

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: PI.- **GILEAD SCIENCES, INC.**-Solicitud de Patente de Invención en Fase Nacional PCT para "**4,6-DIAMINOQUINOLIN-3-CARBONITRILOS SUSTITUIDOS.**"-Clase **C07D 401/14** número **NC2017/0013351.**

1. Me refiero a mi memorial del pasado 15 de agosto de 2018 y a mi memorial de modificación voluntaria al capítulo reivindicatorio presentado hoy, 16 de noviembre de 2018.

2. Dicho lo anterior y de conformidad con lo dispuesto en el Artículo Primero de la Resolución N° 54093 de 18 de septiembre de 2012, y en virtud del Acuerdo para el Procedimiento Acelerado de Estudio de Solicitudes de Patente (PPH) suscrito entre la Superintendencia de Industria y Comercio (SIC) y la Oficina Europea de Patentes (EPO) solicito a su Despacho hacer estudio de patentabilidad de la presente solicitud de conformidad con la guía de procedimiento para este tipo de trámite.

Para la solicitud de referencia específicamente, **solicito se tenga en cuenta el resultado del Informe de Examen Preliminar Internacional (IPER),** aquí presentada bajo la notificación de admisibilidad expedida por esta entidad en relación con la solicitud internacional número PCT/US2016/040520 (publicación internacional número WO2017007689), la cual tiene correspondencia con la solicitud de patente número **EP16738976.6**, presentada y aceptada por la Oficina Europea de Patentes, teniendo en cuenta que la presente solicitud es equivalente a dicha solicitud, tal y como se podrá evidenciar más adelante.

3. La equivalencia entre la materia concedida en la solicitud de patente No. **EP 16 738 976.6** y la materia reclamada en la presente solicitud es explicada tal como se muestra a continuación:

TABLA DE CORRESPONDENCIAS

Reivindicaciones de la presente solicitud (NC 2017/0013351)	Reivindicaciones de la solicitud de patente Europea EP 16 738 976.6	Correspondencia de las reivindicaciones
1	1	Las reivindicaciones son idénticas.
2	2	Las reivindicaciones son idénticas.
3	3	Las reivindicaciones son idénticas.
4	4	Las reivindicaciones son idénticas.
5	5	Las reivindicaciones son idénticas.
6	6	Las reivindicaciones son idénticas.
7	7	Las reivindicaciones son idénticas.
8	8	Las reivindicaciones son idénticas.
9	9	Las reivindicaciones son idénticas.
10	10	Las reivindicaciones son idénticas.
11	11	Las reivindicaciones son idénticas.
12	12	Las reivindicaciones son idénticas.
13	13	Las reivindicaciones son idénticas.
14	14	Las reivindicaciones son idénticas.
15	15	Las reivindicaciones son idénticas.
16	16	Las reivindicaciones son idénticas.
17	17	Las reivindicaciones son idénticas.
18	18	Las reivindicaciones son idénticas.
N.A.	19 a 21	Dichas reivindicaciones de la solicitud de patente europea EP 16 738 976.6 no tienen reivindicaciones correspondientes en el pliego reivindicatorio de la presente solicitud, puesto que la materia definida en las mismas se refiere a usos, considerados materia no patentable de acuerdo a la Decisión Andina 486, por tanto, fueron eliminadas del pliego reivindicatorio de la presente solicitud.

Teniendo en cuenta la tabla de correspondencias aquí presentadas, **amablemente solicito tener en cuenta el examen realizado por la Autoridad Internacional del PCT, y que la Oficina Europea de Patentes tomo como examen de fondo para**

conceder el beneficio de patente a la solicitud presentada ante la EPO, como guía para el estudio de patentabilidad de la solicitud en trámite.

4. De igual forma me permito llamar su atención en cuanto a que la presente solicitud cumple con los requisitos para el reconocimiento de los resultados de exámenes de patentabilidad efectuados a solicitudes extranjeras, establecidos en la Resolución N° 54093 de 2012, de acuerdo con las siguientes condiciones:

- a. Se está solicitando el reconocimiento del examen de patentabilidad realizado por la Autoridad de Búsqueda Internacional del PCT, como parte del trámite de la solicitud internacional PCT/US2016/040520 (publicación internacional número WO2017007689), en el Tratado de Cooperación en materia de Patentes (PCT), que actúa como autoridad reconocida de búsqueda y examen, en donde dicha solicitud internacional tiene equivalencia con la solicitud de patente número **EP 16 738 976.6** presentada en la Oficina Europea de Patentes.
- b. La presente solicitud se encuentra en fase nacional en virtud del PCT y reivindica prioridad, de acuerdo con el artículo 9 de la Decisión 486, en la solicitud de patente europea concedida (**No. EP 16 738 976.6** del 6 de julio de 2015).
- c. La solicitud de patente europea **No. EP 16 738 976.6** tiene correspondencia con la presente solicitud, tal como se evidencia de los capítulos reivindicatorios arriba comparados, la descripción de las solicitudes y los datos bibliográficos.
- d. La intención de concesión por parte de la EPO, hace mención a que las reivindicaciones concedidas corresponden a las reivindicaciones 1-21 (ver Anexo b, *Intention to grant*).

Dichas reivindicaciones son enteramente equivalentes a las 18 reivindicaciones presentadas bajo modificación voluntaria el día de hoy, 16 de noviembre de 2018, y corresponden suficientemente a las reivindicaciones aceptadas, toda vez que las mismas tienen un alcance igual que las aceptadas por la Oficina de Europea de Patentes, tal y como se puede evidenciar en la tabla de correspondencia que realiza el cotejo de los dos capítulos reivindicatorios.

- e. Las 18 reivindicaciones de la solicitud en cuestión presentadas ante la Superintendencia de Industria y Comercio, corresponden a las reivindicaciones protegidas en la solicitud de patente europea **No. EP 16 738 976.6**, y se encuentran del todo contenidas en la materia reclamada ante la Oficina Europea de patentes.

5. Así mismo, por favor encuentre a continuación los datos bibliográficos de la solicitud tal y como se presentó en Europa.

N° de la solicitud	EP 16 738 976.6
Fecha de presentación	60 de junio de 2016
N° Publicación	EP 3 191 470 A1
Solicitante	GILEAD SCIENCES INC
Inventores	Elizabeth M. Bacon, Gayatri Balan, Chien-Hung Chou, Christopher T. Clark, Jeromy J. Cottell, Musong Kim, Thorsten A. Kirschberg, John O. Link, Gary Phillips, Scott D. Schroeder, Neil H. Squires, Kirk L. Stevens, James G. Taylor, William J. Watkins, Nathan E. Wright y Sheila M. Zipfel
Título de la invención	COT MODULATORS AND METHODS OF USE THEREOF

6. Finalmente y en cumplimiento de los requisitos para la tramitación del Procedimiento Acelerado de Estudio de Solicitudes de Patente y la modificación voluntaria al capítulo reivindicatorio, me permito anexar los siguientes documentos:

- Formato único de procedimiento acelerado de exámenes de patentes;
- Copia de la Intención de Concesión (*Intention to grant*) debidamente emitida por la Oficina Europea de Patentes, EPO, donde se detalla el número de la solicitud **EP 16 738 976.6** de fecha 6 de noviembre de 2018, en donde dicha Oficina indica que la patente europea puede ser otorgada bajo las condiciones enumeradas en dicho oficio (ver página 1 de la *Intention to grant*). Adicionalmente, se adjunta su correspondiente traducción.
- Copia del Informe de Examen Preliminar Internacional (IPER) (*Fecha de Emisión del reporte 09 de enero de 2018*) debidamente emitido por la Autoridad de Búsqueda Internacional del PCT, para la solicitud internacional bajo el número PCT/US2016/040520 (publicación internacional número WO2017007689), la cual tiene equivalencia con la solicitud de patente número **EP 16 738 976.6** presentada en la Oficina Europea de Patentes, en donde dicha Entidad Internacional objeta la el nivel inventivo de las reivindicaciones 1 a 18, 28 a 40 y 60-77 originalmente presentadas, frente al estado de la técnica. Adicionalmente, se adjunta la correspondiente traducción.

Me permito señalar que dichas objeciones fueron debidamente superadas con el pliego reivindicatorio modificado, logrando su aceptación posterior, tal como se evidencia en el *Intetion to Grant* emitido por la EPO, y que se encuentra adjunto al presente memorial.

- d) Copia de las reivindicaciones que han sido determinadas como patentables por la EPO (reivindicaciones 1-21) con su correspondiente traducción.
 - e) Copia de la lista de antecedentes citados por el examinador de la EPO.
 - f) Copia de los documentos de literatura no patente citados por el examinador en el IPER.
7. El pago de la tasa que causa el Examen de Patentabilidad se realizó mediante la plataforma virtual SIPI el día de hoy, 16 de noviembre de 2018.
8. De igual forma manifiesto que se ha presentado de manera independiente una modificación voluntaria al capítulo reivindicatorio actualmente presentado en Colombia con el fin de ajustar dicho capitulo al concedido en Europa. El pago por dicha modificación se ha realizado el día de hoy mediante la plataforma virtual SIPI.
9. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,



ALICIA LLORED A RICAURTE

T.P. 53.215

Direcciones para Notificación: notificacionesip@lloredacamacho.com

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Application No. 16 738 976.6 - 1110	Ref. 411-142 PCTEP	Date 06.11.2018
Applicant GILEAD SCIENCES, INC.		

Communication under Rule 71(3) EPC

1. Intention to grant

You are informed that the examining division intends to grant a European patent on the basis of the above application, with the text and drawings and the related bibliographic data as indicated below.

A copy of the relevant documents is enclosed.

1.1 In the text for the Contracting States:

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT
RO RS SE SI SK SM TR

Description, Pages

2-576	as published	
1	filed in electronic form on	30-08-2018

Claims, Numbers

1-15, 16(part 1)	filed in electronic form on	17-11-2017
16(part 2), 17-21	filed in electronic form on	30-08-2018

With the following amendments to the above-mentioned documents proposed by the division

Description, Pages 5, 6, 15, 24, 25, 28-30, 32-40, 49, 75, 77, 88-90, 101, 102

Comments

DESCRIPTION

Pages 5, 6, 15, 24, 25, 28-30, 32-40, 49, 75, 77, 88-90: Inconsistency between claim and description removed (Art. 84 EPC)

Page 6: Article 53(c) EPC

Pages 101, 102: Guidelines F-IV, 4.4

1.2 Bibliographic data

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated contracting states, the registered name(s) of the applicant(s) and the other bibliographic data are shown on **EPO Form 2056** (enclosed).

2. Invitation

You are invited, **within a non-extendable period of four months** of notification of this communication,

2.1 to EITHER approve the text communicated above and verify the bibliographic data (Rule 71(5) EPC)

(1) by filing a translation of the claim(s) in the other two official languages of the EPO

Fee code	EUR
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(2a) by paying the fee for grant including the fee for publication:
minus any amount already paid (Rule 71a(5) EPC):

007	925.00
	925.00

Total amount:	0.00
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(3) by paying additional claims fees under Rule 71(4) EPC;
number of claims fees payable: 0
minus any amount already paid (Rule 71a(5) EPC):

016	0.00
	0.00

Total amount:	0.00
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Important: If the translations of the claims and fees have already been filed and paid respectively in reply to a previous communication under Rule 71(3) EPC, e.g. in the case of resumption of examination after approval (see Guidelines C-V, 6), **agreement as to the text to be granted** (Rule 71a(1) EPC) must be expressed within the same time limit (e.g. by approving the text and verifying the bibliographic data, by confirming that grant proceedings can go ahead with the documents on file and/or by stating which translations of the claims already on file are to be used).

Note 1: See "Notes concerning fee payments" below.

Note 2: Any overpaid "minus" amounts will be refunded when the decision to grant (EPO Form 2006A) has been issued.

Note 3: For the calculation of the grant fee under Article 2(2), No. 7, RFees (old fee structure), the number of pages is determined on the basis of a clean copy of the application documents, in which text deleted as a result of any amendments by the examining division is not shown. Such clean copy is made available via on-line file inspection only.

2.2 OR, in the case of disapproval, to request reasoned amendments or corrections to the text communicated above or keep to the latest text submitted by you (Rule 71(6) EPC).

In this case the translations of the claims and fee payments mentioned under point 2.1 above are NOT due.

The terms "amendment(s)" and "correction(s)" refer only to amendments or corrections of the application documents and not of other documents (e.g. bibliographic data, the designation of the inventor, etc.).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the examining division requesting that you correct this deficiency (Rule 137(4) EPC).

2.3 Bibliographic data

Where you request a change or correction of bibliographic data in response to the Rule 71(3) communication, this will **not** cause the sending of a further communication under Rule 71(3) EPC. You will still have to pay the fees and file translations in reply to the Rule 71(3) communication in the case of 2.1 above, unless you also file a reasoned request for amendments or corrections in response to the Rule 71(3) communication (see case 2.2 above).

3. Loss of rights

If neither of the two possible actions above (see points 2.1 or 2.2) is performed in due time, the European patent application will be deemed to be withdrawn (Rule 71(7) EPC).

4. Further procedure

4.1 In the case of point 2.1 above

- 4.1.1 The decision to grant the European patent will be issued, and the **mention of the grant** of the patent will be published in the European Patent Bulletin, if the requirements concerning the translation of the claims and the payment of all fees are fulfilled and there is agreement as to the text to be granted (Rule 71a(1) EPC).

Note on payment of the renewal fee:

If a renewal fee becomes due before the next possible date for publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (Rule 71a(4) EPC).

Under Article 86(2) EPC, the obligation to pay renewal fees to the European Patent Office terminates with the payment of the renewal fee due in respect of the year in which the mention of the grant of the European patent is published.

Note on payment of the designation fee(s):

If the designation fee(s) become(s) due after the communication under Rule 71(3) EPC, the mention of the grant of the European patent will not be published until these fees have been paid (Rule 71a(3) EPC).

- 4.1.2 After publication, the **European patent specification** can be downloaded free of charge from the EPO publication server <https://data.epo.org/publication-server>.

4.1.3 Filing of translations in the contracting states

As regards translation requirements prescribed by the contracting states under Article 65(1) EPC, please consult the website of the European Patent Office

www.epo.org → Law & practice → Legal texts, National law relating to the EPC

www.epo.org → Law & practice → All Legal texts → London Agreement

In the case of a valid extension or validation

As regards translation requirements prescribed by the extension or validation states, please consult the website of the European Patent Office

www.epo.org → Law & practice → Legal texts, National law relating to the EPC

Failure to supply a prescribed translation in a contracting state, or in an extension or validation state may result in the patent being deemed to be void *ab initio* in the state concerned (Art. 65(3) EPC).

4.2 In the case of 2.2 above

If the present communication under Rule 71(3) EPC is based on an auxiliary request and, within the time limit, you maintain the main request or a higher ranking request which is not allowable, the application will be refused (Art. 97(2) EPC).

If the examining division gives its consent to the requested amendments or corrections, it will issue a new communication under Rule 71(3) EPC; otherwise, it shall resume the examination proceedings (Rule 71(6) EPC).

5. Filing of a divisional application

Any divisional application relating to this European patent application must be filed directly with the European Patent Office in Munich, The Hague or Berlin and will be in the language of the proceedings for the present application, or if the latter was not in an official language of the EPO, the divisional application may be filed in the language of the present application as filed (see Article 76(1) and Rule 36(2) EPC). Any such divisional application must be filed while the present application is still pending (Rule 36(1) EPC; Guidelines A-IV, 1.1.1).

6. Notes concerning fee payments

6.1 Making payments

For payments made via deposit account, please note that as from 1 December 2017 debit orders will only be carried out if filed in an electronically processable format (xml), using an accepted means of filing as laid down in the Arrangements for deposit accounts (ADA), published in the Supplementary publication in the Official Journal.

All relevant information related to the modes of payment of fees to the EPO can be retrieved from the EPO website at "**Making Payments**".

6.2 Information concerning fee amounts

Procedural fees are usually adjusted every two years, on even years, with effect from 1 April. Therefore, before making a payment, parties should verify the amounts actually due on the date of payment using the applicable version of the Schedule of fees and expenses, published as a Supplement to the Official Journal of the EPO, available on the EPO website (www.epo.org) at www.epo.org/schedule-of-fees. The "Schedule of fees" table allows the viewing, downloading and searching of individual fee amounts, both current and previous.

6.3 Note to users of the automatic debiting procedure

The fee for grant, including the fee for publication, and any additional claims fees due under Rule 71(4) EPC will be debited automatically on the date of filing of the translations of the claims, or on the last day of the period of this communication. However, if the designation fee(s) become(s) due as set out in Rule 71a(3) EPC and/or a renewal fee becomes due as set out in Rule 71a(4) EPC, these should be paid separately by another permitted way of payment in order not to delay the publication of the mention of the grant. The same applies in these circumstances to the payment of extension and validation fees. The same applies in these circumstances to the payment of extension and validation fees.

Note: If a waiver is expressed in response to a Rule 71(3) communication (see OJ EPO 2015, A52), the fee for grant, including the fee for publication/printing, and any additional claims fees will not be debited automatically. These fees must be paid separately by another means of payment allowed under the Rules relating to Fees.

Examining Division:

Chairman:	Kleidermigg, Oliver
2nd Examiner:	Sahagún Krause, H
1st Examiner:	Cortés Suárez, José



Pfau, Andrea
For the Examining Division
Tel. No.: +49 89 2399 - 6731

Enclosures: Text intended for grant

EPO Form 2056

Application No.:

16 738 976.6

IV.2. Patent classification

The classification indicated on the published patent application remains unchanged. It is as follows:

INV. C07D401/14 C07D405/14 C07D413/14 C07D401/12 C07D409/14 C07D417/14
C07D471/04 C07D495/04 A61K31/4709 A61P29/00 A61P35/00

IV.3. Title of the invention

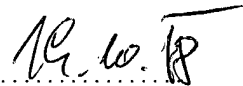
The title indicated on the published patent application remains unchanged. It reads as follows:

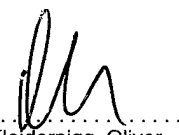
COT-MODULATOREN UND VERFAHREN ZUR VERWENDUNG DAVON

COT MODULATORS AND METHODS OF USE THEREOF

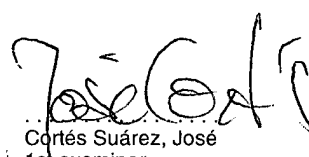
MODULATEURS DE COT ET PROCÉDÉS D'UTILISATION ASSOCIÉS

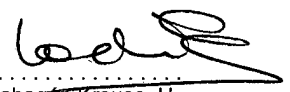
IV.4. Documentation


Date


Kleidernigg, Oliver
Chairman

Enclosure(s):


Cortés Suárez, José
1st examiner


Sahagún Krause, H
2nd examiner



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Solicitud No. 16 738 976.6 - 1110	Ref. 411-142 PCTEP	Fecha 06.11.2018
Solicitante GILEAD SCIENCES, INC.		

Comunicado bajo la Regla 71(3) de la EPC

1. Intención de concesión

Se le informa que la división de exámenes tiene la intención de conceder una patente europea con base en la solicitud arriba mencionada, con el texto y los dibujos y los datos bibliográficos relacionados que se indican a continuación.

Se anexa una copia de los documentos relevantes.

1.1. En el texto para los Estados Contratantes

AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU
LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

Descripción, páginas

2 – 576	tal como fue publicada	
1	presentada en formato electrónico en	30-08-2018

Reivindicaciones, números

1 – 15, 16 (parte 1)	presentada en formato electrónico en	17-11-2017
16 (parte 2), 17 – 21	presentada en formato electrónico en	30-08-2018

Con las siguientes modificaciones a los documentos anteriormente mencionados propuestos por la división

Descripción, páginas 5, 6, 15, 24, 25, 28-30, 32-40, 49, 75, 77, 88-90, 101, 102



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Comentarios

DESCRIPCIÓN

Páginas 5, 6, 15, 24, 25, 28-30, 32-40, 49, 75, 77, 88-90: Se removi6 la inconsistencia entre la reivindicaci6n y la descripci6n (Art. 84 EPC)

Página 6: Art6culo 53(c) EPC

Páginas 101, 102: Guías F-IV, 4.4

1.2 Datos bibliogr6ficos

El t6tulo de la invenci6n en los tres idiomas oficiales de la Oficina Europea de Patentes, la clasificaci6n internacional de patentes, los estados contratantes designados, los nombres registrados de los solicitantes y los dem6s datos bibliogr6ficos se muestran en el **Formulario 2056 de la EPO** (adjunto).

2. Invitaci6n

Se le invita, **dentro de un periodo de cuatro meses (sin posibilidad de extensi6n)**, posteriores a la notificaci6n de este comunicado, a

2.1 aprobar el texto que se present6 anteriormente y verificar los datos bibliogr6ficos (Regla 71(5) de la EPC)

- (1) presentando una traducci6n de las reivindicaciones en los otros dos idiomas oficiales de la EPO

	C6digo de tasa	EUR
(2a) pagando la tasa de concesi6n incluyendo la tasa de publicaci6n:		
Menos cualquier cantidad que ya se haya pagado (Regla 71a(5) de la EPC)	007	925.00
		925.00
	Total:	0.00

- (3) pagando la tasa por reivindicaciones adicionales de acuerdo con la Regla 71(4) de la EPC;

	016	0.00
N6mero de reivindicaciones a pagar: 0		
		0.00
Menos cualquier cantidad que ya se haya pagado (Regla 71a(5) de la EPC)		
	Total:	0.00



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Importante: Si ya se han presentado las traducciones de las reivindicaciones y se han pagado las tasas en respuesta a una comunicación previa de acuerdo con la Regla 71(3) de la EPC; por ejemplo en el caso de una reanudación del examen tras la aprobación (ver guías C-V 6) debe expresarse el **acuerdo con respecto al texto a ser concedido** (Regla 71a(1) de la EPC) dentro del mismo límite de tiempo (por ejemplo, aprobando el texto y verificando los datos bibliográficos, confirmando que los pasos de la concesión pueden realizarse con los documentos en el expediente y/o estableciendo qué traducciones de las reivindicaciones en el expediente serán usadas).

Nota 1: Ver “Notas con respecto a los pagos de las tasas” más adelante.

Nota 2: Cualquier cantidad de pago extra “menos” será reembolsada cuando se emita la decisión de concesión (Formulario 2006A de la EPO).

Nota 3: Para el cálculo de la tasa de concesión de acuerdo con el Artículo 2(2), No. 7, TasasR (estructura de tasas antigua), el número de páginas se determina con base en la copia en limpio de los documentos de la solicitud, donde no se muestra el texto que se ha eliminado como resultado de cualquier modificación por parte de la división de exámenes. Dicha copia limpia se encuentra disponible únicamente por medio de la inspección en línea.

2.2 O, en el caso de desaprobación, solicitar las modificaciones o correcciones razonables al texto que se ha comunicado anteriormente o mantener el último texto enviado por usted (Regla 71(6) de la EPC)

En este caso, no se deben pagar las traducciones de las reivindicaciones y las tasas mencionadas bajo el punto 2.1 mencionado arriba.

Los términos “modificaciones” y “correcciones” solo se refieren a modificaciones o correcciones de los documentos de la solicitud y no a otro tipo de documentos (por ejemplo, datos bibliográficos designación del inventor, etc.).

Si presenta modificaciones, debe identificarlas e indicar la base para ellas en la solicitud tal como se presentó. El incumplimiento de cualquiera de los requisitos puede dar lugar a una comunicación de la división examinadora que solicita que corrija esta deficiencia (Regla 137 (4) EPC).

2.3 Datos bibliográficos

Cuando solicite un cambio o corrección de los datos bibliográficos en respuesta a la comunicación de acuerdo con la Regla 71 (3), esto **no** causará el envío de una comunicación adicional conforme a la Regla 71 (3) de la EPC. En todo caso deberá pagar las tasas y las traducciones de archivos en respuesta a la comunicación de acuerdo con la Regla 71 (3) en el caso de 2.1 anterior, a menos que también presente una solicitud razonable de modificaciones o



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correcciones en respuesta a la comunicación de acuerdo con la Regla 71 (3) (ver el caso 2.2 arriba).

3. Pérdida de derechos

Si ninguna de las dos acciones posibles arriba (ver puntos 2.1 o 2.2) se realiza a su debido tiempo, la solicitud de patente europea se considerará retirada (Regla 71 (7) de la EPC).

4. Procedimiento adicional

4.1 En el caso del punto 2.1 anterior

- 4.1.1 La decisión de otorgar la patente europea se emitirá, y la **menção de la concesión** de la patente se publicará en el Boletín de Patentes Europeo, si se cumplen los requisitos relativos a la traducción de las reivindicaciones y al pago de todas las tasas y si existe un acuerdo sobre el texto a otorgar (Regla 71a (1) de la EPC).

Nota sobre el pago de la tasa de renovación:

Si se debe pagar una tasa de renovación antes de la próxima fecha posible para la publicación de la mención de la concesión de la patente europea, la publicación se efectuará solo después de que se haya pagado la tasa de renovación y se haya pagado cualquier tasa adicional (Regla 71a (4) de la EPC).

En virtud del Artículo 86(2) de la EPC, la obligación de pagar tasas de renovación a la Oficina Europea de Patentes termina con el pago de la tasa de renovación debida con respecto al año en que se publica la mención de la concesión de la patente europea.

Nota sobre el pago de la(s) tasa(s) de designación:

Si la(s) tasa(s) de designación vencen después de la comunicación conforme a la Regla 71 (3) de la EPC, la mención de la concesión de la patente europea no se publicará hasta que estas tasas hayan sido pagadas (Norma 71a(3) de la EPC).

- 4.1.2 Después de la publicación, la **descripción de la patente europea** se puede descargar de manera gratuita desde el servidor de publicaciones de la EPO <https://data.epo.org/publication-server>.

4.1.3 Presentación de traducciones en los Estados contratantes



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La falta de suministro de una traducción prescrita en un estado contratante, o en una extensión o estado de validación puede hacer que la patente se considere nula *ab initio* en el estado en cuestión (Art. 65 (3) de la EPC).

4.2 En el caso del punto 2.2 anterior

Si la presente comunicación conforme a la Regla 71 (3) de la EPC se basa en una solicitud auxiliar y, dentro del límite de tiempo, usted mantiene la solicitud principal o una solicitud de clasificación más alta que no está permitida, la solicitud será rechazada (Art. 97 (2) de la EPC).

Si la división examinadora otorga su consentimiento para las modificaciones o correcciones solicitadas, emitirá una nueva comunicación conforme a la Regla 71 (3) de la EPC; de lo contrario, reanudará el procedimiento de examen (Regla 71 (6) de la EPC).

5. Presentación de una solicitud divisional

Cualquier solicitud divisional relacionada con esta solicitud de patente europea debe presentarse directamente en la Oficina Europea de Patentes de Múnich, La Haya o Berlín y se publicará en el idioma del procedimiento de la presente solicitud, o si esta última no estaba en un idioma oficial de la EPO, la solicitud divisional puede presentarse en el idioma de la presente solicitud tal como ha sido presentada (véase el Artículo 76 (1) y la Regla 36 (2) de la EPC). Cualquier solicitud divisional debe presentarse mientras la presente solicitud aún está pendiente (Regla 36 (1) de la EPC; Guías A-IV, 1.1.1).



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6. Notas sobre el pago de tasas.

6.1 Realizar pagos

Para los pagos realizados a través de la cuenta de depósito, tenga en cuenta que a partir del 1 de diciembre de 2017, las órdenes de débito solo se llevarán a cabo si se presentan en un formato procesable electrónicamente (xml), utilizando un medio de presentación aceptado según lo establecido en los Acuerdos para cuentas de depósito (ADA), publicado en la publicación complementaria del Diario Oficial.

Toda la información relevante relacionada con los modos de pago de las tasas a la EPO se puede obtener del sitio web de la EPO en "**Realizar pagos**".

6.2 Información sobre las tasas

Las tasas procesales generalmente se ajustan cada dos años, en años pares, entrando en vigencia a partir del 1 de abril. Por lo tanto, antes de realizar un pago, las partes deben verificar los montos realmente adeudados en la fecha de pago utilizando la versión aplicable del Programa de honorarios y gastos, publicado como Suplemento al Diario Oficial de la EPO, disponible en el sitio web de la EPO (www.epo.org) en www.epo.org/schedule-of-fees. La tabla "Lista de tasas" permite ver, descargar y buscar los montos de las tasas individuales, tanto actuales como anteriores.

