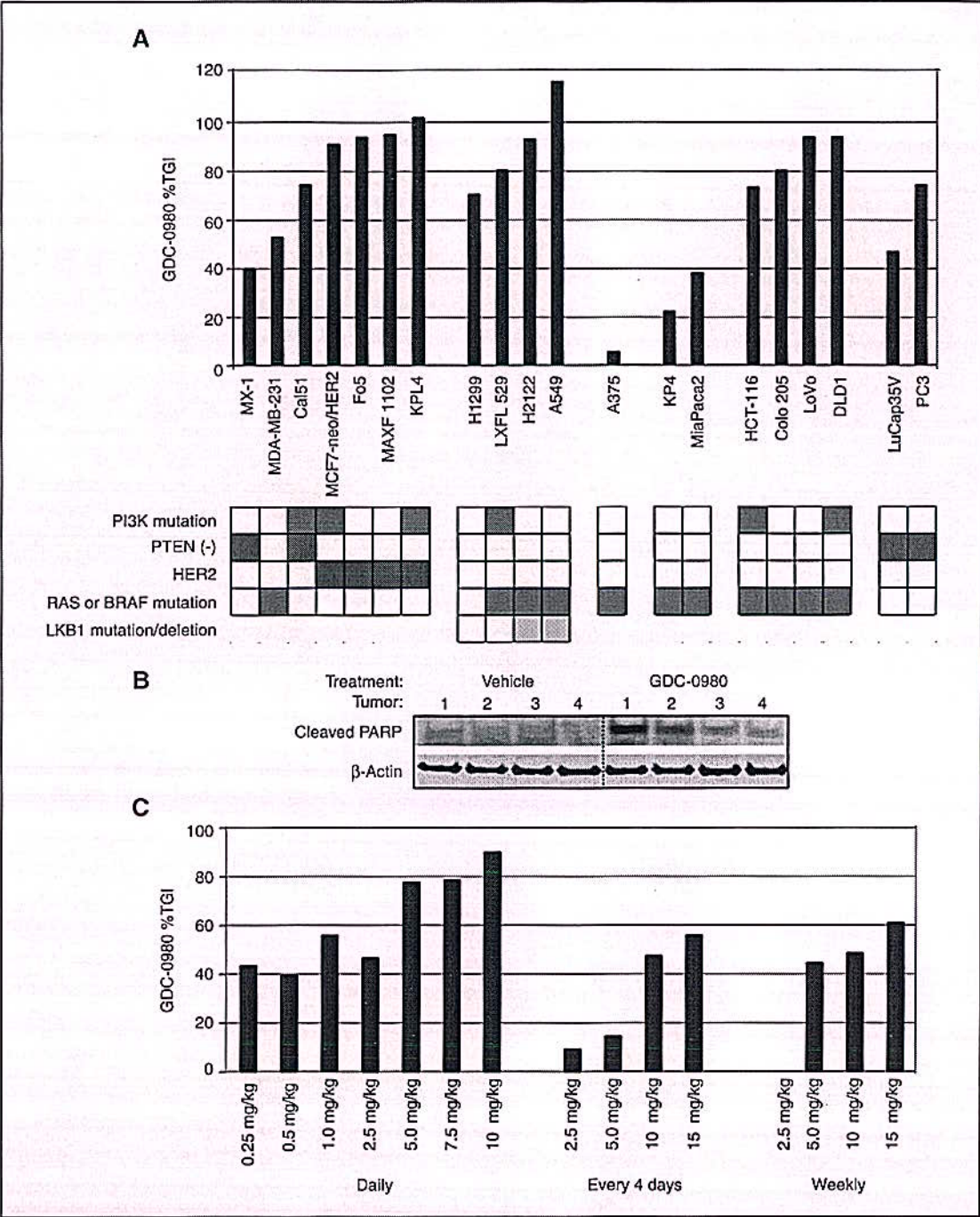