6.3 Nota para los usuarios del procedimiento de débito automático

La tasa por concesión, incluida la tasa por publicación, y cualquier tasa por reivindicaciones adicionales adeudada en virtud de la regla 71 (4) de la EPC se debitará automáticamente en la fecha de presentación de las traducciones de las reivindicaciones, o en el último día del período de esta comunicación. Sin embargo, si la (s) tasa (s) de designación se convierte(n) en vencida(s) como se establece en la Regla 71a (3) de la EPC y / o si se debe una tasa de renovación tal como se establece en la Regla 71a (4) de la EPC, esta debe ser pagada por separado mediante otra forma de pago permitida para no retrasar la publicación de la mención de la concesión. Lo mismo se aplica en estas circunstancias al pago de tasas de extensión y validación. Lo mismo se aplica en estas circunstancias al pago de las tasas de extensión y validación.

Nota: Si se expresa una exención en respuesta a una comunicación de la Regla 71 (3) (ver OJ EPO 2015, A52), la tasa de la concesión, incluyendo la tasa de la publicación / impresión, y las tasas de reclamaciones adicionales no se cargarán automáticamente. Estas tasas deben pagarse por separado por otro medio de pago permitido según las Normas relativas a las tasas.

División de examen:



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IV.2. Clasificación de patente

La clasificación indicada en la solicitud de patente publicada no ha cambiado, y es la siguiente:

INV. C07D401/14 C07D405/14 C07D413/14 C07D401/12 C07D409/14
C07D417/14 C07D471/04 C07D495/04 A61K31/4709 A61P29/00 A61P35/00

IV.3. Título de la invención

El título indicado en la solicitud de patente publicada no ha cambiado, y es el siguiente:

MODULADORES COT Y MÉTODOS DE USO DEL MISMO

IV.4. Documentación

19.10.18
Fecha

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Director

Ilegible Signature
Cortés Suarez, José
Primer examinador

Ilegible Signature
Sahagún Krause, H
Segundo examinador



Selective inhibitors of tumor progression loci-2 (Tpl2) kinase with potent inhibition of TNF- α production in human whole blood

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ABSTRACT

Tpl2 (cot/MAP3K8) is an upstream kinase of MEK in the ERK pathway. It plays an important role in Tumor Necrosis Factor- α (TNF- α) production and signaling. We have discovered that 8-halo-4-(3-chloro-4-fluoro-phenylamino)-6-[(1H-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles (**4**) are potent inhibitors of this enzyme. In order to improve the inhibition of TNF- α production in LPS-stimulated human blood, a series of analogs with a variety of substitutions around the triazole moiety were studied. We found that a cyclic amine group appended to the triazole ring could considerably enhance potency, aqueous solubility, and cell membrane permeability. Optimization of these cyclic amine groups led to the identification of 8-chloro-4-(3-chloro-4-fluorophenylamino)-6-[(1-(1-ethylpiperidin-4-yl)-1H-1,2,3-triazol-4-yl)methylamino]quinoline-3-carbonitrile (**34**). In a LPS-stimulated rat inflammation model, compound **34** showed good efficacy in inhibiting TNF- α production.

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Studies on the complex biology of Tumor Necrosis Factor- α (TNF- α) have revealed that this cytokine is involved in the inflammation process in several disease states such as rheumatoid arthritis (RA), psoriasis, inflammatory bowel disease, and multiple sclerosis.¹ TNF- α also stimulates the production of other pro-inflammatory cytokines such as IL-1 and IL-6, and induces the activity of degradative enzymes such as the matrix metalloproteases (MMPs).² Inhibition of TNF- α with a number of biological agents such as etanercept (Enbrel),³ infliximab (Remicade),⁴ and adalimumab (Humira)⁵ has been a major therapeutic advance in treating RA. These protein therapeutics have changed the treatment paradigm and improved the quality of life of RA patients. Small molecule drug can usually be less expensive and more conveniently administered (orally available) than proteins. Our desire for such therapeutics prompted our investigation of small molecules to inhibit TNF- α production.

Tpl2 (cot/MAP3K8), a serine/threonine kinase, plays a pivotal role in TNF- α production through the MEK-Erk pathway. Studies with Tpl2 knockout mice demonstrated that Tpl2 is essential for lipopolysaccharide (LPS) induced TNF- α production.⁶ Furthermore, Tpl2 kinase is also required for TNF- α signaling.⁷ Inhibition of Tpl2

thus could potentially have the double benefits of blocking both TNF- α production and signaling.

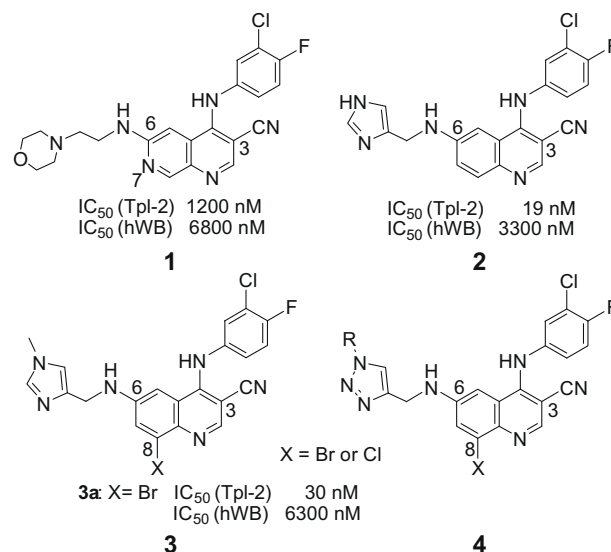
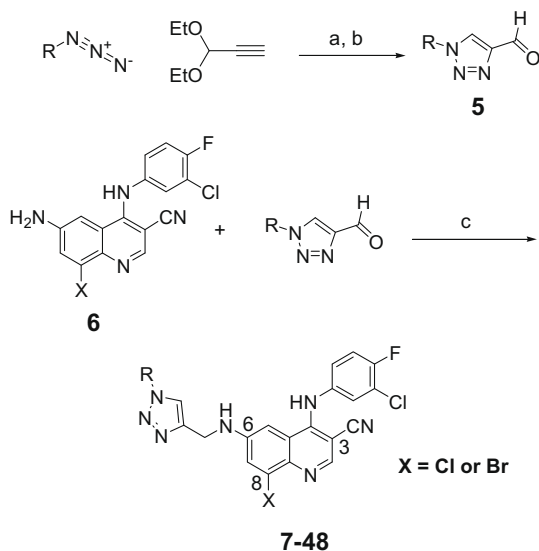


Figure 1. Tpl2 kinase inhibitors.

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Scheme 1. Reagents and conditions: (a) sodium L-ascorbate, copper(II) sulfate; (b) hydrochloric acid; (c) sodium triacetoxyborohydride, 1,2-dichloroethane, rt, 4 h, 20–80%.

Previous studies in our laboratories have shown that 1,7-naphthyridine-3-carbonitriles (**1**, Fig. 1)⁸ and 4,6-diaminoquinoline-3-carbonitriles (**2**)⁹ were potent inhibitors of Tpl2 kinase. We subsequently reported improved selectivity and in vivo efficacy with 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles such as **3** in models of inflammation.¹⁰ 8-Bromo-4-anilino-6-aminoquinoline-3-carbonitriles (**3a**) were over 1000-fold selective against a panel of serine–threonine and tyrosine kinases; however, these compounds show similar potency as compounds **1** and **2** in the inhibition of TNF- α production from LPS-stimulated human whole blood (hWB). As part of our continuing efforts toward the optimization of Tpl2 inhibitors, we herein report on a series of 8-halo-4-anilino-6-[(1*H*-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles (**4**) with improved potency in human blood.

Our preparation of 8-halo-4-anilino-6-[(1*H*-[1,2,3]triazol-4-ylmethyl)-amino]-quinoline-3-carbonitriles employed the synthetic route outlined in Scheme 1. Aldehyde **5** was synthesized through a [3+2] dipolar cyclo-addition of 3,3-diethoxyprop-1-yne and an organic azide,¹¹ which was either purchased commercially or prepared from the azide displacement of an alkyl halide. Under reductive alkylation conditions, aldehyde **5** reacted with 6-amino-8-halo-4-(3-chloro-4-fluorophenylamino)-quinoline-3-carbonitrile **6**, which was prepared through a previously reported multi-step synthesis,¹⁰ to give the target compounds **7–48**.

All compounds prepared for this study were tested for inhibition of Tpl2 kinase using an ELISA assay that measures the amount of direct phosphorylated substrate MEK kinase.^{8,9} Functional inhibition of TNF- α was measured using LPS-stimulated human monocytes and LPS-stimulated human whole blood. As illustrated in Table 1, compounds with an unsubstituted triazole at the 6-position (**7** and **8**) are potent in Tpl2 inhibition (IC_{50} of 40 nM and 18 nM, respectively). They inhibit TNF- α production in LPS-stimulated human whole blood with IC_{50} s of 4.2 μ M, and 7.0 μ M, respectively. Compared to **3**, these compounds show no significant improvement in hWB potency. In addition, **7** and **8** suffer from very low aqueous solubility and permeability.

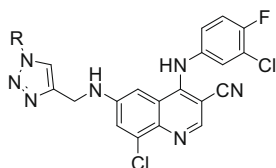
In order to improve the potency and physical properties of **7** and **8**, a variety of groups were introduced to the triazole ring (Table 1). The in vitro data suggest that incorporation of hydrophobic groups on the triazole moiety is not beneficial to the binding activity. For example, in both the C-8 chloride and bromide series, compounds with a hydrophobic group (**9–11** and **15–16**) demonstrated more than three-fold loss of potency in Tpl-2 binding affinity; in human blood assay, the IC_{50} s of these compounds were greater than 20 μ M in hWB. However, when an acidic group such as a carboxylic acid was employed, the resulting analogs were highly potent in Tpl2 inhibition as indicated by **12** and **17**, which showed Tpl2 inhibition IC_{50} s of 9.6 nM and 7.0 nM, respectively, four-fold more potent than **7** and **8**. In addition, both compounds also have much improved aqueous solubility and **17** exhibited high permeability in PAMPA assay (1.8×10^{-6} cm/s). Unfortunately these improved potencies and physical properties did not translate into increased potency in hWB as neither **12** nor **17** had IC_{50} s of below 20 μ M.

Table 1
Inhibition of Tpl2 kinase by compounds with N1-substitutions at the triazole tail

Compound	X	R	Inhibition of LPS-induced TNF- α production			Solubility (μ g/mL, pH 7.4)	PAMPA ($\times 10^{-6}$ cm/s)
			Tpl2 IC_{50} (nM)	IC_{50} (monocytes) (μ M)	IC_{50} (hWB) (μ M)		
7	Cl	H	40	0.39	7.0	0	0
8	Br	H	18	1.8	4.2	0	0
9	Cl	<i>t</i> -Butyl	170	0.69	>20	1.0	0.1
10	Cl	Phenyl	120	3.9	>20	1.0	0
11	Cl	Benzyl	140	5.0	>20		
12	Cl	CH ₂ COOH	9.6	5.0	>20	73	0.04
13	Cl	2-Pyrrolidinylethyl	40	0.27	4.4	28.8	0.57
14	Cl	3-Pyridinylmethyl	10	0.05	5.5	34.7	0.18
15	Br	<i>t</i> -Butyl	61	1.8	>20		
16	Br	Phenyl	40,000	NA	NA	0	1.2
17	Br	CH ₂ COOH	7.0	5.0	>20	64	1.8
18	Br	2-Pyrrolidinylethyl	23	0.32	5.9	35.7	0.72
19	Br	3-Pyridinylmethyl	20	0.22	3.9	28.2	0.13

Table 2

Inhibition of Tpl2 kinase by compounds with N1-substitutions at the triazole tail



Compounds	R	IC ₅₀ (nM) Tpl2	IC ₅₀ (μM) (hWB)
20		4	2.8
21		2.0	9
22		7.6	>20
23		3	>20
24		17	19
25		20	12.8
26		13	10.0
27		22	5.4
28		28	5.7
29		8.0	7.1
30		22	3.1
31		18	5.5
32		5.3	3.9
33		2.2	4.1
34		1.6	0.3
35		8.2	0.9
36		9.1	1.1
37		10	0.4

On the other hand, compounds with groups containing a basic nitrogen atom showed comparable or slightly increased potency over **7** and **8** in both the enzymatic assay and secondary assays. Specifically, when a pyridine moiety was applied, compounds tended to show better aqueous solubility and cell permeability profiles (**14** and **19**). For example, while compound **7** has very poor

solubility and permeability, **14**, with a 3-pyridinylmethyl group at the triazole, is moderately soluble in aqueous buffer (34.7 μg/mL at pH 7.4) and showed moderate cell permeability (0.18×10^{-6} cm/s in PAMPA). In addition, **14** is four-fold more potent than **7** in the enzymatic assay of Tpl2 inhibition. Further optimization of **14** led to **20** (Table 2), which showed a 10-fold increase in Tpl2 inhibition and two-fold improvement in human whole blood activity. However, in a pharmacokinetic study, **20** showed high clearance (88 mL/min/kg) in rats. One of the metabolic soft spots was the benzylic methylene between the pyridyl and triazole rings. To avoid the methylene linker, the pyridine ring was directly connected to the triazole ring and **23** was prepared. Without the methylene between the pyridine ring and triazole, **23** showed lower clearance (28 mL/min/kg) in rats than **20**, however, this compound showed diminished activity in human monocytes and hWB, despite the improved potency (IC₅₀ = 3.0 nM) in the enzymatic assay.

We then turned our attention to amine groups as substitutions on the triazole. A series of compounds with a cyclic or non-cyclic amine moiety at the triazole were synthesized and studied. Some representative examples (compounds **24–37**) are listed in Table 2. We found that compounds with an acyclic amine substitute on the triazole had reduced activity in hWB (for example, IC₅₀s for **24**, **25**, and **26** are greater than 10 μM). On the other hand, cyclic amine analogs (**27–37**) were generally more potent in both assays. Some of them showed significantly improved potency in hWB (**34–37**). Specifically, **34**, with a 1-ethyl-4-piperidinyl substitution at the triazole, was 25-fold more potent than **7** in Tpl-2 binding in the enzymatic assay (IC₅₀ 1.6 nM) and 23-fold more potent in hWB (IC₅₀ 0.3 μM). In addition, compared to **7**, this series of compounds (**31–37**) exhibited moderate to significant improvements in aqueous solubility and cell membrane permeability (Table 3). For example, **35**, with a 4-piperidinyl group at the triazole, is quite soluble in aqueous buffer (50 μg/mL at pH 7.4) and showed moderate cell permeability (0.48×10^{-6} cm/s in PAMPA).

Several active compounds from Table 2 were tested in a panel of kinases, including some known to be involved in TNF-α production (e.g., p38 and MK2) (Table 4). All the compounds reported here are highly selective against these kinases. It is noteworthy to mention that even though **7** weakly inhibits EGFR with an IC₅₀ of 1.0 μM, compounds with N-1 substitutions at the triazole ring showed much improved selectivity (10–30-fold improvement) between Tpl2 and this receptor tyrosine kinase. The selectivity of Tpl2 against EGFR for compound **34** is 6875-fold.

In a pharmacokinetics study, compound **34** was orally administered in rats with 100 mg/kg dosing and showed a C_{max} of 517 ng/mL (0.89 μM) and AUC of 4841 ng h/mL, above its IC₅₀ in hWB. The compound was then tested in the LPS-induced TNF-α production model in female Sprague-Dawley rats. With a 25 mg/kg po dose, **34** inhibited LPS-induced TNF-α production by 83% compared to the vehicle group.

Table 3

Physical properties of selected 8-bromo-4-(3-chloro-4-fluoro-phenylamino)-6-[[1-substituted-1-[1,2,3]triazol-4-ylmethyl]-amino]-quinoline-3-carbonitriles

Compound	Solubility ^a (μg/mL, pH 4.5)	Solubility ^a (μg/mL, pH 7.4)	PAMPA (×10 ⁻⁶ cm/s)
7	3.0	0	0
32	>100	50	0.03
33	N/A	1.0	0.39
34	N/A	4.0	0.36
35	>100	50	0.48
37	22	1.0	0.43

^a The compound to be tested was dissolved in DMSO (20 mg/mL) and a small portion of the solution was added to an aqueous buffer at either pH of 4.5 or 7.4. The mixture was incubated for 18 h at ambient temperature prior to quantitation using a UV plate reader.¹²

Table 4

In vitro selectivity profile of selected 8-bromo-4-(3-chloro-4-fluoro-phenylamino)-6-[[1-substituted-1-[1,2,3]triazol-4-ylmethyl]-amino]-quinoline-3-carbonitriles

	EGFR (μ M)	P38 (μ M)	Src (μ M)	CAMKII (μ M)	MK2 (μ M)	PKA (μ M)	PKC (μ M)
8	1.0	4	41	>100	49	>100	>100
34	11	16	53	39	63	>200	>200
35	30	>200	72	2.6	>200	171	>200
36	22	4.7	35	75	>200	>200	>200
37	22	16	110	70	131	116	>200

In summary, through exploring substitutions at the N1 position of triazole, we have discovered a series of potent and selective Tpl2 inhibitors. Incorporation of a basic amine group gave a number of compounds with moderate improvements in physicochemical properties and improved potency in human blood, for example, **34** is more than 20-fold more potent than **7**. Compound **34** was orally efficacious in reducing TNF- α production in a LPS-induced rat model. This series of inhibitors demonstrates potential as orally available compounds for the treatment of RA and other inflammatory diseases.

Acknowledgments

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Identification of a novel class of selective Tpl2 kinase inhibitors: 4-Alkylamino-[1,7]naphthyridine-3-carbonitriles

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Abstract—We have previously reported the discovery and initial SAR of the [1,7]naphthyridine-3-carbonitriles and quinoline-3-carbonitriles as Tumor Progression Loci-2 (Tpl2) kinase inhibitors. In this paper, we report new SAR efforts which have led to the identification of 4-alkylamino-[1,7]naphthyridine-3-carbonitriles. These compounds show good in vitro and in vivo activity against Tpl2 and improved pharmacokinetic properties. In addition they are highly selective for Tpl2 kinase over other kinases, for example, EGFR, MEK, MK2, and p38. Lead compound 4-cycloheptylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (**30**) was efficacious in a rat model of LPS-induced TNF- α production.

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1. Introduction

Tumor Progression Loci-2 (Tpl2) kinase has been shown to be involved in both production and signaling of TNF- α .¹ TNF- α is a pro-inflammatory cytokine and its role in several inflammatory diseases, most notably rheumatoid arthritis (RA), is well established.² Thus, Tpl2 is an attractive target for the treatment of RA, a chronic incapacitating disease that currently affects more than five million people worldwide. The use of kinase inhibitors in the treatment of chronic diseases like RA has not been clinically proven.³ Many compounds have failed in phase 1 clinical trials due to adverse effects. The toxicity observed could be a result of poor selectivity against other kinases.

Several members of the mitogen-activated protein kinase (MAP kinase) family, particularly the serine/threonine kinase p38 (Fig. 1), have been investigated by several companies. Four compounds have progressed to the clinic,³ AMG-548, BIRB-796, SCIO-469⁴ (SCIO-323), and VX-702.⁴ Two of these, AMG-548 and BIRB-796, have failed to advance further due to toxicity concerns.

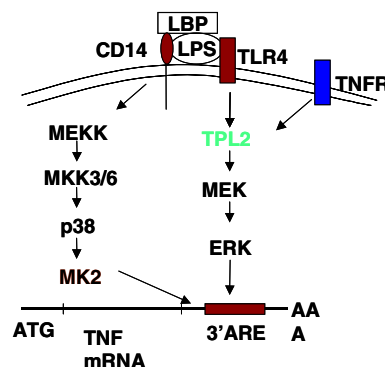


Figure 1. Tpl2 is essential for TNF- α production and TNF- α signaling.

Keywords: Tpl2 kinase; [1,7]Naphthyridine-3-carbonitriles; Quinoline-3-carbonitriles.

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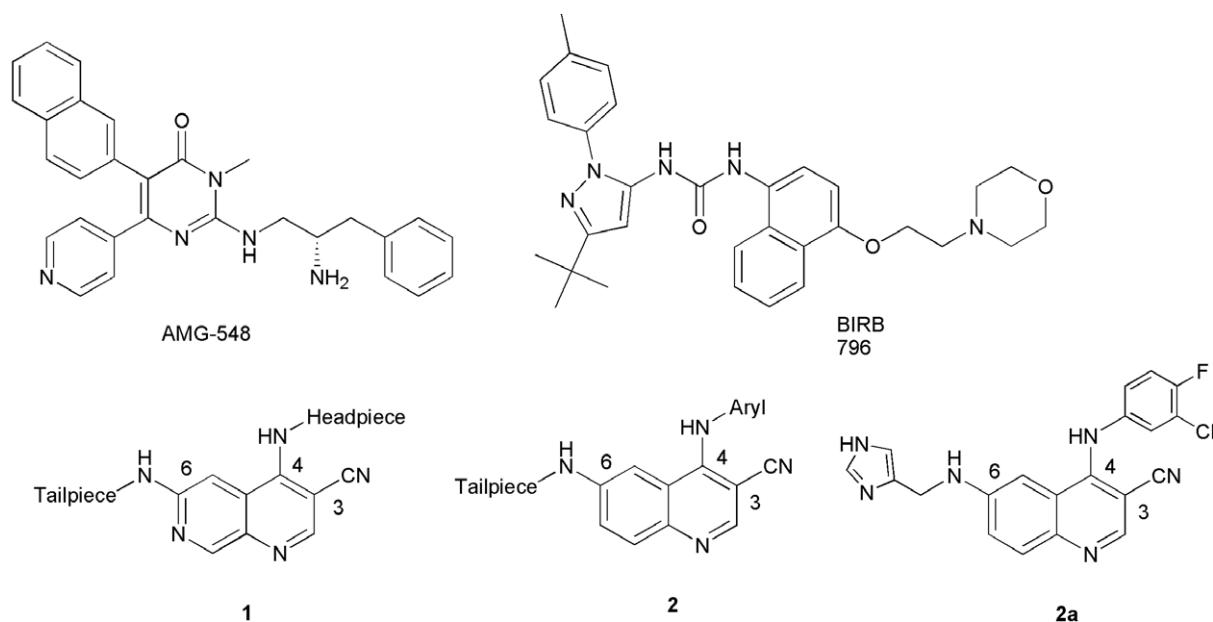
Tpl2 is a serine/threonine kinase in the MAP3K family that is upstream of MEK in the ERK pathway (Fig. 1).¹ Tpl2 is the only known human kinase that has a proline instead of a conserved glycine at the first glycine in the GXGXXG motif of the ATP binding loop.^{5a} Tpl2 is also not inhibited by the pan kinase inhibitor staurosporine.^{5b} Therefore, we believe that Tpl2 has some distinct structural features that will confer selectivity against other kinases and circumvent unwanted side effects.

In our previous work on Tpl2^{6,7} we have reported the [1,7]naphthyridine-3-carbonitriles (**1**) and quinoline-3-carbonitriles (**2**) as potent, reversible, ATP-competitive Tpl2 inhibitors with selectivity against certain kinases. The naphthyridine carbonitriles **1** were identified via 'directed screening' of our kinase library.⁶ Further SAR evaluation of **1** indicated that, unlike other kinases, the inhibitory activity of Tpl2 depended on substitution at the C-6 position (tailpiece). In the literature, the C-6 position of naphthyridine-3-carbonitriles⁸ and 1,3-quinazolines⁹ has been shown to be useful for optimization of solubility and permeability of kinase inhibitors, but

70% inhibition of TNF- α production in the LPS-mouse model when dosed ip at 25 mg/kg. However, compound **2a** has poor selectivity against epidermal growth factor receptor (EGFR) tyrosine kinase. A pharmacokinetic (PK) study of **2a** revealed that it also showed poor bio-availability (*F*) and very high total clearance (Cl) in rats (*F* = 1%; Cl = 97 mL/min/kg). In this article, we report the design and synthesis of carbonitriles that contain novel headpieces and show selectivity against EGFR, improved PK properties and activity in the LPS mouse model.

2. Chemistry

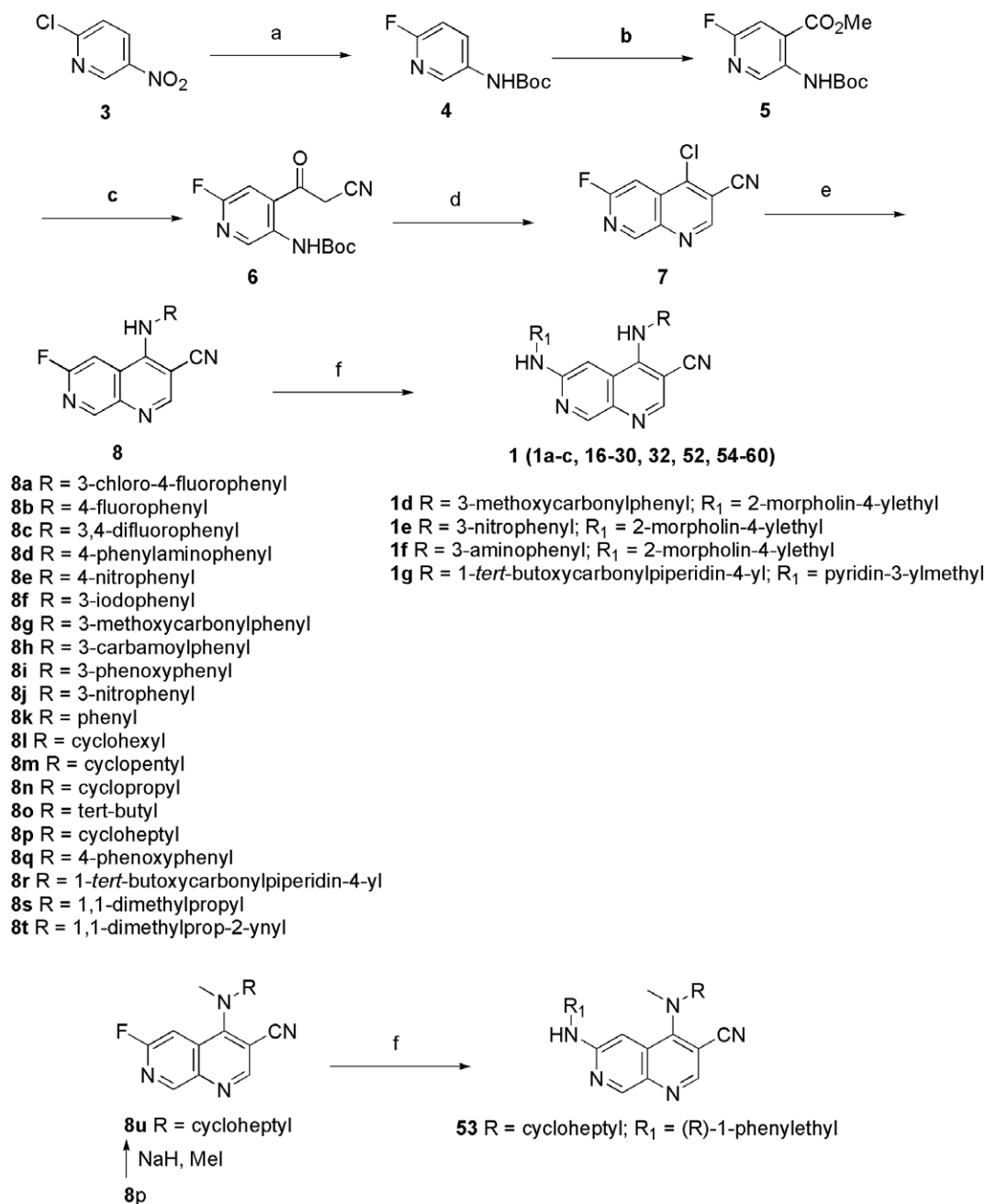
The synthesis of compounds with the general structure **1** was accomplished in a manner reported by Wissner et al.¹⁰ As shown in Scheme 1 the key intermediate **7** was prepared in seven steps from commercially available 2-chloro-5-nitropyridine **3**. Compound **7** and appropriate headpiece amines were either refluxed in ethanol or irradiated by microwave to give intermediates **8**. The 6-fluoro groups of these intermediates were displaced



Compound	Tailpiece	Headpiece
1a	morpholin-4-yl-ethyl	3-Chloro-4-fluorophenyl
1b	pyridin-3-yl-methyl	3-Chloro-4-fluorophenyl
1c	(1R)-1-phenylethyl	3-Chloro-4-fluorophenyl

to have minimal effect on isolated kinase activity. However, several potent tailpieces were identified for class **1** compounds (**1a–c**). Further investigation showed that the closely related quinoline-3-carbonitriles (**2**) had very similar SAR to carbonitriles **1**.⁷ A detailed study of the C-6 substitution in carbonitriles **2**⁷ led to the identification of a potent Tpl2 inhibitor, compound **2a**, with selectivity against several kinases (MEK, PKA, p38, Src, CAMKII, MK2, PKC and S6). In vivo, **2a** showed

with a variety of tailpiece amines using microwave irradiation or heating in pyridine to give final products **1** (Tables 1–3, 5, 6). Compound **21** (Table 1) was prepared by addition–elimination reaction of intermediate **7** with methyl-3-amino benzoate to give **8g**, subsequent reaction with 4-(2-aminoethyl)morpholine to afford **1d**, and finally hydrolysis of the methyl ester to give **21**. Compound **24** (Table 1) was synthesized in three steps from intermediate **8j**. Displacement of the fluoro group

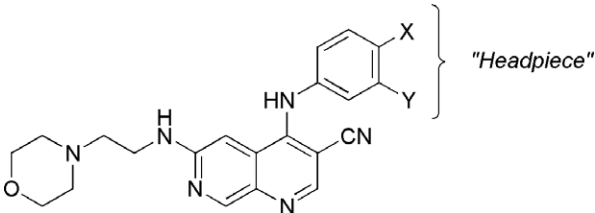


Scheme 1. Synthesis of [1,7]naphthyridine-3-carbonitriles. Reagents and conditions: (a) i—KF, DMSO, 70 °C, 18 h; ii—SnCl₂·2H₂O, EtOAc; iii—Boc₂O, *t*-BuOH 40 °C, 3 h, 53%, three steps; (b) i—*n*-BuLi, TMEDA, ether, −78 °C; then CO₂; ii—TMSCH₂N₂, CHCl₃/MeOH, 51%, two steps; (c) CH₃CN, *n*-BuLi, THF, −78 °C, 76%; (d) i—DMF–DMA, rt, 1 h; ii—oxalyl chloride, DMF, CH₂Cl₂, 59%, two steps; (e) RNH₂, 2-ethoxyethanol or EtOH, reflux; or RNH₂, DME, 140 °C microwave, 10 min; (f) R₁NH₂, THF or DMA or neat, 150–190 °C microwave; or R₁NH₂, pyridine, 80 °C.

with 4-(2-aminoethyl)morpholine generated **1e**. The latter upon reduction with SnCl₂ yielded **1f**, which upon treatment with methyl sulfonylchloride gave **24**. Compound **53** (Table 5) was prepared by N-methylation of **8p** followed by reaction of intermediate **8u** with (R)-1-phenylethylamine. Compound **58** (Table 6) was prepared by condensing intermediate **7** with *N*-Boc piperidine to give **8r**, subsequent reaction with pyridin-3-ylmethylamine to give **1g**, and removal of the Boc group using HCl in dioxane to give **58**.