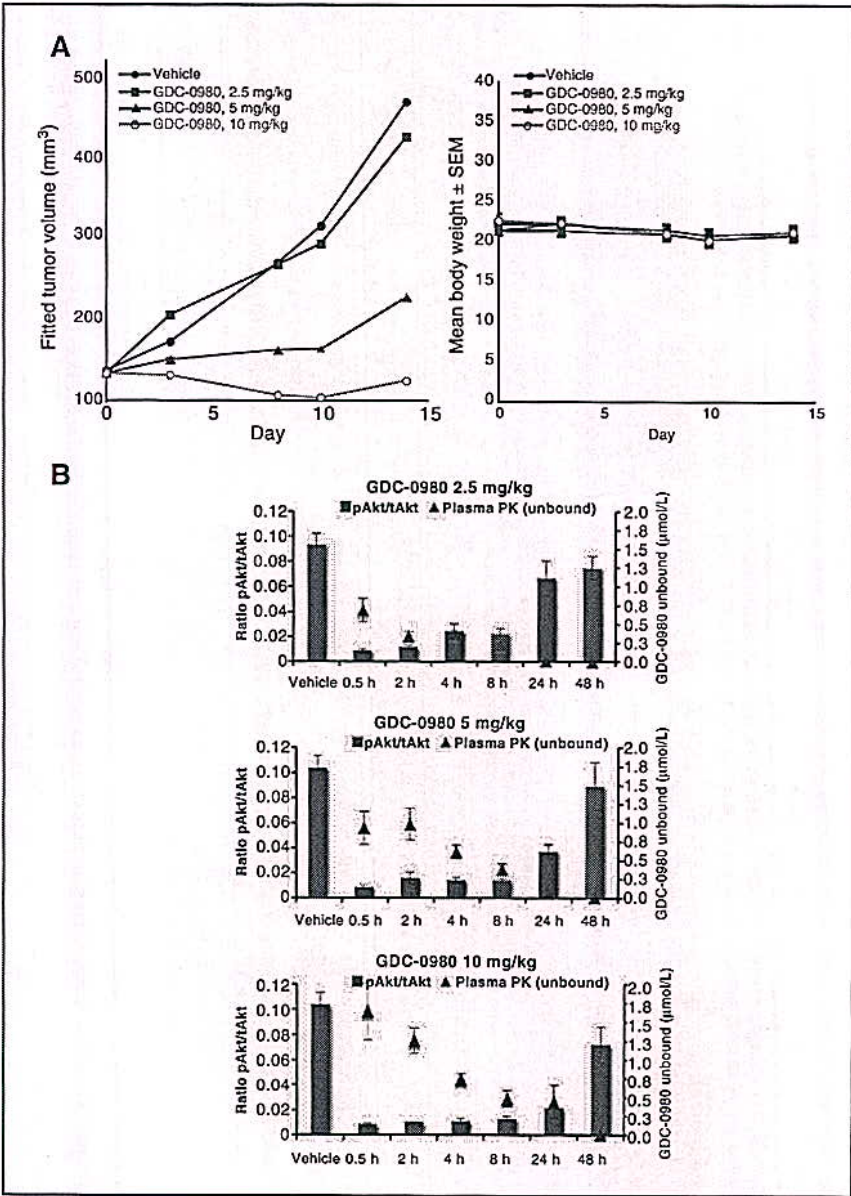