Target compounds with the general structure **2** were made according to Scheme 2.⁷ Synthesis of compound

33 (Table 3) started with commercially available *para*-nitroaniline **9**. The tailpiece was first installed by treating **9** with 4-(2-chloroethyl)morpholine, and the nitro compound (**10a**) obtained was then reduced to **10b**. The latter was alkylated by Michael addition–elimination to give the 2-cyano-3-ethoxyacrylate **11b**. Cyclization in Dowtherm followed by chlorination gave **13b**. Nucleophilic displacement of chloride **13b** with 4-phenoxyphenylamine yielded **33**. Since this method was not suitable for analoging at the 6 position it was not further pursued. An alternate route using common intermediate **13a** was used to synthesize all other such compounds. Cyanoquinoline **13a**⁷ was obtained in three steps from

Table 1. Tpl2 activity of 6-(2-morpholin-4-ylethylamino)-4-arylamino-[1,7]naphthyridine-3-carbonitriles


Compound	X	Y	Tpl2 IC ₅₀ (μM)
1a	F	Cl	1.5
16	F	H	10.7
17	F	F	3.6
18	NHPh	H	7.6
19	NO ₂	H	7.0
20	H	I	7.1
21	H	COOH	>10
22	H	CONH ₂	>10
23	H	OPh	11.5
24	H	NHSO ₂ CH ₃	>10

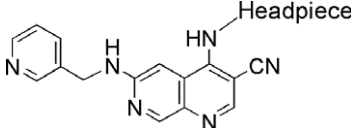
commercially available *para*-nitroaniline **9**.^{8j} Refluxing intermediate **13a** with various amines in ethanol or irradiation of these mixtures by microwave gave intermediates **14(a–d)**. The C-6 nitro group was reduced to give intermediates **15(a–d)**. Tailpiece installation was carried out by reductive amination of **15** with a variety of aldehydes to give **2** (Tables 3–5). Two of the aldehydes used

were not commercially available. Morpholin-4-ylacetaldehyde needed for the synthesis of **31** was prepared *in situ* from its dimethyl acetal¹¹ by heating with aqueous HCl overnight. Refluxing 2-picoline-*N*-oxide with selenium dioxide in pyridine gave the 1-oxypyridine-2-carbaldehyde¹² required for the synthesis of **44** and **36**.

3. Results and discussion

All compounds were studied for inhibition of isolated Tpl2 enzyme via quantification of MEK phosphorylation in an ELISA format. For selected compounds, EGFR selectivity was measured by looking at the inhibition of EGFR kinase autophosphorylation in A431 cells overexpressing this enzyme, via quantification of P-EGFR. Functional inhibition of TNF-α production was measured using LPS-stimulated monocytes and LPS-stimulated human blood. Compounds studied *in vivo* were first assessed for selectivity against other kinases (including p38α, CAMKII, PKC, PKA, MK2, Src, S6K, IKKβ, and Jnk-1).

A brief SAR study of headpieces in the naphthyridine-3-carbonitrile series (**1**)⁶ had revealed the 4'-fluoro-3'-chloro aniline as the optimal arylamino substitution at the 4-position. However, the effect of polar functionality had not been explored. Thus, the headpiece moiety was varied starting with the lead compound (**1a**) in this series. As shown in Table 1, all these compounds showed

Table 2. Tpl2 activity and EGFR selectivity for 6-[(pyridine-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitriles


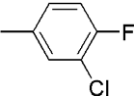
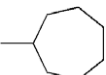
Compound	Headpiece	Tpl2 IC ₅₀ (μM)	LPS-induced TNF-α inhibition		EGFR(A431) IC ₅₀ (μM)
			Monocyte IC ₅₀ (μM)	Human blood IC ₅₀ (μM)	
1b		0.05	1.1	>20	<5
25		0.4	1.1	20	<5
26		0.47	0.5	4.7	>40
27		0.32	0.6	7.7	>40
28		2.8			>40
29		0.63	0.6	4.2	>40
30		0.16	0.5	4.5	>40

Table 3. Tpl2 activity of 4-arylamino-[1,7]naphthyridine-3-carbonitriles versus 4-arylaminoquinoline-3-carbonitriles

R ₁	R	X = N	Tpl2 IC ₅₀ (μM)	X = CH	Tpl2 IC ₅₀ (μM)
		1a	1.5	31	1.0
		32	0.38	33	0.25
		1c	0.05	34	0.018

Table 4. Tpl2 activity of 4-cyclopentylaminoquinoline-3-carbonitrile and 4-*tert*-butylaminoquinoline-3-carbonitrile

Compound	R	Tpl2 IC ₅₀ (μM)	Compound	R ₁	Tpl2 IC ₅₀ (μM)
35		21	44		1.3
36		1.3	45		1.7
37		1.7	46		2.9
38		2.2	47		7.3
39		2.9	48		1.6
40		7.3	49		3
41		1.6			
42		3			
43		19.7			

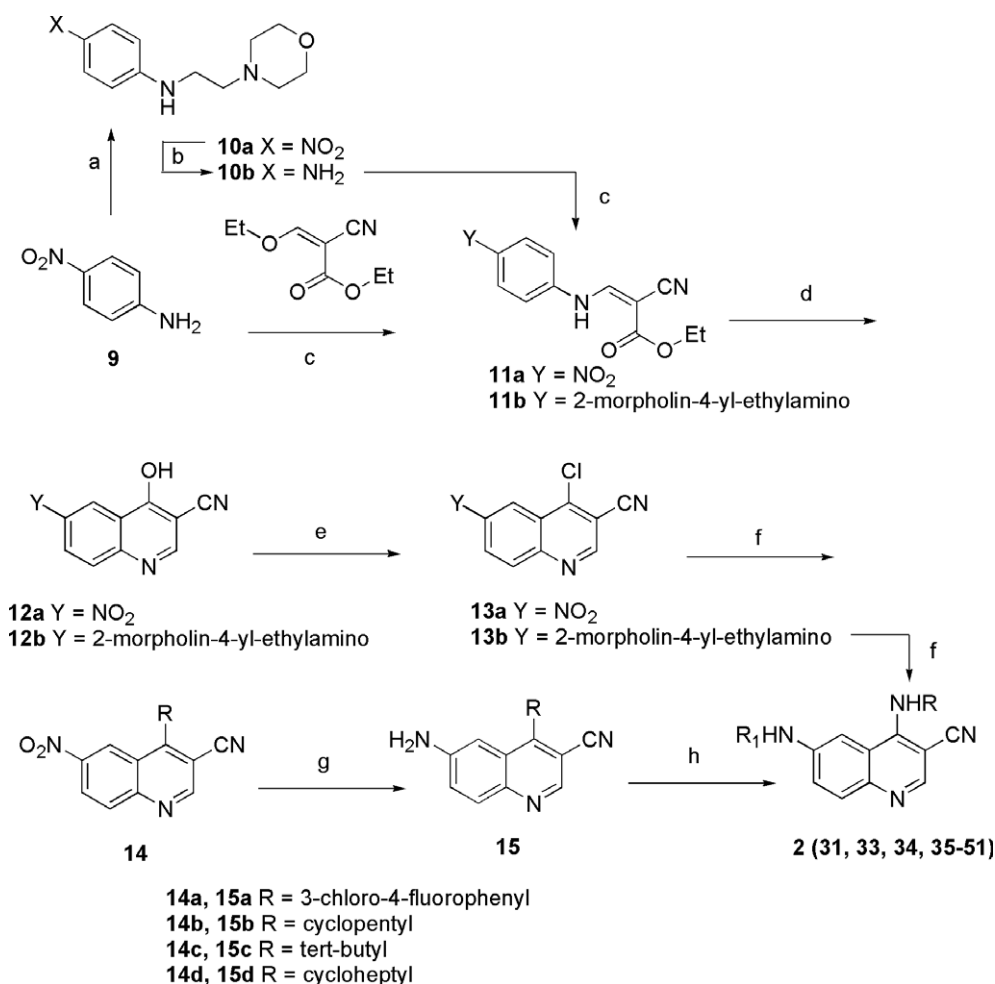
Table 5. SAR mapping of 4-alkylamino-[1,7]naphthyridine-3-carbonitriles

Compound	R1	R	X	Y	Tpl2 IC ₅₀ (μM)
30			N	H	0.16
50			C	H	2.6
27			N	H	0.32
51			C	H	2.4
52			N	H	0.92
53			N	CH ₃	>40
54			N	H	8
55			N	H	7.6
56			N	H	>40
57			N	H	5.2

diminished activity. This result is not unexpected since a common feature seen in the ATP binding site for the naphthyridine-3-carbonitriles⁸ and 1,3-quinazolines⁹ is that the headpiece lies in a mostly lipophilic pocket buried deep within the active site with limited bulk tolerance. Next we looked at the cyclohexylamino headpiece (Table 2). Here the more potent 3-pyridylmethylamino group was used as a tailpiece. We found that compound **26** was equipotent to **25**. This result was a pleasant surprise since an alkyl headpiece usually leads to diminished activity in this core when applied to other kinase targets. We expected that this unusual SAR would grant selectivity over other kinases.

Further analoging of **26** and evaluation in other assays showed that the 4-alkylamino substituted [1,7]naphthyridine-3-carbonitriles had moderate activity in the Tpl2 enzymatic assay but improved activity in the monocyte and human blood assays compared to the 4-arylamino analogs (Table 2). Most importantly, these compounds show excellent selectivity against other kinases, particularly EGFR. In our earlier compounds, lack of selectivity against EGFR had been a concern.

In the past, we had found that the [1,7]naphthyridine-3-carbonitriles (**1**) and quinoline-3-carbonitriles (**2**) had very similar SAR for Tpl2 inhibition. For example, in Table 3, compounds **1a**, **32**, and **1c** of the [1,7]naphthyridine-3-carbonitrile series have similar Tpl2 activity to the corresponding compounds **31**, **33**, and **34** in the quinoline-3-carbonitriles series.⁷ Since the SAR tracks between the two different cores, we made a library of quinoline carbonitrile compounds combining the *tert*-butyl and cyclopentylamino headpiece with our most potent tailpieces from the naphthyridine carbonitrile series.^{6,7} (Table 4). Unfortunately all compounds showed diminished activity. These findings prompted us to do further SAR mapping (Table 5). First, the quinoline analogs of naphthyridines **27** and **30** were evaluated. Both compounds (**50** and **51**) showed 10-fold lower Tpl2 activity than their naphthyridine analogs. It appears that N7 is important for activity in the 4-alkylamino series. Next, the importance of the 4-NH group was tested. Methylating the C-4 nitrogen atom in compound **52** abolished activity (**53**). One hypothesis was that perhaps the 4-alkylamino analogs were binding in a flipped mode compared to the 4-arylamino analogs, with the key interaction made by the 3-cyano group



Scheme 2. Synthesis of quinoline-3-carbonitriles. Reagents and conditions: (a) 4-(2-chloroethyl)morpholine hydrochloride, 170 °C, 14 h; (b) 10% Pd/C, H₂, rt, 24 h; (c) Cs₂CO₃, DMF, rt, 2 h; (d) Dowtherm A, 260 °C, 4–5 h; (e) (COCl)₂, DMF (cat.), DCE; or POCl₃, reflux, 2–12 h; (f) RNH₂, EtOH, microwave or conventional heating; (g) SnCl₂·2H₂O, EtOH, microwave (or conventional heating) or Fe, NH₄Cl, MeOH/H₂O, heat; (h) R₁CHO, NaCNBH₃, HOAc, EtOH.

replaced by one with the N7 atom. The 4-alkylamino groups would then be behaving as the tailpieces. A few compounds were made to test the hypothesis. The 6-(3-chloro-4-fluorophenylamino)-4-cyclohexylamino-[1,7]naphthyridine-3-carbonitrile (**54**) and the 4-(3-chloro-4-fluorophenylamino)-6-(alkylamino)-[1,7]naphthyridine-3-carbonitriles (**55–57**) did not show any appreciable activity, indicating that our hypothesis was probably incorrect.

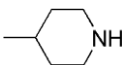
In an attempt to improve the activity of the alkylamino series a few additional analogs were prepared (Table 6). Insertion of heteroatoms into the alkyl headpiece was not accepted (**26** vs **58**). Introduction of an alkynyl group was tolerated (**60**). Although there was no significant improvement in activity, we were able to identify a second compound (**59**) with comparable whole blood activity to lead **30**. This compound was twofold less potent in the Tpl2 enzymatic and monocyte assay.

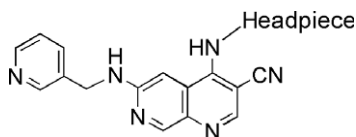
With this new alkylamino class of compounds we have identified a series that is selective against EGFR and demonstrates improved human blood activity (Tables 2 and 6). Selected compounds were next

evaluated for IV PK in rats (Table 7). Compound **30** showed good exposure and moderate clearance and was taken forward for in vivo testing. It was examined in the LPS-induced mouse model at an ip dose of 10 mg/kg. Compound **30** caused an 86% inhibition of TNF- α production compared to vehicle in female C57Bl/6 mice (Fig. 2). Upon screening for selectivity over a panel of kinases in both in vitro and cellular assays (Table 8), it showed >200-fold selectivity against MK2 and p38, two other kinases involved in TNF- α production. It also had >200-fold selectivity against MEK and other serine-threonine kinases. Table 8 also shows a comparison of Tpl2 activity (in vitro and in vivo), kinase selectivity profiles, and PK parameters of lead candidate **30** versus our earlier lead **2a**. Compound **30** has an improved selectivity profile, lower clearance, and improved exposure and bioavailability in rats. In addition, **30** showed better in vivo activity at 10 mg/kg (ip) compared to **2a** at 25 mg/kg (ip) in the LPS mouse model.

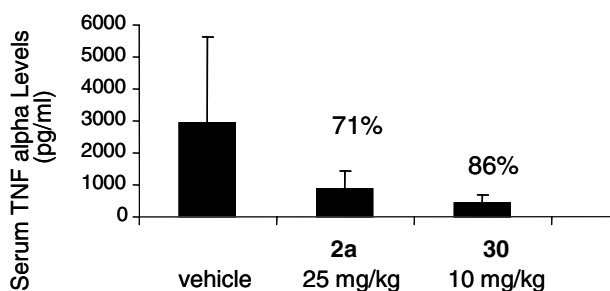
In summary, we have identified a novel class of selective Tpl2 inhibitors, the 4-alkylamino-[1,7]naphthyridine-3-carbonitriles. These compounds are potent in cellular

Table 6. Tpl2 activity and EGFR selectivity for 6-[(pyridine-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitriles

Compound	Headpiece	Tpl2 IC ₅₀ (μM)	LPS-induced TNF-α inhibition		EGFR(A431) IC ₅₀ (μM)
			Monocyte IC ₅₀ (μM)	Human blood IC ₅₀ (μM)	
58		>40			
59		0.36	0.8	4.5	>40
60		0.27	2	NA	

**Table 7.** Rat PK parameters for potent 4-alkylamino naphthyridine carbonitriles

Compound	Clearance (mL/min/kg)	AUC iv/dose (h kg ng/mL/mg)
29	50	328
27	28	732
30	30	1041

**Figure 2.** Effect of compounds **2a** (ip) and **30** (ip) on LPS/D-Gal-induced TNF-α release.

and human blood assays indicating their functional ability to inhibit release of TNF-α. In addition, they show good in vivo activity; a result, we believe, of their im-

proved pharmacokinetic properties. On the basis of this data the alkylamino-naphthyridine-carbonitriles have been selected for further investigation.

4. Experimental

4.1. Chemistry

Reactions were run using commercially available starting materials and solvents, without further purification. Proton NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer using TMS (δ 0.0) as a reference. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson-Microlit. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ± 0.4 of the theoretical values. Low resolution mass spectra were obtained using a Micromass Platform Electrospray Ionization Quadrupole mass spectrometer. High resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 Tesla superconducting magnet (Magnex Scientific Ltd, UK) and an external Bruker APOLLO electrospray ionization

Table 8. Kinase selectivity profile (IC₅₀, μM), Tpl2 activity and PK parameters for compounds **30** and **2a**

Compound	MEK ^a	p38 ^a	Src ^a	CAMKII ^a	MK2 ^a	PKA ^a	PKC ^a	S6 ^a	P-p38 ^b	EGFR ^c
30	>40	>40	38	NA	NA	>40	>40	>40	NA	>20
2a	2.4	30	NA	NA	NA	5.84	>10	>10	2.11	0.03
Compound	Tpl2 IC ₅₀ (μM)	LPS-induced TNF-α inhibition		PK parameters in rat						
		Monocyte IC ₅₀ (μM)	Human blood IC ₅₀ (μM)	Clearance (Cl/min/kg)	AUC iv/dose (h kg ng/mL/mg)	Bioavailability (%)				
30	0.16	0.5	4.5	30	1041	14				
2a	0.019	0.6	3.3	97	171	1				

NA, not active at 40 μM.

^a Enzymatic assays.

^b In human monocytes.

^c In A431 cells.

(ESI) source. The microwave procedures were carried out with a Biotage microwave. Preparative HPLC was run using a Waters reverse phase preparative HPLC with Xterra C18 5 μ M, 30 $\times$ 100 mm column. The flow rate was 40 mL/min and mobile phase A was water; mobile phase B was CH₃CN; triethylamine or formic acid was used as a modifier.

4.1.1. General procedure for the synthesis of [1,7]naphthyridine-3-carbonitriles (1). Step 1 Method A. In a 250 mL round-bottomed flask fitted with a condenser, 4-chloro-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**7**,¹⁰ 1.25 g, 6.02 mmol) and the appropriate aniline (1.32 mmol) were taken up in 100 mL of 2-ethoxyethanol or ethanol and heated at reflux for 1–10 h, until t.l.c. analysis showed complete disappearance of the 4-chloronaphthyridine. After cooling to room temperature, the reaction mixture was partitioned between EtOAc and 5% Na₂CO₃. The aqueous layer was extracted twice more with EtOAc, and the combined organic layers were washed three times with brine, dried over anhydrous MgSO₄, filtered, and evaporated to give 4-substituted-6-fluoro-[1,7]naphthyridine-3-carbonitriles which were considered of sufficient purity to be used directly in the next step. Method B. In a microwave vial, 4-chloro-6-fluoro-[1,7]naphthyridine-3-carbonitrile (1.25 g, 6.02 mmol) and the appropriate aniline (6.6 mmol) were taken up in DME. The vial was crimp-sealed and heated in a microwave reactor at 140 °C for 10 min. The contents of the vials were transferred to a separatory funnel and worked up as described above for Method A, giving 4-substituted-6-fluoro-[1,7]naphthyridine-3-carbonitriles of sufficient purity to be used directly in the next step. Step 2 Method C. A solution of the appropriate amine (1.7 mmol) and 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) in pyridine (3.3 mL) was heated to 80 °C for 7 days. Pyridine was removed by evaporation and crude products purified. Method D. The 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) was mixed with the appropriate amine (20 mmol) in a microwave vial. The sealed vial was heated in a microwave reactor at 190 °C for 5–10 min (until t.l.c. analysis showed complete consumption of starting material). The contents of the vial were transferred into a separatory funnel and partitioned between 300 mL each EtOAc and brine, and the aqueous layer extracted twice more with EtOAc. The combined organic extracts were washed with brine (3 $\times$), dried over anhydrous MgSO₄, filtered, and evaporated. Method E. The 6-fluoro-[1,7]naphthyridine-3-carbonitrile (1 mmol) (from Step 1) was taken up in a microwave vial in 5.6 mL THF or DMA, with the appropriate amine (20 mmol). The sealed vial was heated in a microwave reactor at 150 °C for 1 h, until t.l.c. analysis showed complete disappearance of the starting material. Then the THF was removed under reduced pressure. The crude products from Step 2 were purified by recrystallization, flash chromatography over silica gel or preparative HPLC (water/acetonitrile, triethylamine or formic acid modifier).

4.1.1.1. 4-(3-Chloro-4-fluorophenylamino)-6-(2-morpholin-4-yl-ethylamino)-[1,7]naphthyridine-3-carbonitrile (1a). Step 1 Method B. 4-(3-Chloro-4-fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8a**). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.32–7.49 (m, 1H) 7.53 (t, *J* = 9.0 Hz, 1H) 7.72 (dd, *J* = 6.3, 2.3 Hz, 1H) 8.16 (s, 1H) 8.69 (s, 1H) 9.08 (s, 1H) 10.14 (s, 1H). Step 2 Method C. 44% yield. ¹H NMR (400 MHz, CDCl₃) δ 2.5 (m, 4H) 2.6 (d, *J* = 11.9 Hz, 2H) 3.2 (m, 2H) 3.7 (m, 4H) 5.5 (t, *J* = 5.1 Hz, 1H) 6.2 (s, 1H) 6.9 (s, 1H) 7.1 (m, 1H) 7.2 (t, *J* = 8.6 Hz, 1H) 7.3 (m, 1H) 8.5 (s, 1H) 9.1 (d, *J* = 0.5 Hz, 1H). Anal. (C₂₁H₂₀ClFN₆O + 0.9H₂O) C, H, N.

4.1.1.2. 4-(3-Chloro-4-fluorophenylamino)-6-(pyridin-3-ylmethylamino)-[1,7]naphthyridine-3-carbonitrile (1b). Intermediate **8a** was treated with pyridin-3-ylmethylamine following the procedure for Step 2 Method D. 24% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 4.58 (d, *J* = 6.3 Hz, 2H) 7.10 (s, 1H) 7.26–7.39 (m, 2H) 7.46 (t, *J* = 9.0 Hz, 1H) 7.54 (t, *J* = 6.3 Hz, 1H) 7.59 (dd, *J* = 6.6, 2.8 Hz, 1H) 7.75 (dt, *J* = 7.8, 1.6 Hz, 1H) 8.29 (s, 1H) 8.44 (dd, *J* = 4.7, 1.6 Hz, 1H) 8.60 (d, *J* = 1.8 Hz, 1H) 8.87 (s, 1H) 9.67 (s, 1H). Anal. (C₂₁H₁₄ClFN₆) C, H, N.

4.1.1.3. 4-[(3-Chloro-4-fluorophenyl)amino]-6-[(1*R*)-1-phenylethyl]amino]-[1,7]naphthyridine-3-carbonitrile (1c). Intermediate **8a** was treated with (1*R*)-1-phenylethylamine following the procedure for Step 2 Method E. 35% yield. ¹H NMR (400 MHz, MeOD) δ 1.51 (d, *J* = 14.3 Hz, 3H), 3.46–3.61 (m, 1H), 4.34–4.56 (br s, 1H), 5.06 (br s, 1H), 6.73 (d, *J* = 15.9 Hz, 1H), 7.54–7.69 (m, 5H), 7.73–7.85 (m, 4H), 7.90 (d, *J* = 15.9 Hz, 1H). HRMS (ESI+) calcd for C₂₃H₁₇ClFN₅ (M+H) 418.1230, found 418.1231.

4.1.1.4. 4-(4-Fluorophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (16). Step 1 Method A. 4-(4-Fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8b**). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.27–7.36 (m, 2H) 7.41–7.49 (m, 2H) 8.19 (s, 1H) 8.63 (s, 1H) 9.05 (s, 1H) 10.09 (s, 1H). Step 2 Method E. 26% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.45 (br s, 4H) 2.58 (br s, 2H) 3.38 (q, *J* = 6.4 Hz, 2H) 3.52–3.64 (m, 4H) 6.60 (t, *J* = 6.2 Hz, 1H) 7.06 (s, 1H) 7.27 (t, *J* = 8.8 Hz, 2H) 7.33–7.43 (m, 2H) 8.22 (s, 1H) 8.82 (s, 1H) 9.60 (s, 1H). HRMS (ESI+) calcd for C₂₁H₂₂FN₆O (M+H) 393.1834, found 393.1833.

4.1.1.5. 4-(3,4-Difluorophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (17). Step 1 Method B. 6-Fluoro-4-(3,4-difluorophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8c**). ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.18–7.36 (m, 1H) 7.43–7.65 (m, 2H) 8.17 (s, 1H) 8.69 (s, 1H) 9.08 (s, 1H) 10.16 (s, 1H). Step 2 Method E. 24% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.45 (br s, 4H) 2.57 (t, *J* = 6.4 Hz, 2H) 3.34–3.42 (m, 2H) 3.51–3.64 (m, 4H) 6.66 (t, *J* = 5.7 Hz, 1H) 7.02 (s, 1H) 7.13–7.24 (m, 1H) 7.43–7.58 (m, 2H) 8.28 (s, 1H) 8.85 (s, 1H) 9.68 (s, 1H). HRMS (ESI+) calcd for C₂₁H₂₁F₂N₆O (M+H) 411.1740, found 411.1737.