Figure 4. Single agent efficacy of GDC-0980 in human xenograft tumor models. A, daily dosing of GDC-0980 in multiple tumor xenograft models. Tumor-bearing mice were dosed orally with GDC-0980 (5 mg/kg) or vehicle each day for 21 days. Data is represented as percent tumor growth inhibition (%TGI) and is a comparison of the 5 mg/kg GDC-0980 treatment groups to vehicle at day 21. The higher the %TGI value, the more significant level of efficacy that was achieved in the study. One hundred %TGI is indicative of complete tumor stasis. Tumor growth delay increases from zero to one hundred %TGI. B, tumors treated with GDC-0980 undergo apoptosis. MCF7-neo/HER2 tumor-bearing mice were treated 4 hours with vehicle or GDC-0980 at 7.5 mg/kg. Four xenograft tumors are represented in each group. C, GDC-0980 is efficacious when dosed intermittently. PC3 tumor-bearing mice were dosed orally on different treatment schedules with vehicle or different amounts of GDC-0980 each day for 21 days. Data is represented as %TGI as in A.

74

Figure 5. GDC-0980 efficacy is linked to pharmacodynamic and pharmacokinetic readouts. A, dose proportional response to GDC-0980 treatment in PC3 human xenograft tumors. Tumor-bearing mice were dosed orally with vehicle or GDC-0980 daily for 14 days at the concentrations indicated. B, increases in GDC-0980 free drug concentration is linked to reductions in PI3K pathway signaling. Tumor-bearing mice were dosed orally with vehicle or GDC-0980 at the concentrations indicated. Phospho-Akt^{Ser473} and total Akt protein concentrations were determined by ELISA in xenograft tumors in a time course after dosing. Data for proteins are shown as a ratio of phospho-Akt^{Ser473} to total Akt (pAkt/tAkt, left axis, gray bars). Free drug concentration for GDC-0980 (GDC-0980 unbound, right axis, blue triangles) is also indicated.



efficacy, GDC-0980 plasma levels and tumor pAkt/tAkt levels can be described by assessing the data at 24 hours. In the 2.5 mg/kg cohort, a dose that results in tumor growth delay in this xenograft model, GDC-0980 plasma levels were undetectable and pAkt/tAkt levels had returned to baseline at 24 hours. At the 5 mg/kg dose, which causes tumor stasis, unbound GDC-0980 was minimally measurable in the plasma at the 24-hour time point, indicating the molecule was clearing. As a consequence, the pAkt/tAkt signal in the xenograft tumor was starting to return to baseline, with approximately 60% reduction at 24 hours. At a GDC-0980 dose of 10 mg/kg, which causes tumor

regression, GDC-0980 was measurable in the plasma at 24 hours and pAkt/tAkt levels were decreased by 75%. Thus, the difference in tumor response to GDC-0980 treatment correlated with the duration of knockdown of pAkt/tAkt, and is most evident when assessing plasma PK and tumor PD effects at 24 hours after drug dosing.

GDC-0980 enhances the antitumor activity of docetaxel *in vivo*

Resistance to anticancer agents has been attributed to increased PI3K/PTEN pathway signaling to promote cell survival, hence inhibitors of the pathway have potential

75

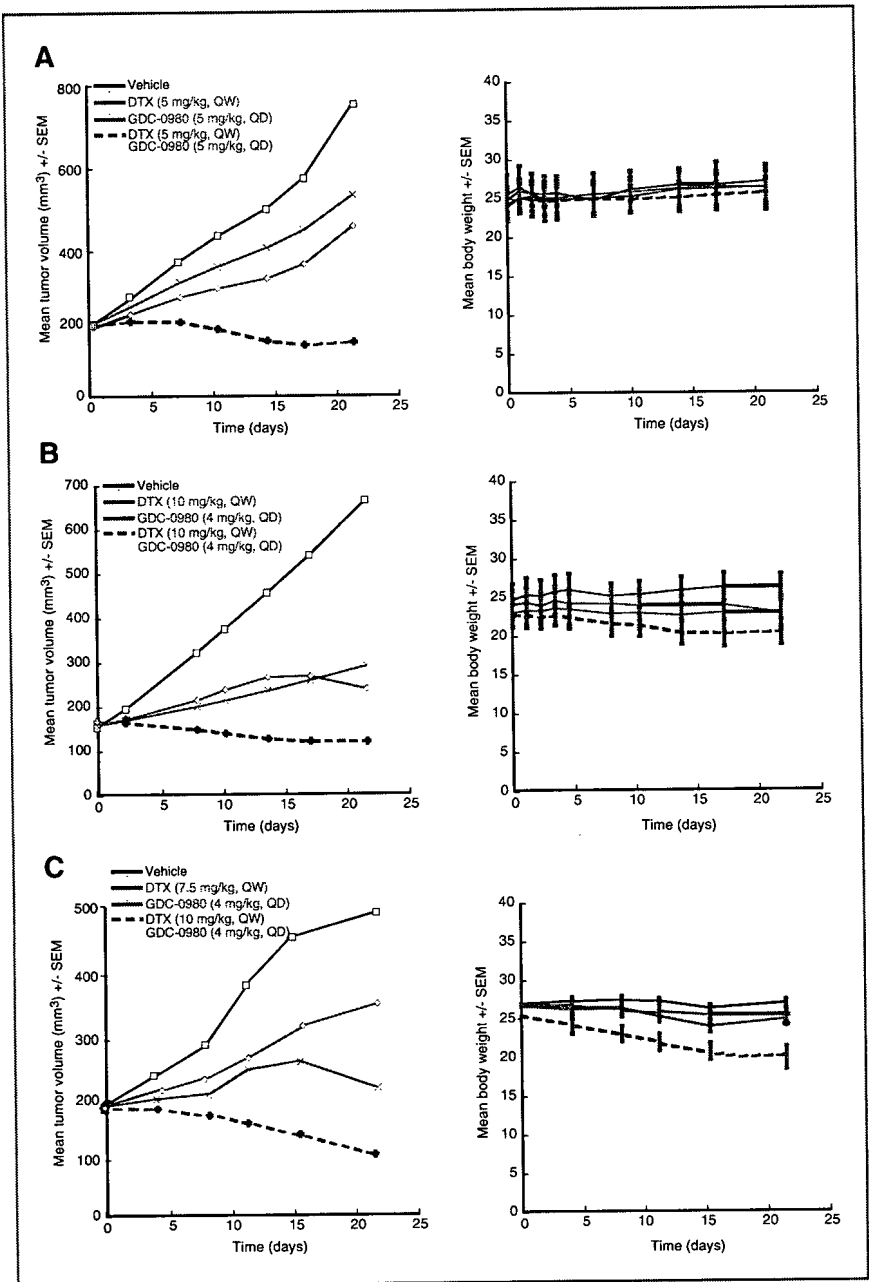


Figure 6. Efficacy of GDC-0980 with docetaxel in human tumor xenograft models. A, increases in efficacy in MX-1 xenografts when GDC-0980 is dosed in combination with DTX. GDC-0980 was dosed orally at 5 mg/kg daily (QD) as a single agent or in combination with DTX. In this study, DTX was dosed weekly (QW) at 5 mg/kg. B, A549 xenograft study with single agent GDC-0980 at 4 mg/kg QD, DTX at 10 mg/kg QW, and the combination. C, MCF7-neo/HER2 tumors treated with GDC-0980 at 4 mg/kg QD, DTX at 7.5 mg/kg QW, or the drugs in combination.

use in combination with these therapies (26). The chemotherapeutic docetaxel (DTX) is an established treatment for a variety of cancers, and interferes with cell division by binding to microtubules (27, 28). The *in vitro* combination of GDC-0980 and DTX at their IC₅₀ concentrations caused an increase in apoptosis compared with either agent alone (Supplementary Fig. S3). Antitumor

efficacy of GDC-0980 was investigated *in vivo* in 3 xenograft models in combination with DTX: (i) MX-1 is a breast cancer model that lacks PTEN protein expression, (ii) A549 is a NSCLC model that contains an activating K-RAS mutation and is negative for LKB1 protein expression, (iii) MCF7-neo/HER2 is a breast cancer model that overexpresses HER2 and harbors the PIK3CA E545K