4.1.1.6. 6-(2-Morpholinoethylamino)-4-(4-(phenylamino)phenylamino)-[1,7]naphthyridine-3-carbonitrile (18). Step 1 Method A. 6-Fluoro-4-(4-(phenylamino)phenylamino)-[1,7]naphthyridine-3-carbonitrile (**8d**). ^1H NMR (400 MHz, DMSO- d_6) δ 6.86 (tt, J = 7.3, 1.0 Hz, 1H) 7.07–7.17 (m, 4H) 7.21–7.32 (m, 4H) 8.21 (s, 1H) 8.37 (s, 1H) 8.58 (s, 1H) 9.02 (s, 1H) 9.99 (s, 1H). Step 2 Method E. 13% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.45 (br s, 4H) 2.58 (t, J = 6.4 Hz, 2H) 3.37 (q, J = 6.7 Hz, 2H) 3.55–3.64 (m, 4H) 6.53 (t, J = 5.4 Hz, 1H) 6.85 (t, J = 7.2 Hz, 1H) 7.05–7.17 (m, 5H) 7.17–7.31 (m, 4H) 8.17 (s, 1H) 8.31 (s, 1H) 8.79 (s, 1H) 9.53 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{27}\text{H}_{28}\text{N}_7\text{O}$ (M+H) 466.2350, found 466.2350.

4.1.1.7. 6-(2-Morpholin-4-yl-ethylamino)-4-(4-nitrophenylamino)-[1,7]naphthyridine-3-carbonitrile (19). Step 1 Method A gave crude **8e**, which was taken to the next step. Step 2 Method E. 18% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.35–2.42 (m, J = 3.8 Hz, 4H) 2.46–2.50 (m, 2H) 3.34–3.39 (m, 2H) 3.52–3.57 (m, 4H) 6.78 (s, 1H) 6.89 (t, J = 5.6 Hz, 1H) 7.23 (d, J = 9.1 Hz, 2H) 8.18–8.23 (m, J = 9.7, 3.0, 2.7 Hz, 2H) 8.54 (s, 1H) 8.98 (s, 1H) 10.12 (s, 1H). HRMS calcd for $\text{C}_{21}\text{H}_{21}\text{N}_7\text{O}_3$ (M+H), 420.1779, found 420.1777.

4.1.1.8. 4-(3-Iodophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile (20). Step 1 Method A. 6-Fluoro-4-(3-iodophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8f**). ^1H NMR (400 MHz, DMSO- d_6) δ 7.25 (t, J = 8.0 Hz, 1H) 7.40 (ddd, J = 8.1, 2.2, 0.9 Hz, 1H) 7.67 (dt, J = 7.8, 1.7 Hz, 1H) 7.75 (t, J = 1.8 Hz, 1H) 8.15 (s, 1H) 8.72 (s, 1H) 9.09 (s, 1H) 10.10 (s, 1H). Step 2 Method E. 17% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.43 (s, 4H) 2.55 (t, J = 6.4 Hz, 2H) 3.36 (q, J = 6.2 Hz, 2H) 3.53–3.61 (m, 4H) 6.66 (t, J = 5.4 Hz, 1H) 6.97 (s, 1H) 7.19 (t, J = 7.8 Hz, 1H) 7.25–7.31 (m, 1H) 7.57 (d, J = 8.1 Hz, 1H) 7.62–7.65 (m, 1H) 8.31 (s, 1H) 8.86 (s, 1H) 9.60 (s, 1H). Anal. ($\text{C}_{21}\text{H}_{21}\text{IN}_6\text{O}$) C, H, N.

4.1.1.9. 3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzoic acid (21). Compound **7** was treated with methyl 3-aminobenzoate following the procedure for Step 1 Method A to give methyl 3-(3-cyano-6-fluoro-[1,7]naphthyridine-4-ylamino)benzoate (**8g**). ^1H NMR (400 MHz, DMSO- d_6) δ 3.88 (s, 3H) 7.62 (t, J = 7.7 Hz, 1H) 7.64–7.69 (m, J = 8.5, 1.9, 1.5 Hz, 1H) 7.88–7.93 (m, 2H) 8.19 (s, 1H) 8.72 (s, 1H) 9.10 (s, 1H) 10.22 (s, 1H). Above compound was treated with 4-(2-aminoethyl)morpholine following the procedure for Step 2 Method E to give methyl 3-(3-cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzoate (**1d**). ^1H NMR (400 MHz, DMSO- d_6) δ 2.40–2.46 (m, 4H) 2.55 (t, J = 6.4 Hz, 2H) 3.34–3.39 (m, 2H) 3.54–3.60 (m, 4H) 3.87 (s, 3H) 6.67 (t, J = 5.2 Hz, 1H) 7.01 (s, 1H) 7.56 (dt, J = 4.4, 1.2 Hz, 2H) 7.79–7.83 (m, 2H) 8.32 (s, 1H) 8.87 (s, 1H) 9.73 (s, 1H). To **1d** (2 mmol) in tetrahydrofuran (12 mL) were added methyl alcohol (4.5 mL) and lithium hydroxide (1 N, 4.5 mL). After 12 h the solvents were evaporated and the crude mixture was purified by preparative HPLC to give 3-(3-cyano-6-(2-morpholino-

ethylamino)-[1,7]naphthyridine-4-ylamino)benzoic acid (**21**) in 54% overall yield. ^1H NMR (400 MHz, MeOD) δ 3.13–3.23 (m, 6H) 3.72 (t, J = 6.1 Hz, 2H) 3.84–3.90 (m, 4H) 7.09 (s, 1H) 7.48–7.55 (m, 2H) 7.90–7.96 (m, 2H) 8.29 (s, 1H) 8.90 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_3$ (M+H), 419.1826, found 419.1821.

4.1.1.10. 3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)benzamide (22). Step 1 Method A. 3-(3-Cyano-6-fluoro-[1,7]naphthyridine-4-ylamino)benzamide (**8h**). ^1H NMR (400 MHz, DMSO- d_6) δ 7.00–7.08 (m, 1H) 7.49–7.59 (m, 2H) 7.81–7.92 (m, 2H) 8.04 (s, 1H) 8.21 (s, 1H) 8.70 (s, 1H) 9.09 (s, 1H) 10.18 (s, 1H). Step 2 Method E. 37% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.38–2.48 (m, 4H) 2.52–2.59 (m, 2H) 3.34–3.41 (m, 2H) 3.53–3.62 (m, 4H) 6.64 (s, 1H) 7.05 (s, 1H) 7.40–7.52 (m, 3H) 7.73–7.81 (m, 2H) 8.02 (s, 1H) 8.29 (s, 1H) 8.86 (s, 1H) 9.69 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{23}\text{N}_7\text{O}_2$ (M+H), 418.1986, found 419.1980.

4.1.1.11. 6-(2-Morpholinoethylamino)-4-(3-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (23). Step 1 Method A. 6-Fluoro-4-(3-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (**8i**). ^1H NMR (400 MHz, DMSO- d_6) δ 6.94–7.18 (m, 6H) 7.33–7.51 (m, 3H) 8.15 (s, 1H) 8.68 (s, 1H) 9.05 (s, 1H) 10.12 (s, 1H). Step 2 Method E. 27% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.44 (br s, 4H) 2.57 (t, J = 6.3 Hz, 2H) 3.34–3.43 (m, 2H) 3.49–3.64 (m, 4H) 6.64 (t, J = 5.8 Hz, 1H) 6.90 (dd, J = 3.8, 1.5 Hz, 2H) 7.00 (s, 1H) 7.03–7.11 (m, 3H) 7.14 (t, J = 7.5 Hz, 1H) 7.33–7.47 (m, 3H) 8.28 (s, 1H) 8.84 (s, 1H) 9.64 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{27}\text{H}_{27}\text{N}_6\text{O}_2$ (M+H) 467.2190, found 467.2188.

4.1.1.12. N-(3-(3-Cyano-6-(2-morpholinoethylamino)-[1,7]naphthyridine-4-ylamino)phenyl)methanesulfonamide (24). Compound **7** was treated with 3-nitroaniline following the procedure for Step 1 Method A to give 6-fluoro-4-(3-nitrophenylamino)-[1,7]naphthyridine-3-carbonitrile (**8j**). ^1H NMR (400 MHz, DMSO- d_6) δ 7.74 (t, J = 8.1 Hz, 1H) 7.80–7.85 (m, 1H) 8.12–8.16 (m, 1H) 8.17 (s, 1H) 8.21 (t, J = 2.0 Hz, 1H) 8.80 (s, 1H) 9.14 (s, 1H) 10.35 (s, 1H). The above compound was treated with 4-(2-aminoethyl)morpholine following the procedure for Step 2 Method E to give 6-(2-morpholinoethylamino)-4-(3-nitrophenylamino)-[1,7]naphthyridine-3-carbonitrile (**1e**). 19% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.37–2.48 (m, 4H) 2.52–2.59 (m, 2H) 3.35–3.41 (m, 2H) 3.54–3.61 (m, 4H) 6.71–6.80 (m, 1H) 6.96 (s, 1H) 7.66–7.71 (m, 2H) 8.00–8.05 (m, 1H) 8.06–8.08 (m, 1H) 8.40 (s, 1H) 8.92 (s, 1H) 9.89 (s, 1H). A mixture of **1e** (120 mg, 0.29 mmol) and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (348 mg, 1.54 mmol) in ethyl alcohol (12 mL) was heated to reflux for 2.5 h. After cooling to room temperature, water (10 mL) was added followed by sodium bicarbonate (500 mg). The mixture was stirred at room temperature for 1 h. Ethyl acetate extraction followed by column chromatography (3–5% methyl alcohol in dichloromethane) yielded 43 mg of 4-(3-aminophenylamino)-6-(2-morpholinoethylamino)-[1,7]naphthyridine-3-carbonitrile, (**1f**) 39% yield. ^1H

NMR (400 MHz, DMSO- d_6) 2.40–2.48 (m, 4H) 2.53–2.60 (m, 2H) 3.34–3.40 (m, 2H) 3.54–3.63 (m, 4H) 5.18–5.27 (m, 2H) 6.39–6.47 (m, 3H) 6.57 (s, 1H) 7.01–7.08 (m, 2H) 8.22 (s, 1H) 8.81 (s, 1H) 9.43 (s, 1H). Compound **1f** (111 mg, 0.29 mmol) was suspended in methylene chloride (10 mL). Triethylamine (44 μ L, 0.32 mmol) was added and the mixture was cooled to 0 °C. Methylsulfonyl chloride (24 μ L, 0.31 mmol) was added and the mixture was stirred at room temperature for 12 h. Another 44 μ L triethylamine and 48 μ L methylsulfonyl chloride were added and the reaction mixture was stirred for 12 h. Work-up and preparative HPLC yielded 45.5 mg of product **24** (34% yield). ^1H NMR (400 MHz, DMSO- d_6) δ 2.39–2.47 (m, 4H) 2.56 (t, J = 6.8 Hz, 2H) 2.99–3.03 (m, 3H) 3.27–3.42 (m, 2H) 3.55–3.61 (m, 4H) 6.63 (t, J = 6.1 Hz, 1H) 7.00–7.04 (m, 1H) 7.04 (s, 1H) 7.08 (d, J = 8.6 Hz, 1H) 7.12–7.16 (m, 1H) 7.36 (t, J = 8.3 Hz, 1H) 8.26 (s, 1H) 8.84 (s, 1H) 9.69 (s, 2H). HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{25}\text{N}_7\text{O}_3\text{S}$ (M+H), 468.1812, found 468.1810.

4.1.1.13. 4-Phenylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (25). Step 1 Method B. 6-Fluoro-4-phenylamino-[1,7]naphthyridine-3-carbonitrile (**8k**). ^1H NMR (400 MHz, DMSO- d_6) δ 7.25–7.33 (m, 3H) 7.42 (t, J = 7.7 Hz, 2H) 8.13 (s, 1H) 8.52 (s, 1H) 8.96 (s, 1H) 10.15 (s, 1H). Step 2 Method D. 38% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 4.56 (d, J = 6.3 Hz, 2H) 7.10–7.16 (m, 1H) 7.22–7.30 (m, 3H) 7.33 (dd, J = 7.6, 4.8 Hz, 1H) 7.41 (t, J = 7.8 Hz, 2H) 7.44–7.54 (m, 1H) 7.75 (d, J = 7.8 Hz, 1H) 8.25 (s, 1H) 8.39–8.48 (m, 1H) 8.59 (s, 1H) 8.85 (s, 1H) 9.62 (s, 1H). Anal. ($\text{C}_{21}\text{H}_{16}\text{N}_6$ + 0.8 H_2O) C, H, N.

4.1.1.14. 4-Cyclohexylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (26). Step 1 Method B. 4-Cyclohexylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8l**). ^1H NMR (400 MHz, DMSO- d_6) δ 1.48–1.84 (m, 8H) 2.01–2.11 (m, 2H) 4.53 (d, J = 5.1 Hz, 1H) 7.86 (d, J = 8.8 Hz, 1H) 8.28 (s, 1H) 8.57 (s, 1H) 8.92 (s, 1H). Step 2 Method D. 63% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.05–1.88 (m, 8H) 2.05 (s, 2H) 4.23 (s, 1H) 4.48–4.68 (m, 2H) 7.07–7.13 (m, 1H) 7.26–7.44 (m, 3H) 7.77 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.43 (d, J = 6.1 Hz, 1H) 8.61 (s, 1H) 8.71 (s, 1H). Anal. ($\text{C}_{21}\text{H}_{22}\text{N}_6$ + 1.8 H_2O) C, H, N.

4.1.1.15. 4-Cyclopentylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (27). Step 1 Method B. 4-Cyclopentylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8m**). ^1H NMR (400 MHz, DMSO- d_6) δ 1.59–1.66 (m, 2H) 1.74–1.88 (m, 4H) 2.03–2.12 (m, 2H) 4.69–4.74 (m, 1H) 7.88 (d, J = 7.3 Hz, 1H) 8.30 (s, 1H) 8.59 (s, 1H) 8.93 (s, 1H). Step 2 Method D. 50% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.71–1.84 (m, 2H) 2.02–2.12 (m, 1H) 3.32 (s, 6H) 4.58 (d, J = 6.3 Hz, 2H) 7.06–7.14 (m, 1H) 7.28–7.36 (m, 2H) 7.41 (d, J = 7.6 Hz, 1H) 7.77 (dd, J = 7.8, 1.8 Hz, 1H) 8.19 (s, 1H) 8.43 (dd, J = 4.7, 1.6 Hz, 1H) 8.61 (d, J = 2.0 Hz, 1H) 8.72 (s, 1H). Anal. ($\text{C}_{20}\text{H}_{20}\text{N}_6$ + 1.2 H_2O) C, H, N.

4.1.1.16. 4-Cyclopropylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (28). Step 1

Method B. 4-Cyclopropylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8n**). ^1H NMR (400 MHz, DMSO- d_6) δ 0.89–1.01 (m, 4H) 3.09–3.18 (m, J = 6.2, 3.9 Hz, 1H) 8.11 (s, 1H) 8.54 (s, 1H) 8.65 (s, 1H) 8.95 (s, 1H). Step 2 Method D. 22% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 0.80–0.89 (m, J = 3.5, 3.5 Hz, 1H) 0.95 (dd, J = 6.8, 1.8 Hz, 2H) 4.54 (d, J = 6.3 Hz, 2H) 7.02–7.08 (m, 1H) 7.26–7.40 (m, 2H) 7.76 (dd, J = 7.8, 1.5 Hz, 1H) 8.15 (d, J = 2.3 Hz, 1H) 8.23 (s, 1H) 8.43 (dd, J = 4.8, 1.5 Hz, 1H) 8.60 (d, J = 2.0 Hz, 1H) 8.73 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{18}\text{H}_{16}\text{N}_6$ (M+H), 317.1509, found 317.1504.

4.1.1.17. 4-tert-Butylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (29). Step 1 Method B. 4-tert-Butylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8o**). ^1H NMR (400 MHz, DMSO- d_6) δ 1.62 (s, 9H) 7.26 (s, 1H) 8.17 (s, 1H) 8.66 (s, 1H) 8.96 (s, 1H). Step 2 Method D. 37% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.42–1.62 (m, 9H) 4.58 (d, J = 5.8 Hz, 2H) 6.58 (s, 1H) 6.89 (s, 1H) 7.25–7.38 (dd, J = 7.7, 4.9 Hz, 1H) 7.45–7.53 (m, 1H) 7.71–7.81 (m, 1H) 8.15 (s, 1H) 8.26 (s, 1H) 8.43 (d, J = 4.8 Hz, 1H) 8.62 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{20}\text{N}_6$ (M+H), 333.1822, found 333.1824.

4.1.1.18. 4-Cycloheptylamino-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (30). Step 1 Method B. 4-Cycloheptylamino-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8p**). ^1H NMR (400 MHz, DMSO- d_6) δ 1.11–1.58 (m, 6H) 1.60–1.87 (m, 4H) 2.03–2.12 (m, 2H) 4.29 (s, 1H) 7.84–7.96 (m, 1H) 8.26 (s, 1H) 8.57 (s, 1H) 8.92 (s, 1H). Step 2 Method D. 33% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.46–1.84 (m, 10H) 1.95–2.11 (m, 2H) 4.38–4.52 (m, 1H) 4.58 (d, J = 6.6 Hz, 2H) 7.11 (d, 1H) 7.22–7.45 (m, 3H) 7.77 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.43 (dd, J = 4.7, 1.4 Hz, 1H) 8.61 (s, 1H) 8.71 (s, 1H). Anal. ($\text{C}_{22}\text{H}_{24}\text{N}_6$ + 2.0 H_2O) C, H, N.

4.1.1.19. 6-[(2-Morpholin-4-ylethyl)amino]-4-[(4-phenoxyphenyl)amino]-[1,7]naphthyridine-3-carbonitrile (32). Step 1 Method A. 6-Fluoro-4-(4-phenoxyphenylamino)-[1,7]naphthyridine-3-carbonitrile (**8q**). ^1H NMR (400 MHz, DMSO- d_6) δ 7.1 (m, 2H) 7.1 (m, 3H) 7.4 (m, 4H) 8.2 (s, 1H) 8.6 (s, 1H) 9.1 (s, 1H) 10.1 (m, 1H). Step 2 Method C. 44% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 2.5 (m, 4H) 2.6 (t, J = 6.8 Hz, 2H) 3.4 (m, 2H) 3.6 (m, 4H) 6.6 (t, J = 5.6 Hz, 1H) 7.0 (m, 2H) 7.1 (m, 4H) 7.4 (m, 3H) 8.2 (s, 1H) 8.3 (s, 1H) 8.8 (s, 1H) 9.6 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{27}\text{H}_{26}\text{N}_6\text{O}_2$ (M+H), 467.2190, found 467.2196.

4.1.1.20. 4-Cycloheptylamino-6-(R)-1-phenylethylamino-[1,7]naphthyridine-3-carbonitrile (52). Intermediate **8p** was treated with (R)-1-phenylethylamine following the procedure for Step 2 Method D. 7% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.41 (d, J = 6.8 Hz, 3H) 1.46–1.89 (m, 12H) 4.32–4.52 (m, 1H) 4.89–5.10 (m, 1H) 7.10–7.21 (dd, J = 7.3, 7.3 Hz, 1H) 7.22–7.44 (m, 5H) 7.85 (s, 1H) 8.18 (s, 1H) 8.38 (s, 2H). HRMS (ESI+) calcd for $\text{C}_{24}\text{H}_{27}\text{N}_5$ (M+H), 386.2339, found 386.2335.

4.1.1.21. 4-(Cycloheptylmethylamino)-6-(*R*)-1-phenylethylamino-[1,7]naphthyridine-3-carbonitrile (53). To a solution of 4-(cycloheptylmethylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8p**, 0.2 g, 0.7 mmol) in DMF (3 mL) was added NaH (60% in mineral oil, 40 mg, 0.8 mmol) at room temperature. After stirring for 10 min 0.2 mL of MeI was added. The reaction mixture was stirred for 4 h, then quenched with water. The aqueous solution was filtered to give 4-(cycloheptylmethylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8u**) as a beige solid (150 mg, 71% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.50–1.84 (m, 12H) 3.32 (s, 3H) 7.69 (d, $J = 2.0$ Hz, 1H) 8.14 (s, 1H) 8.47 (s, 1H). Intermediate **8u** was treated with (*R*)-1-phenylethylamine following the procedure for Step 2 Method D to give compound **53**. 10% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.37–1.85 (m, 12H) 1.84–2.17 (m, 2H) 3.32 (s, 3H) 4.31–4.49 (m, $J = 4.0$ Hz, 1H) 4.96–5.08 (m, $J = 7.8$ Hz, 1H) 6.89–6.99 (m, 1H) 7.10–7.32 (m, 5H) 7.47 (d, $J = 7.3$ Hz, 2H) 8.13 (s, 1H) 8.66 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{25}\text{H}_{29}\text{N}_5$ (M+H), 400.2496, found 400.2499.

4.1.1.22. 6-(3-Chloro-4-fluorophenylamino)-4-(cyclopentylamino)-[1,7]naphthyridine-3-carbonitrile (54). A mixture of 3-chloro-4-fluorobenzeneamine (550 mg, 3.78 mmol), 4-(cyclopentylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8m**, 28 mg, 0.1 mmol), and cesium carbonate (506 mg, 1.55 mmol, 15.5 equiv) in DMF (4 mL) was heated to 200 °C in a microwave tube for 1 h using a microwave reactor. Preparative HPLC purification yielded 15 mg product (36% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.52–1.68 (m, 2H) 1.70–1.91 (m, 4H) 1.99–2.16 (m, 2H) 4.58–4.76 (m, 1H) 7.33 (t, $J = 9.1$ Hz, 1H) 7.45–7.56 (m, 1H) 7.76 (s, 1H) 7.80 (d, $J = 7.1$ Hz, 1H) 7.87–7.97 (m, 1H) 8.32 (s, 1H) 8.90 (s, 1H) 9.64 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{17}\text{ClFN}_5$ (M+H), 382.1229, found 382.1237.

4.1.1.23. 4-(3-Chloro-4-fluorophenylamino)-6-cyclopentylamino-[1,7]naphthyridine-3-carbonitrile (55). Step 1 Method A. 4-(3-Chloro-4-fluorophenylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8a**). Step 2 Method D. 41% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.43–1.63 (m, 4H) 1.65–1.78 (m, 2H) 1.91–2.07 (m, $J = 9.4$ Hz, 2H) 3.84–4.02 (m, 1H) 6.81–6.98 (m, 2H) 7.30–7.40 (m, 1H) 7.41–7.53 (m, 1H) 7.56–7.65 (m, 1H) 8.26 (s, 1H) 8.83 (s, 1H) 9.63 (s, 1H). Anal. ($\text{C}_{20}\text{H}_{17}\text{ClFN}_5 + 1.1 \text{ H}_2\text{O}$) C, H, N.

4.1.1.24. 6-*tert*-Butylamino-4-(3-chloro-4-fluorophenylamino)-[1,7]naphthyridine-3-carbonitrile (56). Intermediate **8a** was treated with *tert*-butylamine following the procedure for Step 2 Method E. 21% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.32–1.51 (m, 9H) 6.40–6.48 (m, 1H) 6.94 (s, 1H) 7.28 (s, 1H) 7.37–7.59 (m, 2H) 8.27 (s, 1H) 8.85 (s, 1H) 9.67 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{17}\text{ClFN}_5$ (M+H), 370.1229, found 370.1229.

4.1.1.25. 4-(3-Chloro-4-fluoro-phenylamino)-6-cycloheptylamino-[1,7]naphthyridine-3-carbonitrile (57). Intermediate **8a** was treated with cycloheptylamine

following the procedure for Step 2 Method D. 19% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.27–2.03 (m, 12H) 3.66–3.83 (m, 1H) 6.72–6.87 (m, 2H) 7.27–7.38 (m, 1H) 7.45 (t, $J = 9.1$ Hz, 1H) 7.52–7.62 (m, 1H) 8.26 (s, 1H) 8.83 (s, 1H) 9.61 (s, 1H). Anal. ($\text{C}_{22}\text{H}_{21}\text{ClFN}_5 + 0.6 \text{ CH}_3\text{OH}$) C, H, N.

4.1.1.26. 4-(Piperidin-4-ylamino)-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (58). Compound **7** was treated with 4-aminopiperidine-1-carboxylic acid *tert*-butyl ester following the procedure for Step 1 Method B to give 4-(3-cyano-6-fluoro-[1,7]naphthyridin-4-ylamino)piperidine-1-carboxylic acid *tert*-butyl ester (**8r**). Crude **8r** was treated with pyridin-3-ylmethylamine following the procedure for Step 2 Method D to give 4-{3-cyano-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-4-ylamino}-piperidine-1-carboxylic acid *tert*-butyl ester (**1g**). Crude **1g** (0.285 mg, 0.621 mmol) from above was treated with 4 M HCl in dioxane (21 mL) at 0 °C. The reaction mixture was stirred overnight at 0 °C and then purified using preparative HPLC to give compound **58**. 10% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.61–1.86 (m, 2H) 2.03 (d, $J = 13.1$ Hz, 2H) 2.71 (t, $J = 12.4$ Hz, 2H) 3.18 (d, $J = 12.4$ Hz, 3H) 4.33 (s, 1H) 4.58 (d, $J = 6.3$ Hz, 2H) 7.13–7.20 (m, 1H) 7.26–7.39 (m, 2H) 7.53 (d, $J = 7.3$ Hz, 1H) 7.77 (d, $J = 7.8$ Hz, 1H) 8.19 (s, 1H) 8.29 (s, 1H) 8.42 (d, $J = 4.6$ Hz, 1H) 8.61 (s, 1H) 8.73 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{21}\text{N}_7$ (M+H), 360.1931, found 360.1925.

4.1.1.27. 4-(1,1-Dimethylpropylamino)-6-[(pyridin-3-ylmethyl)amino]-[1,7]naphthyridine-3-carbonitrile (59). Step 1 Method B. 4-(1,1-Dimethylpropylamino)-6-fluoro-[1,7]naphthyridine-3-carbonitrile (**8s**). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 0.90 (t, $J = 7.5$ Hz, 3H) 1.56 (s, 6H) 2.02 (q, $J = 7.3$ Hz, 2H) 7.12 (s, 1H) 8.18 (s, 1H) 8.65 (s, 1H) 8.97 (s, 1H). Step 2 Method D. The reaction mixture was heated at 140 °C for 10 min. 19% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 0.82 (t, $J = 7.5$ Hz, 3H) 1.47 (s, 6H) 1.79–2.02 (m, 2H) 4.59 (d, $J = 5.8$ Hz, 2H) 6.44 (s, 1H) 6.90 (s, 1H) 7.32 (dd, $J = 8.1, 4.8$ Hz, 1H) 7.49 (t, $J = 6.4$ Hz, 1H) 7.76 (d, $J = 7.8$ Hz, 1H) 8.25 (s, 1H) 8.43 (d, $J = 3.5$ Hz, 1H) 8.61 (d, $J = 1.3$ Hz, 1H) 8.77 (s, 1H). Anal. ($\text{C}_{20}\text{H}_{22}\text{N}_6 + 1.3 \text{ H}_2\text{O}$) C, H, N.

4.1.1.28. 4-(1,1-Dimethylprop-2-ynylamino)-6-[(pyridin-3-ylmethyl)-amino]-[1,7]naphthyridine-3-carbonitrile (60). Step 1 Method B. The reaction mixture was heated at 140 °C for thirty minutes to give **8t**. Crude **8t** was taken forward to the next step. Step 2 Method D. The reaction mixture was heated at 140 °C for ten minutes. 10% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 1.80 (s, 6H) 3.50–3.61 (m, 1H) 4.57 (d, $J = 6.6$ Hz, 2H) 6.95 (d, $J = 14.4$ Hz, 2H) 7.29–7.37 (m, 1H) 7.52 (t, $J = 6.4$ Hz, 1H) 7.77 (dd, $J = 7.8, 2.0$ Hz, 1H) 8.31 (s, 1H) 8.38–8.45 (dd, $J = 4.7, 1.6$ Hz, 1H) 8.62 (s, 1H) 8.80 (s, 1H). Anal. ($\text{C}_{20}\text{H}_{18}\text{N}_6 + 0.4 \text{ H}_2\text{O}$) C, H, N.