hot-spot mutation. In these *in vivo* studies DTX was administered intravenously once per week (QW) for 3 weeks while GDC-0980 was dosed orally daily (QD). Single agent GDC-0980 and single agent DTX treatments caused tumor growth inhibition characterized by either stasis or tumor growth delay in all xenograft models tested. However, the combination of GDC-0980 and DTX resulted in tumor regressions in all 3 xenograft model evaluated (Fig. 6A–C). Most notably, the combination treatment of GDC-0980 and DTX in the MCF7-neo/HER2 xenograft model resulted in a 90% objective response rate based on the number of partial and complete regressions (Fig. 6C). This enhanced efficacy in combination occurred at well-tolerated doses with no significant weight loss in all 3 xenograft models tested (Fig. 6A–C).

Discussion

The PI3K/PTEN pathway is a central signaling network that is perturbed in a number of cancers, thus PI3K pathway inhibitors are currently being evaluated in clinical trials in patients with advanced solid tumors (16). GDC-0980 is a novel, potent small molecule inhibitor with selectivity for class I PI3K and mTOR kinase, and has recently entered clinical trials. In the preclinical studies presented here, GDC-0980 strongly inhibited the PI3K pathway and decreased tumor cell viability in a broad set of cancer cell lines. Across all tumor types mutations in BRAF or RAS were negative predictors of GDC-0980 potency. Cell lines that have BRAF or RAS mutations may be more resistant to GDC-0980 because they are more dependent on MAPK pathway signaling. The additional property of inhibiting mTOR kinase in addition to PI3K may be responsible for the greater cell potency of GDC-0980 relative to GDC-0941. Recent studies have shown that inhibition of mTOR kinase can result in strong antiproliferative effects in cancer lines (29).

Viability changes were reflected with both cell cycle and apoptotic effects produced by GDC-0980, while apoptosis was not observed with an mTOR kinase inhibitor, indicating that PI3K is sufficient for cell survival. Interestingly, despite the trend for mutations in BRAF or RAS

as negative predictors for response to GDC-0980 potency, there are certain KRAS mutant lines (e.g., LoVo) where GDC-0980 induces apoptosis in cultured cells that correspond to strong tumor growth inhibition *in vivo*. Thus the negative predictive value may lie in cancer types such as pancreatic or melanoma or other predictors of sensitivity may be required in addition to RAS and RAF status. GDC-0980 has dose-dependent exposure that is consistent with good solubility and absorption. In xenograft models the knockdown of pathway signaling for 8 to 24 hours resulted in the strongest efficacy, and PD biomarker knockdown was inversely correlated with plasma concentrations of GDC-0980. The dual nature of GDC-0980 as a blockade of 2 nodes in the PI3K signaling network resulted in the strongest knockdown of phospho-protein markers when compared with either a Class I PI3K inhibitor GDC-0941, or an mTOR kinase inhibitor. This effective dual blockade of the pathway by GDC-0980 also resulted in compelling potency of 200 or more nmol/L in 25% of cancer cell lines, and significant *in vivo* efficacy of >50% TGI in 15 of 20 xenograft tumor models. In addition, GDC-0980 and docetaxel combined to enhance the antitumor efficacy in tumor xenograft models. In summary, GDC-0980 is a potent small molecule inhibitor of class I PI3K and mTOR kinase with promise for clinical trials in cancer as either a single agent or in combination with antimitotic agents.

Disclosure of Potential Conflicts of Interest

J. Wallin, K. Edgar, J. Guan, C. Lewis, J. Lesnick, J. Nonomiy, J. Pang, L. Salphati, A. Olivero, D. Sutherland, L. Ross, M. Berry, R. Kassees, W. Prior, L. Lee, M. Lackner, C. O'Brien, J. Spoerke, D. Sampath, M. Belvin, and L. Friedman are employed at Genentech, Inc.

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