4.1.2. General procedure for the synthesis of quinoline-3-carbonitriles (2). Step 1 Method F. In a microwave vial, 4-chloro-6-nitro-quinoline-3-carbonitrile (**13a**,^{8j} 0.4 g,

1.71 mmol) and the appropriate amine (2.05 mmol) were taken up in 2 mL EtOH. The vial was crimp-sealed and heated in a microwave reactor at 150 °C for 45 min. The solvent was removed and the residue was partitioned between ether and H₂O. This gave a suspension, which was filtered, washed with H₂O, and dried on a high vacuum pump overnight to give a solid, which was used in the next step without purification.

Method G. 4-Chloro-6-nitroquinoline-3-carbonitrile (**13a**,^{8j} 2.33 g, 10 mmol) and the appropriate amine (1.74 g, 12 mmol) were suspended in ethanol (60 mL) and heated at reflux for 3 h or until t.l.c. analysis showed that the reaction was complete. After cooling, the solvent was removed in vacuo and the residue was stirred with ether/saturated aqueous NaHCO₃ (100 mL/75 mL) for 2.5 h to triturate. The solid was collected by suction filtration and used in the next step.

Step 2 Method H. In a microwave vial, 4-amino-6-nitroquinoline-3-carbonitrile (**14**, 0.97 mmol) and SnCl₂·2H₂O (1.09 g, 4.83 mmol) were taken up in 2 mL EtOH. The vial was sealed and heated in a microwave reactor at 110 °C for 10 min, until LC/MS analysis showed complete disappearance of the nitroquinoline. The reaction mixture was poured into ice water, neutralized with saturated NaHCO₃, and extracted with EtOAc (2× 150 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated. These products were used in the next step without purification.

Method I. 4-Amino-6-nitroquinoline-3-carbonitrile (**14**, 7.29 mmol) was suspended in ethanol (85 mL), then SnCl₂·2H₂O (8.3 g, 36.5 mmol) was added and the reaction mixture heated at reflux for 2.5 h or until complete by t.l.c. The reaction mixture was diluted with 100 mL of water, and then solid NaHCO₃ was added until the pH was basic (~11). The solution was extracted with chloroform, washed with brine, treated with activated charcoal, dried over MgSO₄, and solvent evaporated. These products were used in the next step without purification.

Step 3 Method J. In a 50 mL round-bottomed flask, the 6-aminoquinoline-3-carbonitrile (**15**, 0.29 mmol) was taken up in 3 mL EtOH, and the appropriate aldehyde (0.37 mmol) was added. Acetic acid was added to bring the pH of the solution to 4, and the mixture stirred for 15 min. NaCNBH₃ (12.5 mg, 0.20 mmol) was then added, and the reaction mixture allowed to stir from 4 to 24 h at 25–30 °C. Ethanol was then removed under reduced pressure and the crude product was purified by preparative HPLC.

4.1.2.1. 4-[(3-Chloro-4-fluorophenyl)amino]-6-[(2-morpholin-4-ylethyl)amino]quinoline-3-carbonitrile (31**).** Step 1 Method G was used to synthesize 4-(3-chloro-4-fluorophenylamino)-6-nitroquinoline-3-carbonitrile (**14a**) in 80% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.28–7.35 (m, 1H) 7.47 (t, *J* = 9.0 Hz, 1H) 7.56 (dd, *J* = 6.7, 2.7 Hz, 1H) 7.99 (d, *J* = 9.1 Hz, 1H) 8.49 (dd, *J* = 9.4, 2.5 Hz, 1H) 8.64 (s, 1H) 9.47 (d, *J* = 2.3 Hz, 1H) 10.72

(s, 1H). Step 2 Method I was used to obtain 6-amino-4-(3-chloro-4-fluorophenylamino)quinoline-3-carbonitrile (**15a**) in 90% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.78 (s, 2H) 7.12–7.19 (m, 2H) 7.25 (dd, *J* = 8.8, 2.3 Hz, 1H) 7.34–7.42 (m, 2H) 7.70 (d, *J* = 9.1 Hz, 1H) 8.34 (s, 1H) 9.36 (s, 1H). 4-(2,2-Dimethoxyethyl)morpholine¹¹ was heated with 37% aqueous HCl (0.6 mL for 0.5 mmol) overnight at 70 °C. This reaction mixture was treated with intermediate **15a** following the procedure for Step 3 Method J. 60% yield. ¹H NMR (400 MHz, MeOD) δ 2.49 (m, 2H), 2.51–2.53 (m, 2H), 3.18–3.30 (m, 4H), 3.49–3.52 (m, 2H), 3.57 (m, 2H), 6.88 (s, 1H), 7.02–7.23 (m, 3H), 7.25–7.33 (m, 1H), 7.58 (d, *J* = 9.1 Hz, 1H), 8.18 (s, 1H). HRMS (ESI+) calcd for C₂₂H₂₁ClFN₅O (M+H), 426.1491; found 426.1497.

4.1.2.2. 4-(3-Chloro-4-fluorophenylamino)-6-[(pyridin-3-ylmethyl)amino]quinoline-3-carbonitrile (34**).** Intermediate **15a** was treated with pyridine-3-carbaldehyde following the procedure for Step 3 Method J. 95% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.35 (dd, *J* = 3.3, 0.8 Hz, 1H) 6.39 (dd, *J* = 3.3, 1.8 Hz, 1H) 6.79 (t, *J* = 5.6 Hz, 1H) 7.21–7.27 (m, 3H) 7.35 (dd, *J* = 9.1, 2.5 Hz, 1H) 7.43 (t, *J* = 9.0 Hz, 1H) 7.48 (dd, *J* = 6.7, 2.7 Hz, 1H) 7.60 (dd, *J* = 1.8, 0.8 Hz, 1H) 7.70 (d, *J* = 9.1 Hz, 1H) 8.33 (s, 1H). HRMS (ESI+) calcd for C₂₁H₁₄ClFN₄O (M+H) 393.0913, found 393.0917.

4.1.2.3. 6-Benzylamino-4-cyclopentylaminoquinoline-3-carbonitrile (35**).** Step 1 Method F was used to synthesize 4-cyclopentylamino-6-nitroquinoline-3-carbonitrile (**14b**) in 66% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.59–1.68 (m, 2H) 1.76–1.93 (m, 4H) 2.05–2.15 (m, 2H) 4.72–4.80 (m, 1H) 7.97 (d, *J* = 9.1 Hz, 1H) 8.40 (s, 1H) 8.46 (dd, *J* = 9.1, 2.3 Hz, 1H) 8.66 (s, 1H) 9.56 (d, *J* = 2.3 Hz, 1H). Step 2 Method H was used to obtain 6-amino-4-cyclopentylaminoquinoline-3-carbonitrile (**15b**) in 50% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.60 (s, 2H) 1.77 (d, *J* = 3.0 Hz, 4H) 2.01–2.11 (m, 2H) 4.64 (d, *J* = 5.8 Hz, 1H) 5.47 (s, 2H) 7.00–7.06 (m, *J* = 7.6 Hz, 1H) 7.11–7.16 (m, 1H) 7.25 (d, *J* = 1.8 Hz, 1H) 7.54 (d, *J* = 8.6 Hz, 1H) 8.15 (s, 1H). Step 3 Method J gave product **35** in 90% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.57–1.67 (m, 2H) 1.73–1.83 (m, 4H) 2.02–2.11 (m, 2H) 4.42–4.47 (m, *J* = 5.8 Hz, 2H) 4.62–4.70 (m, 1H) 6.72 (t, *J* = 5.7 Hz, 1H) 7.05–7.13 (m, 2H) 7.22–7.28 (m, 2H) 7.33 (t, *J* = 7.5 Hz, 2H) 7.44 (d, *J* = 7.3 Hz, 2H) 7.55 (d, *J* = 8.8 Hz, 1H) 8.21 (s, 1H). HRMS (ESI+) calcd for C₁₈H₂₀N₆ (M+H), 321.1822; found 321.1822.

4.1.2.4. 4-Cyclopentylamino-6-[(1-oxypyridin-2-ylmethyl)amino]quinoline-3-carbonitrile (36**).** Intermediate **15b** was treated with 1-oxypyridine-2-carbaldehyde¹² following the procedure for Step 3 Method J. 28% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.59 (s, 2H) 1.69–1.78 (m, 4H) 2.00–2.08 (m, 2H) 4.59–4.68 (d, *J* = 6.6 Hz, 2H) 6.76 (t, *J* = 6.8 Hz, 1H) 7.03 (d, *J* = 7.8 Hz, 1H) 7.20–7.43 (m, 5H) 7.59 (d, *J* = 8.8 Hz, 1H) 8.19 (d, *J* = 7.3 Hz, 2H) 8.32 (d, *J* = 6.3 Hz, 1H). HRMS (ESI+) calcd for C₂₁H₂₁N₅O (M+H), 360.1819; found 360.1819.

4.1.2.5. 6-(2-Cyanobenzylamino)-4-cyclopentylaminoquinoline-3-carbonitrile (37). Intermediate **15b** was treated with 2-cyanobenzaldehyde following the procedure for Step 3 Method J. 55% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.55–1.65 (m, 2H) 1.66–1.82 (m, 4H) 1.98–2.10 (m, 2H) 4.58–4.68 (m, 3H) 6.69–6.76 (dd, $J = 5.8, 5.8$ Hz, 1H) 6.97 (d, $J = 7.8$ Hz, 1H) 7.11 (d, $J = 2.0$ Hz, 1H) 7.24 (dd, $J = 9.1, 2.5$ Hz, 1H) 7.45–7.52 (m, 1H) 7.56–7.70 (m, 3H) 7.87 (d, $J = 7.8$ Hz, 1H) 8.19 (s, 1H). Anal. ($\text{C}_{23}\text{H}_{21}\text{N}_5 + 1.0 \text{ H}_2\text{O}$) C, H, N.

4.1.2.6. 6-(3-Cyanobenzylamino)-4-cyclopentylaminoquinoline-3-carbonitrile (38). Intermediate **15b** was treated with 3-cyanobenzaldehyde following the procedure for Step 3 Method J. 95% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.54–1.64 (m, 2H) 1.68–1.79 (m, 4H) 2.00–2.11 (m, 2H) 4.49–4.54 (m, $J = 6.1$ Hz, 2H) 4.59–4.66 (m, 1H) 6.79–6.86 (dd, $J = 5.8, 5.8$ Hz, 1H) 6.92 (d, $J = 6.3$ Hz, 1H) 7.03 (d, $J = 2.0$ Hz, 1H) 7.21 (dd, $J = 9.1, 2.3$ Hz, 1H) 7.51–7.59 (m, 2H) 7.74 (dd, $J = 18.8, 8.0$ Hz, 2H) 7.90 (s, 1H) 8.17 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{23}\text{H}_{21}\text{N}_5$ (M+H), 368.1870; found 368.1866.

4.1.2.7. 4-Cyclopentylamino-6-(3-methanesulfonylbenzylamino)quinoline-3-carbonitrile (39). Intermediate **15b** was treated with 3-methanesulfonylbenzaldehyde following the procedure for Step 3 Method J. 40% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.55–1.63 (m, 2H) 1.69–1.81 (m, 4H) 2.01–2.11 (m, 2H) 3.18 (s, 3H) 4.58 (d, $J = 5.8$ Hz, 2H) 4.60–4.66 (m, 1H) 6.83 (t, $J = 6.1$ Hz, 1H) 6.96 (d, $J = 7.3$ Hz, 1H) 7.10 (d, $J = 2.3$ Hz, 1H) 7.23 (dd, $J = 8.8, 2.3$ Hz, 1H) 7.54–7.64 (m, 2H) 7.77–7.84 (m, 2H) 8.00 (t, $J = 1.6$ Hz, 1H) 8.17 (s, 1H). Anal. ($\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_2\text{S} + 1.3\text{H}_2\text{O}$) C, H, N.

4.1.2.8. 4-[(3-Cyano-4-cyclopentylaminoquinolin-6-ylamino)methyl]benzenesulfonamide (40). Intermediate **15b** was treated with 4-formyl-benzenesulfonamide following the procedure for Step 3 Method J. 86% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.61 (t, $J = 4.8$ Hz, 2H) 1.71–1.81 (m, 4H) 2.01–2.11 (m, 2H) 4.53 (d, $J = 6.1$ Hz, 2H) 4.61–4.67 (m, 1H) 6.79 (t, $J = 5.9$ Hz, 1H) 6.98 (d, $J = 7.8$ Hz, 1H) 7.09 (d, $J = 2.5$ Hz, 1H) 7.21 (dd, $J = 9.1, 2.3$ Hz, 1H) 7.29 (s, 2H) 7.56 (d, $J = 9.1$ Hz, 1H) 7.61 (d, $J = 8.3$ Hz, 2H) 7.75–7.80 (m, 2H) 8.17 (s, 1H). Anal. ($\text{C}_{22}\text{H}_{23}\text{N}_5\text{O}_2\text{S} + 1.0 \text{ CH}_3\text{OH}$) C, H, N.

4.1.2.9. 4-Cyclopentylamino-6-[(2H-pyrazol-3-ylmethyl)amino]quinoline-3-carbonitrile (41). Intermediate **15b** was treated with 2H-pyrazole-3-carbaldehyde following the procedure for Step 3 Method J. 28% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.62 (s, 2H) 1.79 (s, 4H) 2.05–2.14 (m, 2H) 4.40 (d, $J = 5.3$ Hz, 2H) 4.64–4.72 (m, 1H) 6.27 (d, $J = 1.8$ Hz, 1H) 6.38 (t, $J = 5.3$ Hz, 1H) 7.03 (d, $J = 7.8$ Hz, 1H) 7.16 (s, 1H) 7.26 (dd, $J = 9.0, 2.15$ Hz, 1H) 7.55 (d, $J = 8.8$ Hz, 1H) 7.60 (s, 1H) 8.17 (s, 1H) 8.20 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{20}\text{N}_6$ (M+H), 333.1822; found 333.1818.

4.1.2.10. 4-Cyclopentylamino-6-[(1-methyl-1H-imidazol-2-ylmethyl)amino]quinoline-3-carbonitrile (42). Intermediate **15b** was treated with 1-methyl-1H-imidaz-

ole-2-carbaldehyde following the procedure for Step 3 Method J. 35% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.60–1.66 (m, 2H) 1.74–1.84 (m, 4H) 2.08–2.16 (m, 2H) 3.76 (s, 3H) 4.68 (d, $J = 5.1$ Hz, 3H) 6.65 (t, $J = 5.2$ Hz, 1H) 6.98 (d, $J = 7.8$ Hz, 1H) 7.14 (d, $J = 2.3$ Hz, 1H) 7.22 (dd, $J = 9.2, 2.2$ Hz, 1H) 7.27 (d, $J = 1.8$ Hz, 1H) 7.55 (d, $J = 1.8$ Hz, 1H) 7.61 (d, $J = 9.1$ Hz, 1H) 8.23 (s, 1H) 8.30 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{22}\text{N}_6$ (M+H), 347.1979; found 347.1974.

4.1.2.11. 4-Cyclopentylamino-6-(2-morpholin-4-ylethylamino)quinoline-3-carbonitrile (43). 4-(2,2-Dimethoxyethyl)morpholine ¹¹ was heated with 37% aqueous HCl (0.6 mL for 0.5 mmol) overnight at 70 °C. This reaction mixture was treated with intermediate **15b** following the procedure for Step 3 Method J. 22% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.59–1.65 (m, 2H) 1.75–1.83 (m, 4H) 2.05–2.14 (m, 2H) 2.43–2.48 (m, $J = 8.1$ Hz, 4H) 2.55–2.60 (m, 2H) 3.27–3.34 (m, 4H) 3.57–3.64 (m, 4H) 4.64–4.70 (m, 1H) 5.87 (t, $J = 4.2$ Hz, 1H) 7.03–7.09 (m, 2H) 7.21 (dd, $J = 9.1, 2.3$ Hz, 1H) 7.54 (d, $J = 8.8$ Hz, 1H) 8.16 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}$ (M+H), 366.2288; found 366.2291.

4.1.2.12. 4-(tert-Butylamino)-6-[(1-oxypyridin-2-yl)methyl]aminoquinoline-3-carbonitrile (44). Step 1 Method F was used to synthesize 4-cyclopentylamino-6-nitroquinoline-3-carbonitrile (**14c**). The crude product was taken to the next step. Step 2. To a solution of **14c** (800 mg, 2.95 mmol) in methanol (5 mL) and water (2 mL) were added iron powder (935 mg, 16.7 mmol) and ammonium chloride (1.47 g, 27.7 mmol). The mixture was stirred vigorously and heated to 75 °C for 4 h. After allowing the mixture to cool to rt, EtOAc (25 mL) and saturated aqueous NaHCO_3 (10 mL) were added. The organic layer was dried over MgSO_4 and concentrated to afford 6-amino-4-(tert-butylamino)quinoline-3-carbonitrile (**15c**) as a tan solid in quantitative yield. The product was used without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 1.52 (s, 9H) 5.67 (br s, 2H), 5.99 (br s, 1H), 7.12 (s, 1H), 7.69 (d, $J = 9.1$ Hz, 1H) 8.28 (s, 1H). Intermediate **15c** was treated with 1-oxypyridine-2-carbaldehyde¹² following the procedure for Step 3 Method J, giving product **44** in 60% yield. ^1H NMR (400 MHz, MeOD) δ 1.61 (s, 9H), 3.15–3.26 (m, 2H), 7.57 (s, 1H), 7.74–7.96 (m, 4H), 8.11 (d, $J = 9.1$ Hz, 1H), 8.77 (d, $J = 9.1$ Hz, 1H) 8.99 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{20}\text{H}_{21}\text{N}_5\text{O}$ (M+H), 348.1819; found 348.1821.

4.1.2.13. 4-(tert-Butylamino)-6-[(3-cyanobenzyl)aminol]quinoline-3-carbonitrile (45). Intermediate **15c** was treated with 3-cyanobenzaldehyde following the procedure for Step 3 Method J. 65% yield. ^1H NMR (400 MHz, MeOD) δ 1.48 (s, 9H), 3.32–3.49 (m, 2H), 4.58 (s, 1H), 6.74 (d, $J = 2.3$ Hz, 1H), 7.29 (dd, $J = 9.1, 2.3$ Hz, 1H), 7.45 (m, 1H), 7.55–7.78 (m, 4H), 8.28 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{21}\text{N}_5$ (M+H), 356.1870; found 356.1872.

4.1.2.14. 4-(tert-Butylamino)-6-[[3-(methylsulfonyl)benzyl]amino]quinoline-3-carbonitrile (46). Intermediate **15c** was treated with 3-methanesulfonylbenzaldehyde

following the procedure for Step 3 Method J. 42% yield. ^1H NMR (400 MHz, MeOD) δ 1.61 (s, 9H), 3.03 (s, 3H), 3.16–3.24 (m, 2H), 4.58 (s, 1H) 6.90 (d, J = 2.3 Hz, 1H) 7.37 (dd, J = 9.1, 2.27 Hz, 1H) 7.52 (t, J = 7.7 Hz, 1H), 7.59 (d, J = 9.1 Hz, 1H) 7.68 (d, J = 8.3 Hz, 1H), 7.76 (dd, J = 7.8, 2.0 Hz, 1H), 7.90 (s, 1H) 8.55 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{22}\text{H}_{24}\text{N}_4\text{O}_2\text{S}$ (M+H), 409.1693, found 409.1692.

4.1.2.15. 4-((4-(*tert*-Butylamino)-3-cyanoquinolin-6-yl)amino)methyl)benzenesulfonamide (47). Intermediate **15c** was treated with 4-formylbenzenesulfonamide following the procedure for Step 3 Method J. 55% yield. ^1H NMR (400 MHz, MeOD) δ 1.40 (s, 9H), 3.22–3.34 (m, 2H), 4.56 (s, 1H) 6.92 (s, 1H), 7.43–7.49 (m, 1H), 7.72–7.79 (m, 2H), 7.82 (d, J = 9.1 Hz, 1H), 8.02 (br s, 2H), 8.44 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{23}\text{N}_5\text{O}_2\text{S}$ (M+H), 410.1645; found 410.1640.

4.1.2.16. 4-(*tert*-Butylamino)-6-[(1*H*-pyrazol-5-ylmethyl)amino]quinoline-3-carbonitrile (48). Intermediate **15c** was treated with 2*H*-pyrazole-3-carbaldehyde following the procedure for Step 3 Method J. 70% yield. ^1H NMR (400 MHz, MeOD) δ 1.68 (s, 9H), 3.46 (s, 2H), 4.57 (s, 1H), 6.42 (s, 1H), 7.07 (s, 1H), 7.38 (dd, J = 9.1, 2.3, 1H), 7.71 (s, 1H), 7.79 (d, J = 9.1 Hz, 1H), 8.41 (s, 1H), 8.59 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{18}\text{H}_{20}\text{N}_6$ (M+H), 321.1822, found 321.1822.

4.1.2.17. 4-(*tert*-Butylamino)-6-[(1-methyl-1*H*-imidazol-2-yl)methyl]amino]quinoline-3-carbonitrile (49). Intermediate **15c** was treated with 1-methyl-1*H*-imidazole-2-carbaldehyde following the procedure for Step 3 Method J. 47% yield. ^1H NMR (400 MHz, MeOD) δ 1.58 (s, 9H), 3.26 (s, 2H), 3.85 (s, 3H), 7.20 (s, 1H), 7.32 (s, 1H), 7.38 (dd, J = 9.1, 2.3, 1H), 7.47 (s, 1H), 7.69 (d, J = 9.1 Hz, 1H), 8.67 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{22}\text{N}_6$ (M+H), 335.1979; found 335.1980.

4.1.2.18. 4-(Cycloheptylamino)-6-[(pyridin-3-ylmethyl)amino]quinoline-3-carbonitrile (50). Step 1 Method F was used to synthesize 4-cycloheptylamino-6-nitroquinoline-3-carbonitrile (**14d**) in 70% crude yield. Step 2 Method H was used to prepare 6-amino-4-cycloheptylaminoquinoline-3-carbonitrile (**15d**, 83% crude yield). Step 3 Method J gave compound **50** in 69.5% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.37–1.78 (m, 10H) 1.89–2.05 (m, 2H) 4.31–4.39 (m, 1H) 4.41 (d, J = 5.8 Hz, 2H) 6.65 (t, J = 6.0 Hz, 1H) 6.88 (d, J = 8.3 Hz, 1H) 7.05 (d, J = 2.3 Hz, 1H) 7.15 (dd, J = 9.0, 2.4 Hz, 1H) 7.29 (dd, J = 7.3, 4.3 Hz, 1H) 7.49 (d, J = 9.1 Hz, 1H) 7.71–7.80 (m, 1H) 8.10 (d, J = 6.3 Hz, 1H) 8.39 (dd, J = 4.8, 1.8 Hz, 1H) 8.60 (d, J = 1.8 Hz, 1H). HRMS (ESI+) calcd for $\text{C}_{23}\text{H}_{25}\text{N}_5$ (M+H) 372.2182, found 372.2186.

4.1.2.19. 4-Cyclopentylamino-6-[(pyridin-3-ylmethyl)amino]quinoline-3-carbonitrile (51). Intermediate **15b** was treated with pyridine-3-carbaldehyde following the procedure for Step 3 Method J. 20% yield. ^1H NMR (400 MHz, DMSO- d_6) δ 1.58–1.66 (m, 2H) 1.73–1.81 (m, 4H) 2.01–2.11 (m, 2H) 4.48 (d, J = 5.8 Hz, 2H) 4.61–4.69 (m, 1H) 6.50 (s, 1H) 6.72 (t, J = 6.1 Hz, 1H)

6.98 (d, J = 8.3 Hz, 1H) 7.11 (s, 1H) 7.22 (dd, J = 9.1, 2.3 Hz, 1H) 7.36 (dd, J = 7.5, 5.2 Hz, 1H) 7.56 (d, J = 9.1 Hz, 1H) 7.82 (d, J = 7.8 Hz, 1H) 8.17 (s, 1H) 8.46 (d, J = 3.0 Hz, 1H) 8.67 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{21}\text{N}_5$ (M+H), 344.1870; found 344.1872.

4.1.3. 6-[(2-Morpholin-4-ylethyl)amino]-4-[(4-phenoxyphenyl)amino]quinoline-3-carbonitrile (33). To a 250 mL Erlenmeyer flask equipped with a large stir bar were added 4-nitroaniline (27.6 g, 0.2 mol) and 4-(2-chloroethyl)morpholine hydrochloride (18.6 g, 0.1 mol). The mixture was heated at a sand bath temperature of 170 °C for 14 h. After allowing the reaction mixture to cool to 60 °C, EtOAc (100 mL) was added. The thick mixture was partitioned between EtOAc (600 mL) and water (600 mL). The aqueous phase was extracted with EtOAc (3 $\times$ 200 mL), then adjusted to pH 9 with 1 N NaOH and further extracted with dichloromethane (3 $\times$ 200 mL). The organic phase was washed with brine and dried over Na_2SO_4 to provide 10.1 g (40%) of *N*-(2-morpholinoethyl)-4-nitroaniline (**10a**) isolated as a yellow solid. The material was used without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 2.29–2.58 (m, 4H), 3.15–3.40 (m, 4H), 3.48–3.70 (m, 4H), 6.58–6.76 (m, 2H), 7.18 (t, J = 5.3 Hz, 1H), 7.99 (d, J = 9.4 Hz, 2H).

To a solution of **10a** (8.5 g, 33.7 mmol) in methanol (200 mL) was added a slurry of 10% Pd/C (1.2 g) in EtOAc (100 mL). Hydrogen gas was bubbled through the solution for 1 min. The mixture was then stirred at rt under an atmosphere of hydrogen gas for 24 h, at which time N_2 gas was bubbled into the mixture for 15 min. The mixture was filtered through a pad of Celite and the filtrate concentrated to give 7.4 g (99%) of *N*1-(2-morpholinoethyl)benzene-1,4-diamine (**10b**) as a red syrup. The material was used without further purification. ^1H NMR (400 MHz, CD_2Cl_2 - d_2) δ 2.26–2.41 (m, 2H), 2.43–2.56 (m, 2H), 2.89–3.04 (m, 4H), 3.54–3.63 (m, 4H), 6.33–6.44 (m, 2H), 6.44–6.53 (m, 2H)

To a solution of **10b** (5.0 g, 22 mmol) in toluene (100 mL) was added ethyl 2-cyano-3-ethoxyacrylate (3.8 g, 22 mmol). The solution was heated at 110 °C for 8 h, allowed to cool to rt, and stirred overnight. Hexanes (800 mL) were added with vigorous stirring. The resulting suspension was heated to 60 °C and EtOAc was added slowly until a copious precipitate formed. After allowing to cool to rt the solid was isolated by filtration to give 4.17 g of 2-cyano-3-(4-(2-morpholinoethylamino)phenylamino)acrylic acid ethyl ester (**11b**) as a yellow powder. After standing for 2 h, the mother liquor was filtered to give 3.47 g of a yellow solid for a combined yield of 7.54 g (99%), which was used directly in the next step. Dowtherm A (300 mL) was bubbled with Ar gas for 1 h while being heated to 100 °C. The acrylate **11b** (3.76 g, 10.9 mmol) was added in three portions and the mixture was heated to 259 °C under a stream of Ar. After gently refluxing for 6 h, the reaction mixture was allowed to cool below 50 °C and then poured into 1500 mL of hexanes. Filtration provided 4-hydroxy-6-(2-morpholin-4-yl-ethylamino)quinoline-3-carbonitrile (**12b**) as a tan powder (1.15 g, 37%). ^1H NMR

(400 MHz, DMSO- d_6) δ 2.33–2.59 (m, 4H), 3.13–3.27 (m, 4H), 3.51–3.65 (m, 4H), 6.06 (t, J = 5.3 Hz, 1H), 7.08 (d, J = 2.8 Hz, 1H), 7.17 (dd, J = 8.8, 2.8 Hz, 1H), 7.41 (d, J = 9.1 Hz, 1H), 8.47 (s, 1H).

A solution of the quinoline **12b** (1.0 g, 3.3 mmol) in phosphorus oxychloride (50 mL) was heated at 120 °C for 12 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was stirred in dichloromethane (200 mL) and cooled to 0 °C. Water (100 mL) was added followed by solid Na_2CO_3 , added slowly until the aqueous phase reached pH 9. The organic layer was dried over Na_2SO_4 and concentrated to provide 4-chloro-6-(2-morpholinoethylamino)quinoline-3-carbonitrile (**13a**) (800 mg, 69%) as a light yellow powder. ^1H NMR (400 MHz, DMSO- d_6) δ 2.37–2.54 (m, 4H), 2.55–2.67 (m, 4H), 3.33 (s, 4H), 3.61 (s, 4H), 6.82 (d, 1H), 6.87 (d, J = 2.5 Hz, 1H), 7.52 (dd, J = 9.2, 2.7 Hz, 1H), 7.87 (d, J = 9.1 Hz, 1H), 8.74 (s, 1H).

To a solution of **13b** (135 mg, 0.39 mmol) in 2-ethoxyethanol (5 mL) was added 4-phenoxyaniline (57 mg, 0.39 mmol). The mixture was heated at 135 °C for 36 h. After allowing to cool to rt the reaction mixture was diluted with ether and hexane (1:1, 20 mL), stirred for 4 h, and filtered. The solid was washed with ether and dried on a vacuum pump to give **33** (106 mg, 55%) as an orange solid. ^1H NMR (400 MHz, DMSO- d_6) δ 3.01–3.55 (m, 6H), 3.65–4.19 (m, 6H), 7.03–7.08 (m, 1H), 7.09–7.20 (m, 4H), 7.34–7.50 (m, 5H), 7.55 (m, 1H), 7.82 (d, J = 9.1 Hz, 1H), 8.65 (s, 1H). HRMS (ESI+) calcd for $\text{C}_{28}\text{H}_{27}\text{N}_5\text{O}_2$ (M+H), 466.2238; found 466.2234.

4.2. Pharmacokinetic studies

The animals used in the pharmacokinetic (PK) studies were male adult Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA). All the PK studies were performed at Wyeth Research Laboratories (Andover, MA) under the supervision of the Institutional Animal Care and Use Committee. The dose formulation for intravenous administration was 50% DMSO/50% polyethylene glycol 200 (v/v, 1 mL/kg). The oral dose formulation was an aqueous suspension containing 2% Polysorbate 80 (a.k.a. Tween 80), 0.5% methylcellulose, and 0.06% lactic acid. Blood samples of approximately 0.25 mL were collected into K_2 EDTA coated sampling tubes at 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 h post dose administration. Plasma samples were harvested and stored at –80 °C until analysis.

In a 0.5 mL 96-well plate, 50 μL of plasma sample was precipitated with 100 μL acetonitrile containing 500 ng/mL internal standard. The internal standard is a compound with a similar chemical structure as that of the test article. The samples were vortexed and centrifuged at 5700 rpm for 10 min. Supernatants were subjected to LC-MS/MS analysis. HPLC separation was performed on a Perkin-Elmer Series 200 HPLC system (Perkin-Elmer, Norwalk, CT) using an XTerra MS C18 column (2.1 $\times$ 20 mm, 2.5 μm ; Waters, Milford,

MA). The detection of test articles was performed on a PESCIEX API-3000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, L4K4V8) using TurboIon Spray source. Plasma standard curves were generated by plotting peak area ratio of test article and internal standard against nominal concentrations.

The pharmacokinetic parameters were determined using WinNonlin (version 4.1, Pharsight, Mountain View, CA). Calculations were performed using the non-compartmental analysis approach. The estimation of area under the plasma concentration versus time curve (AUC) was based upon the log trapezoidal rule. The terminal rate constant (λ) was derived from the slope of the terminal log-linear phase of plasma concentrations–time curves. The apparent terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda$. No statistical analysis other than descriptive statistics was conducted.

4.3. Biology

4.3.1. Tpl2 enzymatic assay. Tpl2/Cot activity was directly assayed using GST-MEK1 as a substrate. The phosphorylation on serine residues 217 and 221 of GST-MEK1 was detected by an ELISA. Briefly, 0.4 nM Tpl2 was incubated with 35 nM GST-MEK1 in a kinase reaction buffer of pH 7.2 containing 20 mM Mops, 50 μM ATP, 20 mM MgCl_2 , 1 mM DTT, 25 mM β -glycerophosphate, 5 mM EGTA, and 1 mM sodium orthovanadate for 1 h at 30 °C. Compounds solubilized in 100% DMSO were pre-diluted in assay buffer so that the final concentration of DMSO in the reaction was 1%. The kinase reaction was carried out in 100 μL volume on 96-well plates. The kinase reaction was then stopped by the addition of 100 mM EDTA. The entire reaction mixture was then transferred to the detection plate, a 96-well Immunosorb plate that had been pre-coated with anti-GST antibody (Amersham). After 1 h incubation at room temperature, the detection plate was washed four times with TBST (TBS + 0.05% Tween 20) and then incubated for another hour at room temperature with anti-phospho-MEK1 antibody (Cell Signaling) 1:1000 in 10 mM Mops 7.5, 150 mM NaCl, 0.05% Tween 20, 0.1% gelatin, 0.02% NaN_3 , and 1% BSA. The detection plate was washed again and incubated for 30 min with DELFIA Europium (Eu) labeled goat anti-rabbit IgG (Perkin-Elmer), 1:4000 in the same buffer used for the primary incubation. After a final wash, Eu detection solution was added to each well and the Eu signal was measured in a Wallac Victor Multilabel Counter. Data were imported into Excel and IC_{50} calculations were performed using the Xlfit (IDBS) software package.

4.3.2. Inhibition of $\text{TNF-}\alpha$ in primary human monocytes. Human blood buffy coat (containing 1 mM EDTA) was incubated with RosetteSep human monocyte enrichment antibody cocktail (Stem Cell Technologies #15068). This mixture was then diluted with an equal volume of PBS/2% FBS/1 mM EDTA, layered onto an equal volume of Histopaque-1077 (Sigma #H8889), and centrifuged for 20 min at 1500 rpm. The monocytes were then recovered from the interface, diluted, and

reisolated on a Histopaque-1077 cushion. After this second round of purification, the monocytes were washed twice in PBS/2% FBS/1 mM EDTA, resuspended in RPMI/0.5% FBS to 2×10^6 cells/mL, plated at 800,000 cells per well in 48-well formats, and incubated at 37 °C/5% CO₂. Thirty minutes prior to LPS stimulation, compounds (in 100% DMSO) were added. LPS was then added to 10 ng/mL and the cultures allowed to incubate for an additional ~3 h. Media supernatants were then harvested and analyzed for TNF- α by standard ELISA or electrochemiluminescence on a Sector6000 reader (Meso Scale Discovery).

4.3.3. Inhibition of TNF- α in human blood. Blood was drawn from healthy male volunteers into heparin tubes. At 37 °C, the blood was diluted 1–5 with pre-warmed RPMI/3% FCS, and then dispensed into 96-well-formatted 200 μ L tube strips and capped. Thirty minutes prior to LPS stimulation compounds (in 100% DMSO) were added. Final DMSO concentrations equaled 1.0%. LPS was then added to 10 ng/mL and the cultures are allowed to incubate for an additional ~3 h. Samples were centrifuged in a strip rotor at 6500 rpm, and the plasma supernatants were analyzed by standard ELISA or electrochemiluminescence on a Sector6000 reader (Meso Scale Discovery).

4.3.4. LPS-induced acute TNF- α production in mice. LPS/D-Gal-induced acute TNF- α production in mouse sera: female C57Bl/6 mice, 8–10 weeks of age, were obtained from the Jackson Laboratory. The animals were fed food and water ad libitum and all procedures were approved by the Institutional Animal Care and Use Committee. Compound at 10 or 25 mg/kg, or vehicle, was administered to the mice by the intraperitoneal (ip) route. One hour after the compound, LPS plus D-galactosamine in PBS was administered ip. Final LPS and D-gal concentrations in each animal were 2 and 160 ng/kg, respectively. The mice were euthanized with carbon dioxide 1.5 hour after the LPS/D-Gal injection and bled by cardiac puncture. TNF- α levels were measured in the serum samples using a TNF- α ELISA.

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Models for anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles

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Abstract An *in silico* approach through decision tree (DT) and moving average analysis (MAA) has been employed for development of models for prediction of anti-inflammatory activity. A data set consisting of 44 analogues of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles was selected for this study. These compounds act as anti-inflammatory agents through inhibition of tumor progression loci-2 (Tpl2) kinase and tumor necrosis factor- α (TNF- α) production. A total of 44 descriptors of diverse nature, from a large pool of molecular descriptors calculated through *E-Dragon software* (version 1.0) and an in-house computer program were selected for further analysis. DT was used to determine the importance of molecular descriptors. DT identified two topological indices as most important and classified the analogues involved in the data set with an accuracy of $\sim 91\%$ in training set and $\sim 66\%$ in 10 fold cross validated set. Three independent models were also developed through MAA. Accuracy of prediction of these models varied from 87.5 to 92.5%. The statistical significance of proposed models was assessed through intercorrelation analysis, sensitivity, specificity, and Matthew's correlation coefficient. The proposed models offer a vast potential for providing lead structures for development of potent anti-inflammatory drugs.

Keywords Anti-inflammatory activity · Wiener-type index from Z weighted distance matrix · Gutman molecular topological index · Eccentric distance sum topochemical index · 8-Substituted-4-anilino-6-aminoquinoline-3-carbonitriles

Introduction

Rheumatoid arthritis (RA), which affects about 1% of the world's population, is a serious, chronic, and systemic inflammatory disease (Miwatashi *et al.*, 2005) associated with the erosion of cartilage and bone leading to joint pain, swelling, stiffness, and restricted movement (Gavrin *et al.*, 2005). Although the etiology of RA is not clear, it is known that cytokines play critical role in the inflammatory processes that lead to joint destruction. These cytokines include TNF- α and IL-1, which are detected in synovial fluids from patients with RA and are critical in pathogenesis of the disease (Mbalaviele *et al.*, 2006). Anti-cytokine biotherapeutic approaches have dramatically changed the treatment options for patients with RA and had a major impact on the treatment of RA. However, they are expensive, have to be injected and are only partially effective. As a consequence, there is undiminished interest in identifying novel, orally active compounds that block the production of TNF α (Rousseau *et al.*, 2008).

An alternate approach of inhibiting inflammatory cytokine action is by decreasing their production (Lee *et al.*, 1994). The family of nuclear factor kappaB (NF- κ B) transcription factors governs the regulation of expression of numerous genes in the setting of the inflammatory response (Roman-Blas and Jimenez, 2006). NF- κ B is usually sequestered in the cytoplasm by a group of inhibitory

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proteins termed as inhibitors of NF- κ B (I κ Bs) (Wan and Lenardo, 2010). Most commonly employed drugs like corticosteroids, aspirin, and nonsteroidal anti-inflammatory drugs do block NF- κ B. They accomplish this by inhibiting IKK β -dependent phosphorylation of I κ B α , thereby, preventing its degradation and subsequent activation of NF- κ B (Tripathi and Aggarwal, 2006). Therefore, suppression of NF- κ B activity might provide a functional tool for the development of novel therapeutics for patients suffering from pain and inflammation (Niederberger and Geisslinger, 2008). Further, L-, P-, and E-selectins constitute vital components of the cell adhesion molecules which are essential for leukocyte recruitment in response to tissue injury and inflammation (Gao *et al.*, 2005). Indeed, P-selectin plays major role as a mediator during early phases of inflammation and atherosclerotic plaque formation (Mayadas *et al.*, 1997; Johnson *et al.*, 1997) rendering it a strong therapeutic candidate in atherosclerosis, thrombosis, and inflammation. Evidence suggesting that targeting P-selectin will provide a novel target in a variety of indications, has led the pharmaceutical industry to develop molecules targeting p-selectin (Woollard and Chin-Dusting, 2006). Mitogen-activated protein kinases (MAPKs) are reported to be key intermediate enzymes in the signal transduction cascade from the extracellular environment to the nucleus (Kumar *et al.*, 2001). Tpl2 is a member of the mitogen-activated protein kinase kinase (MAP3K) family (Prem *et al.*, 2009) and is activated by Toll-like receptor agonists and pro-inflammatory cytokines like TNF α and IL-1 (Cohen, 2009). Tpl2 is a key component in activation of T cells and macrophages, resulting in TNF- α production from these cell types (Luciano *et al.*, 2004). Indeed, mice that lack Tpl2 secrete low levels of TNF- α when exposed to bacterial endotoxin LPS (lipopolysaccharide) and are resistant to LPS/D-galactosamine-induced endotoxin shock. Moreover, ablation of Tpl2 protects from TNF- α -induced inflammatory bowel disease and arthritis (Das *et al.*, 2005). Thus, pharmacologic inhibition of Tpl2 is a valid approach to therapeutic intervention in the pathogenesis of RA (Hall *et al.*, 2007).

There is an ever growing need for pharmaceutical industry to reorient their research methodologies that permit the “rational” selection or design of novel compounds to minimize cost of drug development (Estrada *et al.*, 1998). (Quantitative) Structure–activity relationship [(Q)SAR] analysis is one such technique that has the potential to provide a successful solution to this problem (Liu *et al.*, 2004). The goal of QSAR studies is to establish a correlation between biological activity and structure/property relationship by utilizing molecular descriptors such as topological indices (TIs) so as to facilitate drug design (Golbraikh and Tropsha, 2002).

In this study, numerous molecular descriptors of diverse nature have been successfully utilized to build suitable models through DT and MAA for the prediction of anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles.

Methodology

Dataset

A data set comprising 44 analogues (see Fig. 1; Table 1) of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles, which produce anti-inflammatory activity through inhibition of Tpl2 kinase and TNF- α production, was selected for the present investigation (Green *et al.*, 2007). Anti-inflammatory activity has been reported quantitatively as drug concentration required for 50% inhibition of Tpl2 enzyme (IC₅₀), which plays an important role in the production of TNF- α and other proinflammatory cytokines (Green *et al.*, 2007). Analogues having IC₅₀ value of ≤ 25 nM were considered to be active, [labelled as “A” ($N = 14$)] and analogues having IC₅₀ value of > 25 nM were considered to be inactive [labelled as “B” ($N = 30$)] for the purpose of this study.

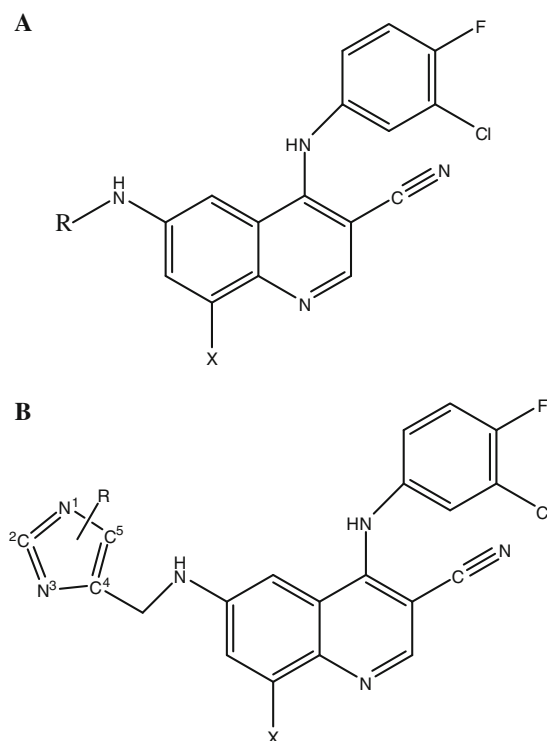


Fig. 1 Basic structures of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles (Green *et al.*, 2007)

Table 1 Relationship between topological indices and inhibition of Tpl2 kinase

Comp ound. no.	Basic ring	X	R	$W(G_{\text{view}})$	S_G	ξ_c^{DS}	Tpl2 (enzyme) inhibition			
							Predicted			Reported
							D^Z	S_G	ξ_c^{DS}	
1.	A	H		1551.767	29444.55	53138.305	+	±	–	+
2.	A	F		1663.216	34083.78	57114.137	+	±	+	+
3.	A	Cl		1654.431	30394	57558.164	+	±	+	+
4.	A	Br		1649.349	30086.52	61866.227	+	±	+	+
5.	A	CF ₃		2096.894	47336.11	71610.414	±	–	±	–
6.	A	OH		1665.549	32897.78	57033.402	±	±	–	–
7.	A	OMe		1801.08	34194.55	61963.035	±	±	±	–
8.	A	SOMe		1877.081	35229.22	70010.406	±	+	±	+
9.	A	SO ₂ Me		1985.237	39503.89	77826.406	±	–	–	–
10.	A	CONMe ₂		2299.675	41753.45	78110.313	–	–	–	–
11.	A	C(O)Me		1955.362	37229.22	66364.547	±	+	±	+
12.	A	CH ₂ CH(OH)CH ₂ OH		2381.675	43586.67	79678.133	–	–	–	–
13.	A	OCH ₂ CH(OH)CH ₂ OH		2576.707	49219.89	91094.922	–	–	–	–
14.	A	Cl		1654.431	30394	69069.453	+	±	±	–
15.	A	Br		2041.838	38466.26	76646.797	±	–	–	–
16.	A	Cl		1848.157	32310.33	67337.953	±	±	±	–
17.	A	Br		1822.649	31731.89	68588.953	±	±	±	–
18.	A	Br		2879.311	49862.22	120300.43	–	–	–	–
19.	A	Cl		2392.302	43385.33	94187.516	–	–	–	–
20.	A	Cl		1843.995	32532.45	66631.984	±	±	±	+
21.	A	Cl		4306.208	103381.1	178295.45	–	–	–	–
22.	A	Cl		4625.922	65364.78	206383.53	–	–	–	–
23.	A	Cl		2033.558	34120.55	74878.891	±	±	–	–
24.	A	Cl		3242.837	54493.22	142103.63	–	–	–	–
25.	A	Cl		2046.653	35746.67	73588.43	±	+	±	+

Table 1 continued

Comp ound. no.	Basic ring	X	R	$W(G_{\text{vew}})$	S_G	ξ_c^{DS}	Tpl2 (enzyme) inhibition			
							Predicted			Reported
							D^Z	S_G	ξ_c^{DS}	
26.	A	Br		1515.134	27116.89	55500.66	–	–	–	–
27.	A	Cl		1644.921	33508.22	58210.563	+	±	+	+
28.	A	Cl		1325.603	26838	43621.984	–	–	–	–
29.	A	Cl		2561.541	46910.45	103569.9	–	–	–	–
30.	A	Br		1646.9	31716.89	61953.59	+	±	+	+
31.	A	Br		3194.577	47833.04	144531.48	–	–	–	+
32.	B	Br	C ⁵ -isopropyl	2248.851	36882.04	83877.297	±	+	–	–
33.	B	Cl	C ⁵ -isopropyl	2254.478	37223.22	80957.477	±	+	–	+
34.	B	Cl	C ⁵ -propyl	2283.478	37442.33	85403.586	±	+	–	+
35.	B	Cl	C ⁵ -ethyl	2046.296	35418	73406.391	±	+	±	+
36.	B	Br	C ⁵ -CO ₂ Me	2445.351	48416.7	96776.133	–	–	–	–
37.	B	Cl	C ⁵ -[3-(dimethylamino)propyl]	3135.595	47032.89	128901.98	–	–	–	–
38.	B	Br	N ¹ -methyl	1850.778	31824.93	69706.148	±	±	±	–
39.	B	Cl	C ² -ethyl-C ⁵ -methyl	2304.763	34130.55	86180.781	–	±	–	–
40.	B	Cl	C ^{2,5} -dimethyl	2063.581	32162.22	74030.43	±	±	–	–
41.	B	Cl	N ³ -methyl	1844.042	32141.22	63985.043	±	±	±	–
42.	B	Br	C ² -phenyl	2986.496	51624.96	131134.3	–	–	–	–
43.	B	Br	N ¹ -[2-(morpholinyl)ethyl]	3836.068	60066.41	181850.53	–	–	–	–
44.	B	Br	N ¹ -[2-(methylsulfonyl)ethyl]	3029.236	51943.53	154210.45	–	–	–	–

+ Active analogue

– Inactive Analogue

± Transitional analogue where activity could not be specifically assigned

Molecular descriptors

The structures of all the compounds belonging to data set were drawn in software SYBYL 7.1 (SYBYL 7.1) using a standard procedure. The 3D-structures were minimized using Powell method with termination gradient set at 0.05 kcal/(mol * Å) and a maximum of 1000 iterations employing Tripos Force Field. Energy minimized 3D-structures of these molecules saved as .mol2 files, were imported into DRAGON software (Todeschini and Consonni, 2003) for generating 663 molecular descriptors of diverse nature such as TIs, GETAWAY, the

Randic molecular profiles, geometrical, WHIM, RDF, 3D-MORSE descriptors and topological charge indices. Most of these descriptors are reviewed in the textbook by Todeschini and Consonni (2000). Besides DRAGON software, an in-house computer program was also employed to calculate additional topological descriptors which were not included in the DRAGON software. Descriptors with constant values inside each group were discarded. For the remaining descriptors, a pairwise correlation analysis for all families of descriptors was carried out. This exclusion method was used to reduce, in a first step, the collinearity and correlation

Table 2 Topological indices

Code	Name of descriptor	Reference
A1	Wiener-type index from Z weighted distance matrix	Barysz <i>et al.</i> (1983)
A2	Gutman MTI by valence vertex degrees	Gutman (1994)
A3	Wiener-type index from mass weighted distance matrix	Barysz <i>et al.</i> (1983)
A4	Wiener-type index from van der Waals weighted distance matrix	
A5	Wiener-type index from electronegativity weighted distance matrix	
A6	Wiener-type index from polarizability weighted distance matrix	
A7	Schultz Molecular Topological Index (MTI)	Schultz (1989)
A8	Schultz MTI by valence vertex degrees	
A9	Gutman Molecular Topological Index	Gutman (1994)
A10	Log of product of row sums	Schultz <i>et al.</i> (1992)
A11	Xu index	Ren (1999)
A12	Quasi-Wiener index (Kirchhoff number)	Mohar <i>et al.</i> (1993)
A13	Hyper-distance-path index	Diudea (1996)
A14	Reciprocal hyper-distance-path index	Diudea (1997)
A15	Detour index	Amic and Trinajstić (1997)
A16	Reciprocal hyper-detour index	Diudea (1997)
A17	Distance/detour index	Randić (1997)
A18	All-path Wiener index	Lukovits (1998)
A19	Total information content on the distance equality information indices	Bonchev and Trinajstić (1978)
A20	Total information content on the distance magnitude information indices	
A21	Total information content index (neighborhood symmetry of 1-order) information indices	Magnuson <i>et al.</i> (1983)
A22	Distance/detour ring index of order 6	Trinajstić (1992)
A23	Molecular connectivity topochemical index	Goel and Madan (1995); Dureja and Madan (2005)
A24	Eccentric adjacency topochemical index	Gupta <i>et al.</i> (2003)
A25	Augmented eccentric connectivity topochemical index	Bajaj (2002)
A26	Superadjacency topochemical index	Bajaj <i>et al.</i> (2004b)
A27	Eccentric connectivity topochemical index	Kumar <i>et al.</i> (2004)
A28	Connective eccentricity topochemical index	Gupta (2002)
A29	Zagreb topochemical index, M1 ^c	Bajaj <i>et al.</i> (2005)
A30	Zagreb topochemical index, M2 ^c	
A31	Wiener's topochemical index	Bajaj <i>et al.</i> (2004a)
A32	Superaugmented eccentric connectivity topochemical index-1	Dureja <i>et al.</i> (2008a)
A33	Molecular connectivity index	Randić (1975)
A34	Eccentric adjacency index	Gupta <i>et al.</i> (2001)
A35	Augmented eccentric connectivity index	Bajaj <i>et al.</i> (2006)
A36	Superadjacency index	Bajaj <i>et al.</i> (2004b)
A37	Eccentric connectivity index	Sharma <i>et al.</i> (1997)
A38	Connective eccentricity index	Gupta <i>et al.</i> (2000)
A39	Zagreb group parameter, M ₁	Gutman <i>et al.</i> (1975); Gutman and Randić (1977)
A40	Eccentric distance sum topochemical index	–
A41	Zagreb group parameter, M ₂	Gutman <i>et al.</i> (1975); Gutman and Randić (1977)
A42	Wiener's index	Wiener (1947)
A43	Superaugmented eccentric connectivity index-1	Dureja and Madan (2007)
A44	Superpendent index	Gupta <i>et al.</i> (1999)

between descriptors. Finally, a total of 44 descriptors (Table 2) from a large pool of molecular descriptors were selected for further analysis with DT and MAA.

Decision tree (DT)

DTs are among the most popular of the new statistical learning techniques being used in the pharmaceutical industry for predicting QSARs (Blower and Cross, 2006). DTs are supervised algorithms that recursively partition the data based on its attributes, until some stopping condition is reached. This recursive partitioning gives rise to a tree-like structure (Singh *et al.*, 2008). In contrast to multiple linear regression analysis that creates a continuous data range, recursive partitioning techniques lead to a distinct binning of data (Andres and Hutter, 2006). DT assigns a probability value (0–1) that a compound is active or inactive; compounds with the probability ≥ 0.5 are designated as active, while others are designated as inactive (Hong *et al.*, 2005). In this study, a classification tree was grown with the R program version 2.1.0 using the RPART library using the default parameters (Gupta and Aires-de-Sousa, 2007).

Moving average analysis

In order to generate single TI-based models for classifying data set into active and inactive analogues, MAA was applied. Index values of all the 44 chosen descriptors were analyzed and suitable models were developed after identification of the active ranges by maximization of moving average with respect to active compounds (<35% = inactive, 35–65% = transitional, >65% = active) (Gupta *et al.*, 2001; Dureja *et al.*, 2008b). Subsequently, each analogue of data set was assigned a biological activity using these models, which was then compared with the reported activity (Green *et al.*, 2007).

Calculation of topological indices

Superpendentic index ($\int^P$)

A pendent-distance-based graph invariant termed as *superpendentic index* and denoted by $\int^P$ is defined as the square root of the sum of products of the non-zero row elements in the pendent matrix. It is expressed as:

$$\int^P = \left\{ \sum_{i=1}^n \prod_{j=1}^m P_{(ij)} \right\}^{0.5} \quad (1)$$

where m and n are maximum possible numbers of i and j , respectively (Gupta *et al.*, 1999).

Wiener-type index from Z weighted distance matrix [$W(G_{\text{vew}})$] (Barysz *et al.*, 1983)

It may be defined as the sum of all the elements in upper triangular Barysz matrix as well as all the diagonal elements thereof as per following:

$$W(G_{\text{vew}}) = \sum d_{ii} + \frac{1}{2} \sum_i \sum_j d_{ij}. \quad (2)$$

where

$$d_{ii} = 1 - \frac{6}{Z_i}. \quad (3)$$

where Z_i is the number of all (valence and inner shell) electrons in the atom i .

The off-diagonal elements of the distance matrix for the vertex- and edge-weighted (multi) graphs are defined as:

$$d_{ij} = \sum_r k_r. \quad (4)$$

where the summation goes over r ($r = 1, 2, \dots$), weighted and unweighted, bonds, while the parameter k_r is given by (Barysz *et al.*, 1983),

$$k_r = \frac{1}{b_r} * \frac{36}{Z_i * Z_j}. \quad (5)$$

$$\text{where } b_r = \begin{pmatrix} 1 & \text{for a single bond} \\ 2 & \text{for a double bond} \\ 3 & \text{for a triple bond} \\ 1.5 & \text{for an aromatic bond} \end{pmatrix} \text{ and } Z_i \text{ and } Z_j$$

are the numbers of all electrons in atoms i and j making up the r -bond (Barysz *et al.*, 1983).

Gutman molecular topological index by valence vertex degrees (S_G)

It may be defined as:

$$S_G = \sum_{i=1}^N \sum_{j=1}^N v_i v_j \cdot D_{ij}. \quad (6)$$

where $v_i v_j \cdot D_{ij}$ is the topological distance between the vertices v_i and v_j weighted by product of the endpoint vertex degrees (Gutman, 1994).

Eccentric distance sum topochemical index (ξ_c^{DS})

It is a topochemical version of *eccentric distance sum index* (Gupta *et al.*, 2002) and may be defined as the summation of product of chemical eccentricity and chemical distance sum of each vertex in a hydrogen suppressed molecular graph having n vertices as:

$$\xi_c^{DS}(G) = \sum_{i=1}^n (E_{ic} * S_{ic}). \quad (7)$$

where S_{ic} is chemical distance sum of vertex i , E_{ic} is chemical eccentricity of vertex i and n is the number of vertices in graph G .

Performance evaluation

The goodness of the models was assessed by calculating sensitivity, specificity, overall accuracy of prediction (Han *et al.*, 2008; Lamanna *et al.*, 2008). The sensitivity and specificity can be defined as:

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FN}),$$

$$\text{Specificity} = \text{TN}/(\text{TN} + \text{FP})$$

where the true positive (TP) is the number of compounds correctly predicted as active, false negative (FN) is the number of compounds incorrectly predicted as inactive, true negative (TN) is the number of compounds correctly predicted as inactive, false positive (FP) is the number of compounds incorrectly predicted as active. Thus, the overall accuracy is defined as:

$$\text{Overall accuracy} = (\text{TP} + \text{TN})/(\text{TP} + \text{FN} + \text{TN} + \text{FP}) * 100\%$$

Using this information, Matthews correlation coefficient (MCC) was calculated as (Matthews, 1975; Cannon *et al.*, 2006):

$$\text{MCC} = \frac{\text{TP} \cdot \text{TN} - \text{FN} \cdot \text{FP}}{\sqrt{(\text{TP} + \text{FN})(\text{TP} + \text{FP})(\text{TN} + \text{FN})(\text{TN} + \text{FP})}}$$

This coefficient returns a value ranging from -1 to $+1$. The higher the value of MCC, the more reliable is the classification result. Further, intercorrelation between Wiener type index from Z weighted distance matrix denoted by $W(G_{\text{vew}})$ (A1), Gutman MTI by valence vertex degrees denoted by S_G (A2), and eccentric distance sum topochemical index denoted by ξ_c^{DS} (A40) was investigated using the index values of analogues of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles. The validation of the DT-based models and self-consistency test were performed by 10-fold cross validation (CV) method (Cyril, 1997). The degree of correlation between developed models was also appraised by the correlation coefficient r . Pairs of indices with $r \geq 0.97$ are considerably highly intercorrelated, those with $0.90 \geq r < 0.97$ are appreciably correlated, those with $0.50 \leq r \leq 0.89$ are weakly correlated and finally the pairs of indices with low r values (<0.50) are not intercorrelated (Nikolic *et al.*, 2003).

Results and discussion

Various studies have revealed that TNF- α is involved in the inflammation process in several disease states such as RA, psoriasis, inflammatory bowel disease, and multiple sclerosis (Wu *et al.*, 2009). Thus, the control of TNF- α has become a major goal in treating the progression of RA (Hu *et al.*, 2006). The prediction of physico-chemical, pharmacological, and toxicological properties of molecules in QSAR/QSPR studies can be obtained from their molecular structure, encoded in a numerical form with the aid of various descriptors, such as graph theoretic and topological indices, and geometrical, electrostatic, and quantum-chemical descriptors (Murcia-Soler *et al.*, 2003).

In this study, DT and MAA were employed to develop models for the prediction of anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles (Green *et al.*, 2007). In order to further test the significance of selected 44 descriptors through exclusion method, a single DT was grown. This recursive partitioning scheme generates rules derived from the numerical data of the available molecular descriptors for each molecule. In this

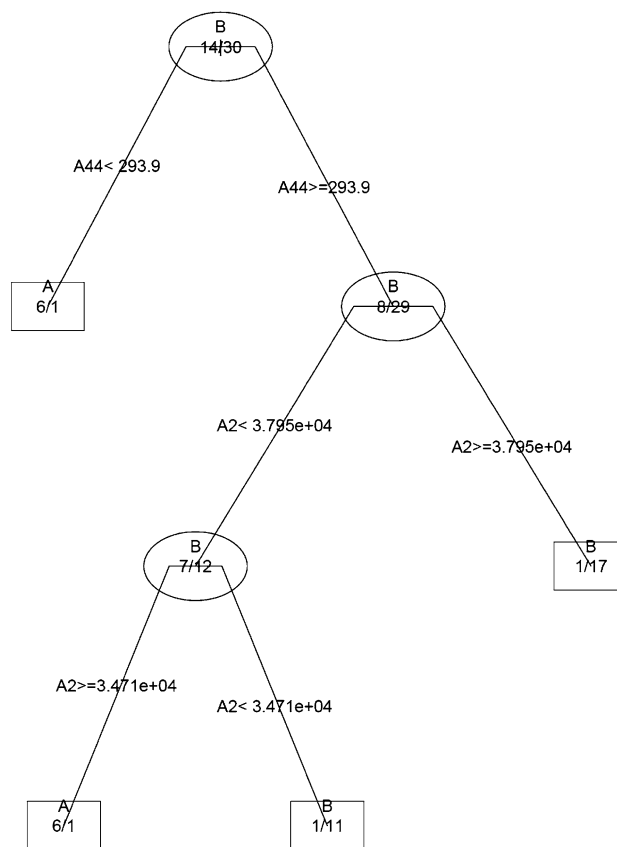


Fig. 2 Topology of decision tree distinguishing active compounds {A} from inactive compounds {B}

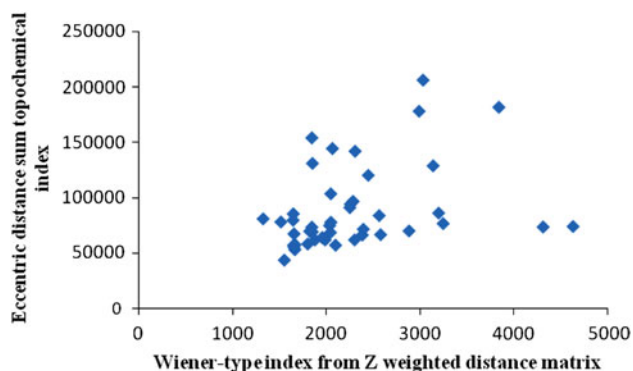
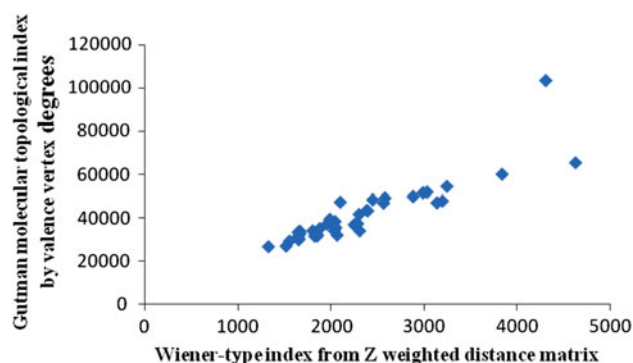
Table 3 Confusion matrix for inhibition of Tpl2 kinase and recognition rate of model based on decision tree

Model	Description	Ranges	Number of compounds predicted		Sensitivity (%)	Specificity (%)	Overall accuracy of prediction	MCC
			Active	Inactive				
Decision tree	Training set	Active	12	2	85.71	93.33	90.9	0.79
		Inactive	28	2				
	Cross validated set	Active	07	07	53.8	74	65.9	0.2
		Inactive	24	6				

case, a classification of data set into active and inactive compounds was desired. DT identified A2 and A44 as the two important topological indices. The resulting topology of the DT is illustrated in Fig. 2, where the respective descriptor is denoted with an alphanumerical abbreviation that refers to Table 2. The index at the root node is most significant and importance of index decreases as the tree increases. Thus, A44 is the most important, followed by A2.

DT classified analogues of the data set in the training set with an accuracy of 90.9% and in the 10-fold cross validated set with an accuracy of 65.9% with regard to anti-inflammatory activity. The goodness of DT-based model was further evaluated by calculating sensitivity, specificity, and MCC. The sensitivity, specificity, and MCC of DT-based model in the training set were found to be 85.71%, 93.33% and 0.79, respectively, and of cross-validated set were 53.8%, 74%, and 0.2, respectively (Table 3; Figs. 3, 4, 5).

All the descriptors selected through preprocessing were subjected to MAA to develop models for prediction of anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles. Though all the analogues in the data set possess varying degree of biological activity but only those analogues having IC_{50} values of ≤ 25 nM were considered to be active for the purpose of this study. Using a single index at a time, MAA provided three independent models with an accuracy of prediction ranging from 87.5 to

**Fig. 3** Intercorrelation between Wiener-type index from Z weighted distance matrix and eccentric distance sum topochemical index**Fig. 4** Intercorrelation between Wiener-type index from Z weighted distance matrix and Gutman molecular topological index by valence vertex degrees

92.5%. The index values of various analogues along with their substituents are presented in Table 1. These three independent models are based on A1, A2, and A40. A1 accounts simultaneously for the presence of heteroatom (s) and multiple bond (s) in the molecule. A2 takes into consideration the relative intricacy (branchedness) of the molecule. A40 takes into consideration the eccentricity, distance sum and presence as well as the relative position of heteroatom(s) in a molecule.

The MAA used in this study aims at the development of suitable models for providing lead molecules through exploitation of the active ranges in the proposed topological models. Proposed models are unique and differ widely

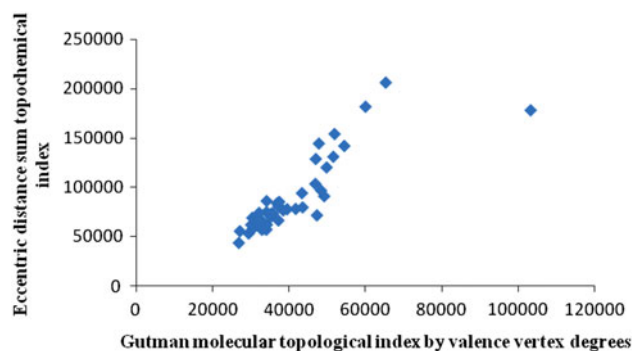
**Fig. 5** Intercorrelation between Gutman molecular topological index by valence vertex degrees and eccentric distance sum topochemical index

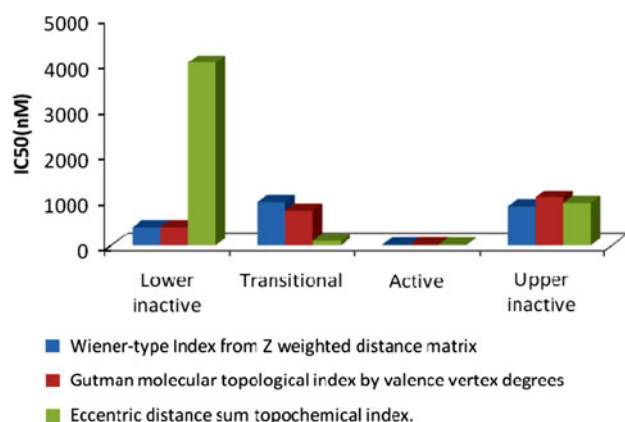
Table 4 MAA base topological models for inhibition of Tpl2 kinase

Model Index	Nature of range in proposed model	Index value	Number of analogues falling in the range		Percent accuracy	Average IC_{50} (nM)*	Overall accuracy of prediction (%)
			Total	Correct			
$W(G_{\text{vew}})$	Lower inactive	<1551.767	2	2	>99.9	395	92.0
	Active	1551.767–1663.216	7	6	85.7	31.42 (15)	
	Transitional	>1663.216–<2299.675	19	N.A.	N.A.	951.157	
	Upper inactive	≥ 2299.675	16	15	93.7	806.312 (858.733)	
S_G	Lower inactive	<29444.55	2	2	>99.9	395	92.5
	Transitional	29444.55–<35229.22	17	N.A.	N.A.	765.529	
	Active	35229.22–37442.33	7	6	85.7	35.42 (19.66)	
	Upper inactive	>37442.33	18	17	94.4	996.16 (1053.58)	
ξ_c^{DS}	Lower inactive	<57114.137	4	3	75	3027.5 (4030)	87.5
	Active	57114.137–61953.59	5	5	>99.9	14	
	Transitional	>61953.59–<74030.43	12	N.A.	N.A.	95.92	
	Upper inactive	≥ 74030.43	23	20	86.95	810.96 (929.55)	

* Values in the brackets indicate average IC_{50} values of correctly predicted analogues of the particular range

from conventional QSAR models. Both the systems of modeling have their own advantages and limitations. In the instant case, the modeling system adopted has distinct advantage of identification of narrow active range(s), which may be erroneously skipped during routine regression analysis in conventional QSAR modeling. Since the ultimate goal of modeling is to provide lead structures, therefore, these active ranges can play vital role in lead identification (Dureja and Madan, 2006).

Retrofit analysis of data (Table 4) reveals that MAA-based models derived from $W(G_{\text{vew}})$, S_G , and ξ_c^{DS} correctly predicted analogues with regard to inhibition of Tpl2 Kinase activity to the tune of 92, 92.5, and 87.5%, respectively. Existence of transitional ranges in the proposed models is ideal and it simply indicates gradual change in the inhibition of Tpl2 Kinase activity. As observed from Table 4 and Fig. 6 the average IC_{50} values

**Fig. 6** Average IC_{50} (nM) values of correctly predicted analogues in various ranges of the proposed topological models

of correctly predicted analogues of active ranges of all the three models varied from only 14 to 19.66 nM indicating *exceptionally high potency*. As observed from Fig. 6, the ratio of average IC_{50} values of active range and inactive ranges of the proposed models for correctly predicted analogues varied from a minimum of 1:20 to a maximum of 1:287. Sensitivity and specificity of the proposed models ranged from 55.55% (ξ_c^{DS}) to 85.7% (S_G) and from 94.4% [$W(G_{\text{vew}})$] to 100% (ξ_c^{DS}), respectively. The value of MCC varied from 0.69 (ξ_c^{DS}) to 0.8 (S_G) indicating robustness of the proposed models.

Intercorrelation analysis (Table 5) revealed that two out of three pairs of indices are weakly correlated.

An in-depth study of proposed models as well as the corresponding structural information involved in the data set clearly reveals that the substitution of amino group at position 6 of the quinoline nucleus with a five/six-membered N-heterocycle is essential for a compound to be biologically active. Absence of this moiety leads to the loss of the required distance sum, heteroatom(s), and multiple bonds as per the model based upon A1, loss of the desired branchedness/intricacy as per A2 model, apart from loss of the requisite eccentricity, distance sum and heteroatom(s) as per the model based upon A40 leading the molecule to fall outside the active range of proposed model(s) with consequent loss of biological activity.

Table 5 Intercorrelation matrix

	$W(G_{\text{vew}})$	S_G	ξ_c^{DS}
$W(G_{\text{vew}})$	1	0.89	0.98
S_G		1	0.85
ξ_c^{DS}			1

A substituent at position 8 of quinoline ring and the presence of an optional substituent at position 1 or 3 of six-membered ring and at position 3 or 5 of five-membered ring is needed for a compound to be within the active range of the proposed model(s) so as to be biologically active. Absence of substitution at both these positions leads to steep decrease in eccentricity, branchedness, and distance sum leading to decrease in the index values so as to fall outside the active range of proposed model(s).

Moreover, the total number of non-hydrogen atoms at both the positions (substituent at position 8 of quinoline ring and optional substituent at position 1 or 3 of six-membered ring, or at position 3 or 5 of five-membered ring) should not exceed 4 and no single substituent at these positions should exceed three non-hydrogen atoms for a molecule to be biologically active. Any additional non-hydrogen atom beyond permissible limits in a molecule simply leads to increase in eccentricity, branchedness, and distance sum with consequent increase in the index values so as to fall outside the active range of proposed model(s).

The link between the quinoline nucleus and five/six-membered N-heterocycle should not be more than 2 non-hydrogen atoms. Any additional non-hydrogen atom beyond this permissible limit will naturally lead to increase in eccentricity and distance sum with consequent increase in the index values so as to fall outside the active range of proposed model(s).

Moreover, substitution in N-heterocycle at positions just next to the attachment of the link (substituent at position 1 or 3 of six-membered ring and at position 3 or 5 of five-membered ring) is only tolerated. Substitution at any other position farther than these positions of the N-heterocycle leads to increase in both eccentricity and distance sum with consequent increase in index values so as to fall outside the active range of proposed model(s) resulting in loss of biological activity.

Conclusion

DT and MAA were successfully employed for development of models for the prediction of anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles. The overall accuracy of prediction of proposed MAA models varied from 87.5% for a model based upon A40 to 92.5% for a based upon A2. DT correctly classified the analogues of data set into active and inactive ones with accuracy of prediction of ~91% in the training set and ~66% in the cross validated set, respectively.

These models offer vast potential for providing lead structures for the development of potent therapeutic agents with regard to anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles through inhibition

of Tpl2 kinase and TNF- α production. However, there is a future need to investigate effect of these anti-inflammatory agents in in vivo and in vitro models of inflammation such as—inhibition of P-selectin up-regulation by LPS, inhibition of inducible cyclooxygenase-2 (cox-2) expression and inhibition of NF- κ B-mediated translocation.

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Inhibitors of Tumor Progression Loci-2 (Tpl2) Kinase and Tumor Necrosis Factor α (TNF- α) Production: Selectivity and in Vivo Antiinflammatory Activity of Novel 8-Substituted-4-anilino-6-aminoquinoline-3-carbonitriles

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Tumor progression loci-2 (Tpl2) (Cot/MAP3K8) is a serine/threonine kinase in the MAP3K family directly upstream of MEK. Recent studies using Tpl2 knockout mice have indicated an important role for Tpl2 in the lipopolysaccharide (LPS) induced production of tumor necrosis factor α (TNF- α) and other proinflammatory cytokines involved in diseases such as rheumatoid arthritis. Initial 4-anilino-6-aminoquinoline-3-carbonitrile leads showed poor selectivity for Tpl2 over epidermal growth factor receptor (EGFR) kinase. Using molecular modeling and crystallographic data of the EGFR kinase domain with and without an EGFR kinase-specific 4-anilinoquinazoline inhibitor (erlotinib, Tarceva), we hypothesized that we could diminish the inhibition of EGFR kinase by substitution at the C-8 position of our 4-anilino-6-aminoquinoline-3-carbonitrile leads. The 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles were prepared from the appropriate 2-substituted 4-nitroanilines. Modifications to the C-6 and C-8 positions led to the identification of compounds with increased inhibition of TNF- α release from LPS-stimulated rat and human blood, and these analogues were also highly selective for Tpl2 kinase over EGFR kinase. Further structure–activity based modifications led to the identification of 8-bromo-4-(3-chloro-4-fluorophenylamino)-6-[(1-methyl-1H-imidazol-4-yl)methylamino]quinoline-3-carbonitrile, which demonstrated in vitro as well as in vivo efficacy in inhibition of LPS-induced TNF- α production.

Introduction

Inflammatory cytokines such as tumor necrosis factor α (TNF- α) and interleukin-1 (IL-1) are believed to be major contributors to inflammatory diseases such as psoriasis, Alzheimer's disease, inflammatory bowel disease, and most notably rheumatoid arthritis (RA).^{1–3} RA is a debilitating systemic disease that affects over 1% of the population worldwide with an annual incidence of 0.04%.³ The disease is characterized by recurring symptoms such as pain, swelling, stiffness, and restricted mobility due to the erosion of joint cartilage, connective tissue, and eventually bone. Furthermore, several mechanisms may contribute to the pathology of RA, adding to the complex treatment of this disease. TNF- α contributes to cartilage breakdown and bone erosion by stimulating the production of other proinflammatory cytokines such as IL-1 and IL-6 and inducing degradative enzymes such as the matrix metalloproteases (MMPs).^{1,4} Disease-modifying antirheumatic drugs that target TNF- α and IL-1 including etanercept (Enbrel), infliximab (Remicade), and adalimumab (Humira) have shown efficacy in the treatment of RA. Routes of administration, contraindications, and/or precautions associated with these agents warrant further investigation toward additional drug therapies.^{5,6}

Several pharmaceutical companies have focused on TNF- α converting enzyme (TACE) for the treatment of inflammatory diseases.⁴ Candidates such as BMS-561392 and TMI-1 (**3**) have shown efficacy in animal models of arthritis and have advanced to clinical trials; however, safety and efficacy concerns eventually led to their discontinuation (Chart 1).⁵

An alternative method toward the inhibition of TNF- α production is through disruption of signal transduction pathways that include several members of the mitogen activated protein kinase (MAP kinase) family, particularly p38 kinase. The identification of the inhibitor SB203580 initiated intense focus in the pharmaceutical industry for the discovery of p38 inhibitors.⁷ Compounds such as VX-745 (Vertex Pharmaceuticals), BIRB-796 (Boehringer-Ingelheim), and AMG-548 (Amgen; see Chart 1) represent the first examples of small-molecule p38 kinase inhibitors to advance into the clinic.⁸ In addition, GlaxoSmithKline, Roche, Scios, Pfizer, and Array BioPharma have also advanced p38 kinase inhibitors to the clinic for RA and other inflammatory diseases.⁹

Upstream activation of the MAP kinases is controlled by tumor progression loci-2 (Tpl2) kinase, a serine/threonine kinase in the MAP3K family.^{10–12} Recent studies using Tpl2 knockout mice confirmed that Tpl2 plays an important role in the lipopolysaccharide (LPS) induced production of TNF- α and other proinflammatory cytokines.¹³ Tpl2 is also required for TNF- α -induced signaling, and thus, an inhibitor of Tpl2 may provide multiple benefits by blocking both TNF- α production and signaling.^{14–17} Tpl2 is not inhibited by staurosporine, a nonspecific kinase inhibitor, and it is the only known human kinase that has a proline instead of a conserved glycine in the

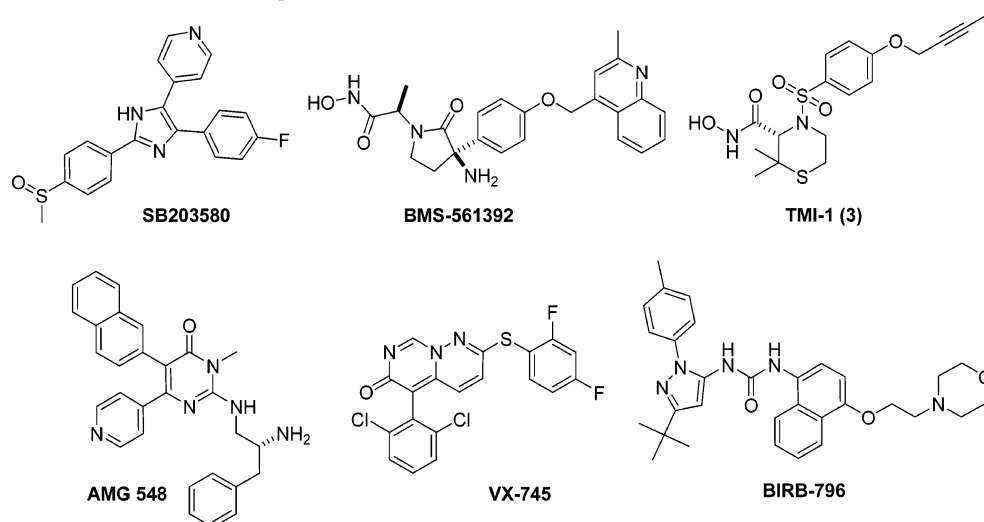
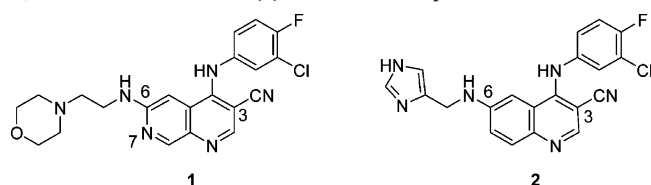
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Chart 1. Chemical Structures of TACE and p38 Kinase Inhibitors of TNF- α Production**Chart 2.** 1,7-Naphthyridine-3-carbonitrile (**1**) and Quinoline-3-carbonitrile (**2**) Inhibitors of Tpl2

glycine-rich ATP binding loop, a factor that may lead to the discovery of highly selective inhibitors.¹⁷

We have previously disclosed a series of 1,7-naphthyridine-3-carbonitriles (**1**) and more recently the related quinoline-3-carbonitrile (cyanoquinoline) Tpl2 inhibitors (**2**, Chart 2).^{18,19} Both of these leads were identified from our in-house kinase inhibitor collection, which comprises mostly tyrosine kinase inhibitors. Initial selectivity for Tpl2 over other kinases, particularly epidermal growth factor receptor (EGFR) tyrosine kinase, was moderate to poor for both the 1,7-naphthyridine-3-carbonitriles and cyanoquinolines. In this report we describe our efforts toward improving selectivity and potency of the cyanoquinoline class of Tpl2 inhibitors.²⁰

Design and Synthesis

The synthesis of most of the cyanoquinoline analogues with variations at X and at R started with the substituted nitroanilines **4a–g** (Scheme 1).²¹ Nitroanilines **4b–g** were reacted with ethyl 2-cyano-3-ethoxyacrylate under basic conditions to give the 2-cyano-3-(arylamino)acrylates **5b–g**.¹⁹ The acrylates were washed liberally with water upon workup and then dried in a vacuum oven for 48 h prior to thermal cyclization in Dowtherm A, which provided quinolones **6b–f** in moderate to good yields. The fluoroquinolone **6b** was converted to a thioether with sodium methylthiolate in 1,3-dimethyltetrahydropyrimidinone (DMPU) at room temperature²² and subsequently oxidized with either 1.2 or 2.2 equiv of *m*-chloroperbenzoic acid (*m*-CPBA) to provide the sulfoxide **6h** and the sulfone **6i**, respectively.²³ After chlorination of **6b–i**, the 3-fluoro-4-chloroaniline headpiece was installed by S_NAr displacement to give nitro intermediates **8b–i**. Demethylation of **8f** with pyridinium hydrochloride in DMF gave the corresponding $-OH$ derivative **8j**.²⁴ Reduction of **8b–j** using tin dichloride²⁵ or catalytic hydrogenation²⁶ afforded the C-6 amino compounds **9b–j**. Attachment of moieties onto the C-6 amino group (tailpieces)

was accomplished by reductive amination²⁷ using a variety of commercially available or readily synthesized aldehydes to provide target 4-anilino-6-aminoquinoline-3-carbonitrile analogues **10–18**, **24–28**, **31–37**, **42–51**, and **55** (see Tables 1–3).

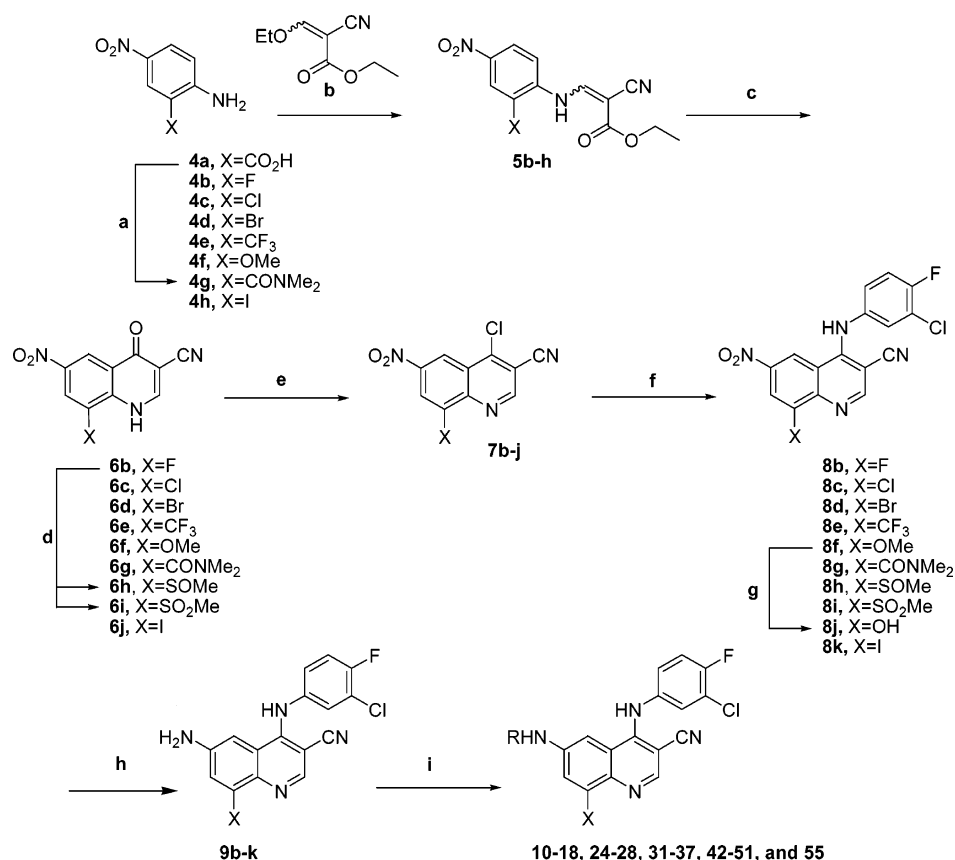
The synthesis of C-8 substituted analogues **19–21** was accomplished according to the chemistry shown in Scheme 2. Iodide **9n** was subjected to a Stille coupling reaction with tributyl(1-ethoxyvinyl)stannane, and the resulting enol ether was hydrolyzed to give **19**. Alkylation of **9d** gave **9l**, which was acetylated, subjected to dihydroxylation, and deprotected to give the dihydroxy intermediate **9m**. Reductive amination of **9m** afforded analogue **20**. Alkylation of the hydroxyl group of **8j** gave the *O*-allyl intermediate **8l**, and reduction of the nitro group and subsequent reductive amination gave **21**.

The syntheses of compounds **22**, **23**, **29**, **30**, and **38–41**, all of which include substituted imidazolyl and pyridinyl tailpieces, are shown in Schemes 3 and 4. A microwave-assisted Wittig homologation²⁸ of trityl-protected 1*H*-imidazole-5-carbaldehyde afforded 2-(1*H*-imidazol-5-yl)acetaldehyde, which was used in a reductive amination reaction with **9d** to give **22**. Acylation of **9d** with imidazolylacetic acid provided **23**. A bromine–magnesium exchange with 3,5-dibromopyridine²⁹ was used to furnish the pyridylaldehydes for the preparation of **29** and **30**. The method of Paul et al. (Scheme 4) was used to prepare **52–54**, leading to the quinolinecarbonitriles **38–41**.³⁰ A Sonogashira coupling reaction followed by reduction of the resulting alkyne and reductive amination was used to convert **56**³¹ to **43**.

Discussion

Molecular Modeling. We had previously studied the structure–activity relationships of a series of cyanoquinolines and found that potency improvement was afforded by variation of the 6-amino tailpiece portion while substitution at other sites on the scaffold ablated Tpl2 inhibition.¹⁹ In that report it was shown that compound **2** was a potent inhibitor of Tpl2 kinase with an IC_{50} value of 30 nM but that it was equally potent against EGFR kinase. Our initial plan was to improve selectivity and potency through variation of the C-4 headpiece moiety;³² however, SAR studies of related 1,7-naphthyridine-3-carbonitriles did not show this approach to be effective.¹⁸

At the early stages of this project the low yield for the expression of Tpl2 and the difficulty in isolating stable and active protein deterred efforts toward X-ray crystallographic studies with this kinase. In addition, no suitable homology model

Scheme 1^a

^a Conditions: (a) BOP, 4-methylmorpholine, Me₂NH₂HCl, CH₂Cl₂, 100%; (b) Cs₂CO₃, DMF, room temp, 2 h, 72–99%; (c) Dowtherm A, 259 °C, 4–5 h, 21–68%; (d) (i) MeSNa, DMPU, room temp, 38%, (ii) MCPBA, CH₂Cl₂, room temp, 3 or 16 h; (e) (COCl)₂, DMF (cat.), DCE or POCl₃, reflux, 12 h, 85–92%; (f) 3-fluoro-4-chloroaniline, EtOH, reflux, 12 h, 41–78%; or DME, 120 °C microwave, 15 min; (g) pyridine·HCl, pyridine, 110 °C; (h) SnCl₂·2H₂O, EtOH, reflux 1 h or microwave, 110 °C, 5 min, or Pd/C, H₂, EtOH, acetic acid, 12 h, 63–90%; (i) RCHO, NaCNBH₃, HOAc, EtOH or DCE, Na(OAc)₃BH, 3 h, 4–76%.

Table 1. Tpl2 and P-EGFR Inhibition SAR

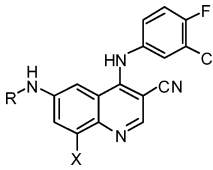
compd	X	IC ₅₀ (nM) ^a		LPS induced TNF-α inhibition ^b	
		Tpl2	P-EGFR	monocytes (nM)	human blood (nM)
2	H	20	30	600	3300
10	F	20	500	510	>10000
11	Cl	20	1800	350	4900
12	Br	10	3300	370	>10000
13	CF ₃	160	>20000	600	>10000
14	OH	11300	NT ^c	NT ^c	NT ^c
15	OMe	130	2080	3000	>10000
16	SOMe	20	5000	2130	13300
17	SO ₂ Me	190	NT ^c	NT ^c	NT ^c
18	CONMe ₂	130	>20000	>1000	>10000
19	C(O)Me	23	156	580	>10000
20	CH ₂ CH(OH)CH ₂ OH	910	NT ^c	NT ^c	NT ^c
21	OCH ₂ CH(OH)CH ₂ OH	830	NT ^c	NT ^c	NT ^c

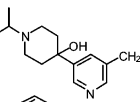
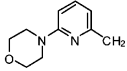
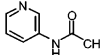
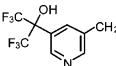
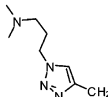
^a Average of at least two runs performed in duplicate with a standard deviation of 20%. ^b Average of at least two runs performed in duplicate with a standard deviation of 25%. ^c NT: not tested.

for Tpl2 was found that could be used for inhibitor–protein docking studies. In order to improve selectivity against EGFR, we used molecular modeling and the crystal structure of the EGFR kinase domain with a 4-anilinoquinazoline inhibitor³³ (erlotinib) to determine an alternative site on our cyanoquinoline core.³⁴ Thus, the sequences of human EGFR and Tpl2 were

aligned and sequence differences were examined. The following residue differences were observed near the ATP binding site (numbering according to the EGFR structure, EGFR/Tpl2): Met⁷⁴²/Cys, Cys⁷⁵¹/Ala, Thr⁷⁶⁶/Met, Leu⁷⁶⁸/Ala, Met⁷⁶⁹/Gly, Pro⁷⁷⁰/Glu, and Thr⁸³⁰/Val. Two residues in particular, Leu⁷⁶⁸ (Ala in Tpl2) and Pro⁷⁷⁰ (Glu in Tpl2), are in the hinge region

Table 2. SAR of Various Tailpiece Analogues



compound	X	Tpl2 (enzyme) IC ₅₀ (nM) ^a	LPS induced TNF-α inhibition ^b		P-EGFR IC ₅₀ (nM) ^a	compound	R	X	Tpl2 (enzyme) IC ₅₀ (nM) ^a	LPS induced TNF-α inhibition ^b		P-EGFR IC ₅₀ (nM) ^a	
			monocytes	human blood (nM)						monocytes	human blood (nM)		
11		Cl	20	350	4900	1800	30		Cl	500			
12		Br	10	370	>10,000	3300	31		Cl	170			
22		Cl	130		>20,000		32		Cl	350			
23		Br	>5000				33		Cl	18	1500	11,400	2900
24		Cl	290				34		Br	590			
25		Br	98	665	>20,000		35		Cl	2	>5000	>20,000	
26		Br	250				36		Cl	200	>5000	>20,000	
27		Cl	280				37		Cl	110			
28		Cl	16	>1000	>20,000	>5000	51		Br	18	74	3500	>5000
29		Cl	>3000				55		Br	20	221	3080	>5000

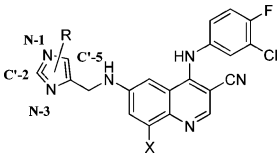
^a Average of at least two runs performed in duplicate with a standard deviation of 20%. ^b Average of at least two runs performed in duplicate with a standard deviation of 25%.

and are the only side chain differences near the C-8 position of erlotinib in the X-ray complex structure. Glide XP^{38,39} was used to dock compound **2** into the binding site of EGFR, and the predicted location of C-8 in the cyanoquinoline inhibitors was shown to overlay with the C-8 position in erlotinib (see Figure 1). Maps of preferred functional group positions within the unliganded EGFR binding site were calculated using MCSS;^{40,41} these calculations indicate that a methyl or larger group attached at C-8 to erlotinib or our cyanoquinoline inhibitors would be expected to have substantial steric clash with the hinge residues in EGFR. It is clear from Figure 1 that a methyl in an optimal position near the hinge residues (shown in cyan) is far too close to be bonded to erlotinib or the cyanoquinoline inhibitor **2** without shifting the inhibitor scaffold out of the pocket somewhat and thereby reducing EGFR inhibition. It was also predicted that substitution at the C-8 position of a cyanoquinoline would have a negligible impact on its binding to Tpl2 because the Leu⁷⁶⁸ in EGFR is replaced by the much smaller Ala side chain in Tpl2, allowing for larger substituents in this area. We did not know with certainty what the three-dimensional similarities were between EGFR kinase and Tpl2 kinase; therefore, we prepared a number of C-8 substituted cyanoquinolines in order to determine what groups would provide detrimental binding to EGFR kinase but maintain or improve Tpl2 inhibition.

Structure–Activity Relationships (SAR). All compounds were examined for inhibition of isolated Tpl2 enzyme via

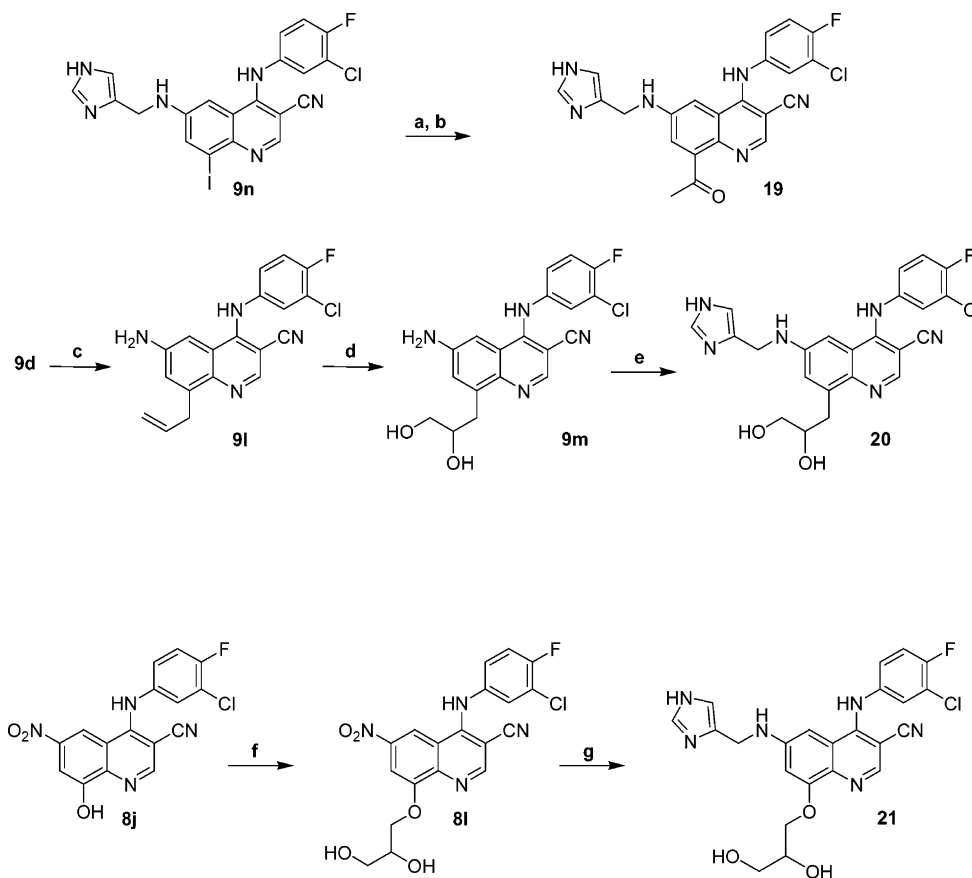
quantification of MEK phosphorylation in an ELISA format.^{18,19} For several compounds, selectivity was measured against EGFR kinase autophosphorylation in A431 cells overexpressing this enzyme.²¹ Functional inhibition of TNF-α production was measured using LPS-stimulated monocytes and LPS-stimulated human blood. Compounds studied in vivo were first assessed for selectivity against other kinases (including p38α, CAMKII, PKC, PKA, MK2, Src, S6K, IKKβ, and Jnk-1).

Our first goal was to study the impact that C-8 substitution would have on potency and selectivity. Toward that end, we chose groups with a range of sizes and polarity and the ability to donate and/or accept a hydrogen bond. We also attempted to make a possible contact with the ribose-binding region of Tpl2 with diols **19–21**. We were pleased to find that for **10**, **11**, and **12** (X = F, Cl, and Br, respectively) not only was potency maintained but more importantly a significant improvement in selectivity (25- to 470-fold) was demonstrated. The potency against Tpl2 was lower for nonhalogenated C-8 compounds **13–15** and **17**. Larger polarizable groups (Cl, Br, SOMe) appear to be favored for both Tpl2 inhibition and EGFR selectivity. A C-8 methyl group appears to be less favored by Tpl2, perhaps because simple van der Waals interactions in the absence of other electronic contributions are detrimental to binding. For example, a cyanoquinoline with a methyl group at C-8 and a 3-pyridyl in place of the imidazole tailpiece (not shown) had a Tpl2 kinase inhibition IC₅₀ of >1 μM. Other halogen C-8

Table 3. SAR and TNF- α Inhibition of Substituted Imidazole Tailpiece Analogues


compd	R	X	Tpl2 (enzyme) IC ₅₀ (nM) ^a	LPS induced TNF- α inhibition ^b	
				monocytes (nM)	human blood (nM)
11	H	Cl	20	580	5200
12	H	Br	10	320	11600
38	C'5-isopropyl	Br	130	2200	>20000
39	C'5-isopropyl	Cl	16	NT ^c	5800
40	C'5-propyl	Cl	25	220	5600
41	C'5-ethyl	Cl	16	NT ^c	7500
42	C'5-CO ₂ Me	Br	87	2000	>20000
43	C'5-[3-(dimethylamino)propyl]	Cl	130	NT ^c	15200
44	N'1-methyl	Br	30	670	6300
45	C'2-ethyl-C'5-methyl	Cl	320	NT ^c	NT ^c
46	C'2,5-dimethyl	Cl	220	NT ^c	NT ^c
47	N'3-methyl	Cl	220	NT ^c	>20000
48	C'2-phenyl	Br	5900	NT ^c	NT ^c
49	N'1-[2-(morpholinyl)ethyl]	Br	41	430	8950
50	N'1-[2-(methylsulfonyl)ethyl]	Br	43	1000	>20000

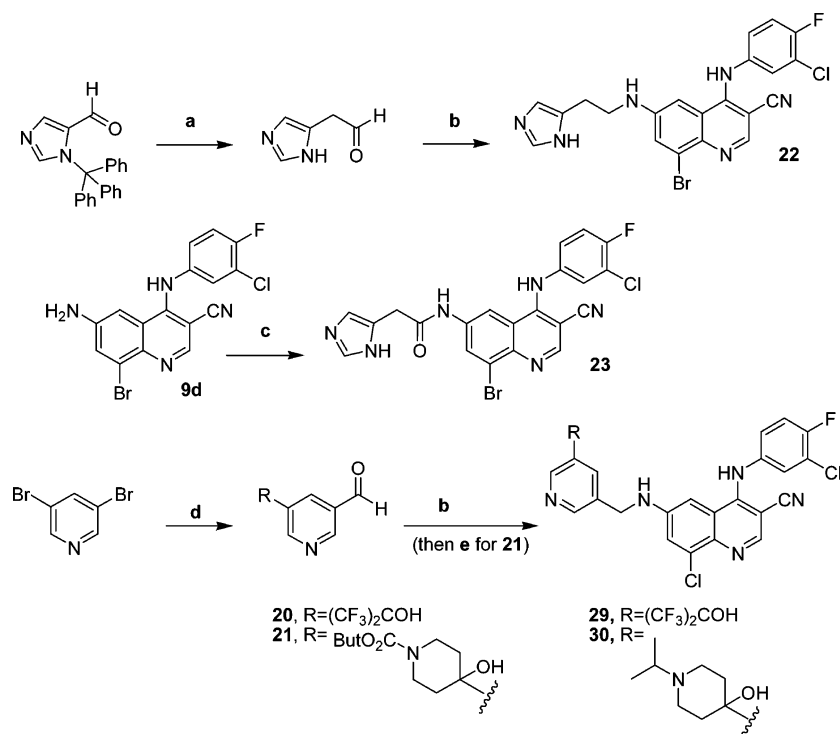
^a Average of at least two runs performed in duplicate with a standard deviation of 20%. ^b Average of at least two runs performed in duplicate with a standard deviation of 25%. ^c NT: not tested.

Scheme 2^a

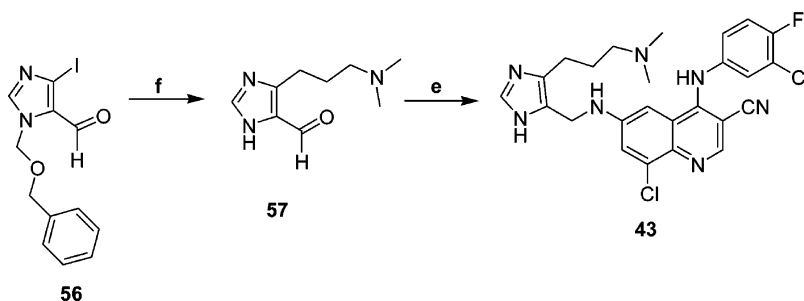
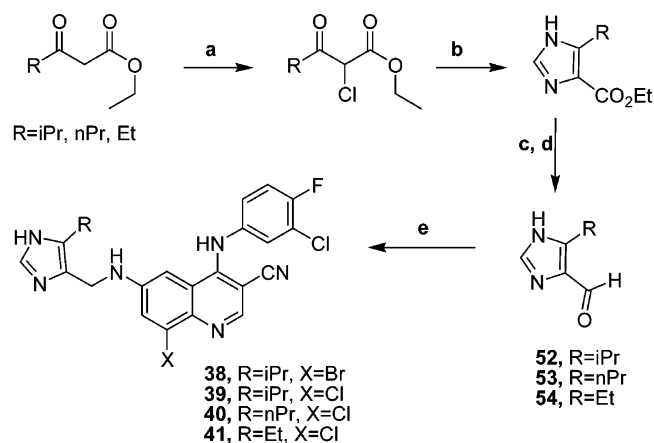
^a Conditions: (a) tributyl(1-ethoxyvinyl)tin, PdCl₂(PPh₃)₂, DMF; (b) 1 N HCl, heat, 14%; (c) allyltributylstannane, PdCl₂(PPh₃)₂, DMF, 48%; (d) (i) (CF₃CO)₂O, pyridine, CH₂Cl₂; (ii) OsO₄, NMO, acetone/H₂O, 1:1; (iii) 5 M LiOH, quantitative; (e) 1H-imidazole-5-carbaldehyde, HOAc, EtOH, Na(OAc)₃BH, 45%; (f) (i) allyl bromide, K₂CO₃, DMF, 75%; (ii) OsO₄, NMO, acetone/H₂O, 47%; (g) (i) SnCl₂·2H₂O, EtOAc; (ii) 1H-imidazole-5-carbaldehyde, HOAc, EtOH, Na(OAc)₃BH, 54%.

substitutions with this same tailpiece (e.g., compound **28**, Table 2) showed potency similar to that of the imidazole-substituted compounds.

The lack of Tpl2 potency for **14** and moderate potency for the corresponding methyl ether **15** indicated that a hydrogen bond donor group at the C-8 position was not tolerated. An

Scheme 3^a

^a Conditions: (a) (i) (methoxymethyl)triphenylphosphonium bromide, NaH, THF; (ii) MeOH, THF, HCl; (b) **9d**, THF, DMF, NaCNBH₃, HOAc, 22–50%; (c) 2-(1*H*-imidazol-5-yl)acetic acid, BOP, NMM, DMF; (d) (i) *i*-PrMgCl, THF, hexafluoroacetone, or *i*-PrMgCl, THF, *tert*-butyl 4-oxopiperidine-1-carboxylate; (ii) *i*-PrMgCl, THF, DMF, 19–22%; (e) (i) TFA, CH₂Cl₂ (1:3), 2 h, 100%; (ii) acetone, THF, EtOH, NaCNBH₃ 90%.

Scheme 4^a

^a Conditions: (a) SO₂Cl₂, CHCl₃, 0 °C to reflux, 2 h, 94%; (b) formamide, H₂O, 5–30%; (c) (i) LAH, THF, 70–90%; (d) MnO₂, acetone–CH₂Cl₂, 30–50%; (e) **9c** or **9d**, THF, DMF, NaCNBH₃, HOAc, 30–55%; (f) (i) *N,N*-dimethylprop-2-yn-1-amine, PdCl₂(PPh₃)₂, DMF, 90 °C, 2.5 h; (ii) Pd/C, H₂, THF, 3 h; (iii) 6 N HCl, MeOH, reflux, 12 h.

interesting SAR result was seen with the sulfoxide **16** and the sulfone **17**: the sulfoxide was nearly 10-fold more potent. This may be attributed to a potential repulsive steric or electronic interaction in that binding region due to the additional oxygen

atom on the sulfone. Alternatively, or in addition to the lack of an additional oxygen atom, the sulfone must be capable of adapting a favorable conformation unavailable to the sulfone **17** or the amide **18**. The smaller ketone analogue **19** was

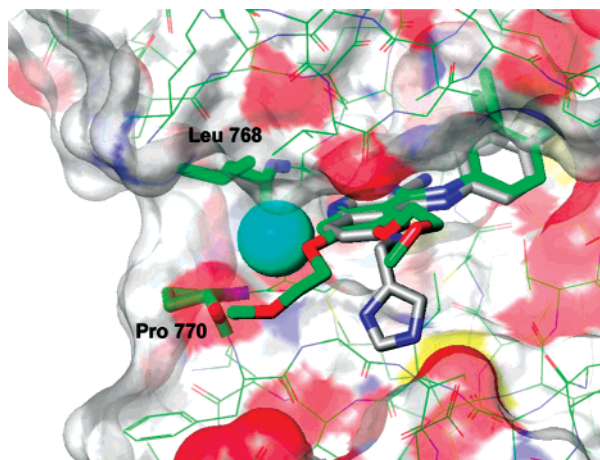


Figure 1. Structure of EGFR with erlotinib bound shown colored by element with carbons in green and a translucent molecular surface on the protein. A model of compound **2** bound to EGFR is also shown in thick lines colored by element with carbons in gray. A calculated optimal position for methyl in the binding site is shown in a space filling model in cyan. Glide XP^{38,39} (Schrodinger, Inc., New York, NY) was used to dock compound **2** into the unliganded binding site of EGFR, and MCSS^{40,41} was used to calculate optimal methyl positions in the binding site.

comparable in potency to the sulfoxide **16** but lacked the selectivity seen for the halogenated analogues. The diols **20** and **21** were also 10-fold less potent than **10–12**, which may be a result of negative steric interactions.

Having discovered that C-8 substitution with halogen maintained potency and improved selectivity, we varied the C-6 tailpiece portion of the cyanoquinoline scaffold in order to further improve enzymatic and cellular potency. In addition, we attempted to address the potential metabolic liability of compound **11**. In vitro metabolic studies with **11** indicated that the compound was moderately metabolized in rat ($t_{1/2}$ = 35 min), mouse ($t_{1/2}$ = 23 min), and human ($t_{1/2}$ = 31 min) liver microsomes with N-dealkylation, desaturation, and hydroxylation being the major metabolic pathways (Figure 2). We therefore chose C-6 replacements with more sterically demanding groups, longer carbon tethers, or groups with different electronic properties to reduce the metabolism at this position.

As seen previously in studies with the related 1,7-naphthyridine-3-carbonitrile series¹⁸ and shown here in Table 2, enzymatic potency was sensitive to C-6 tailpiece changes. Compared with imidazole tailpiece analogue **11**, pyridyl **28** and pyridyl N-oxide **33** had similar enzymatic potencies while pyrazoles **24–26**, the imidazopyridine **27**, the diol **34**, the carboxylic acid **36**, and the amide **37** were an order of magnitude less potent. Compound **28** showed reduced potency in cells and whole blood. The tetrazole analogue **35** was the most potent analogue in the enzymatic assay; however, the ionization of the tetrazole ring most likely rendered this compound much less potent in cellular and blood assays (solubility⁴² of 0 $\mu\text{g/mL}$; PAMPA⁴² permeability of 0 at pH 7.4). Large substituents on the pyridyl ring of compounds **29**, **30**, and **32** were not favored, while smaller groups such as methyl (**31**) did not offer an advantage in potency. The one-carbon-atom homologated derivative **22** was 13-fold less active than **12**, and the imidazolylacetamide **23** was much less potent. Two triazole-containing analogues **51** and **55** had potencies comparable to those of compounds **11**, **12**, **28**, and **33** in the enzyme assay with improved potency in whole blood and were studied further (see below).

We further investigated the effect of substitution patterns on the imidazole tailpiece analogues and evaluated the resulting compounds in cellular and blood assays for the functional ability to inhibit the release of TNF- α with the goal of identifying several compounds for in vivo evaluation. A select group of these analogues, with either Cl or Br at the C-8 position, is shown in Table 3. In the enzymatic assay, N-1', C-5' analogues were the most potent followed C-2' substituted analogues. Substitution at the C-2' position alone reduced Tpl2 inhibition. For example, compounds **39–42**, **44**, **49**, and **50** were slightly to moderately more potent than compounds **45–48**. Although imidazole tailpiece analogues with substituted basic amino groups had improved solubility (36 $\mu\text{g/mL}$ for **43** and 69 $\mu\text{g/mL}$ for **49** compared to 2 $\mu\text{g/mL}$ for **12**), this property enhancement did not translate into increased potency in cells and blood. All compounds in Table 3 had IC₅₀ values of >5 μM against EGFR kinase autophosphorylation in A431 cells.

Selectivity. After identification of compounds with in vitro potency and selectivity for EGFR, seven compounds were chosen for further screening against a panel of kinases and for cytochrome P450 inhibition (3A4 isozyme). The panel included a number of serine–threonine kinases and kinases involved with TNF- α production (p38 α , MK2, IKK β , Jnk1) kinases with diverse biological functions and kinases that were deemed undesirable because of possible off-target toxicities (Table 4). Compounds **10**, **11**, **33**, **40**, **44**, **51**, and **55** demonstrated 200- to 1000-fold selectivity for Tpl2 in this panel and had moderate CYP3A4 inhibition.

Pharmacokinetics. Compounds **11**, **33**, **39**, **40**, **44**, **51**, and **55** were further evaluated for pharmacokinetic profiling in rats. Data for individual oral (po) and intravenous (iv) dosing parameters are shown in Table 5. Compounds **11** and **51** had moderate plasma clearance and moderate volumes of distribution, while compounds **33**, **40**, and **44** had high plasma clearance and moderate (**33**) or high (**40** and **44**) volumes of distribution. Compounds **11**, **33**, **51**, and **55** exhibited low oral bioavailability, possibly due to low aqueous solubility and/or permeability and/or a high first-pass effect. With the exception of **55**, all had solubilities of <10 $\mu\text{g/mL}$ and PAMPA⁴² permeability of <5.0 $\times 10^{-6}$ cm/s at pH 7.4. In contrast, compound **44** had higher oral bioavailability (42%). Compound **44** was advanced for an in vivo oral activity study in a rat model of TNF- α production.

In Vivo Study. Compound **44** was examined for the ability to inhibit the production of TNF- α in the LPS-induced rat model at an oral dose of 50 mg/kg. Compound **44** exhibited a statistically significant inhibition of TNF- α in female Sprague-Dawley rats (Figure 3). In this same study compound **3** showed an 85% inhibition of TNF- α , consistent with a previously determined ED₅₀ of 5 mg/kg in the corresponding mouse model.⁴ The average plasma concentration of compound **44** at 60 and 150 min postchallenge were 1.1 and 0.55 μM , respectively, which was closer to the cellular inhibition concentration of **44** (0.67 μM) than to the blood inhibition concentration (6.3 μM). Further biophysical and animal model studies will help to discern the pharmacodynamic relationship of potency in blood, plasma concentration, and in vivo efficacy.⁴³

Conclusions

Using X-ray crystallographic data and molecular modeling, we discovered a key structural addition that resulted in an improved selectivity profile for the cyanoquinoline series.

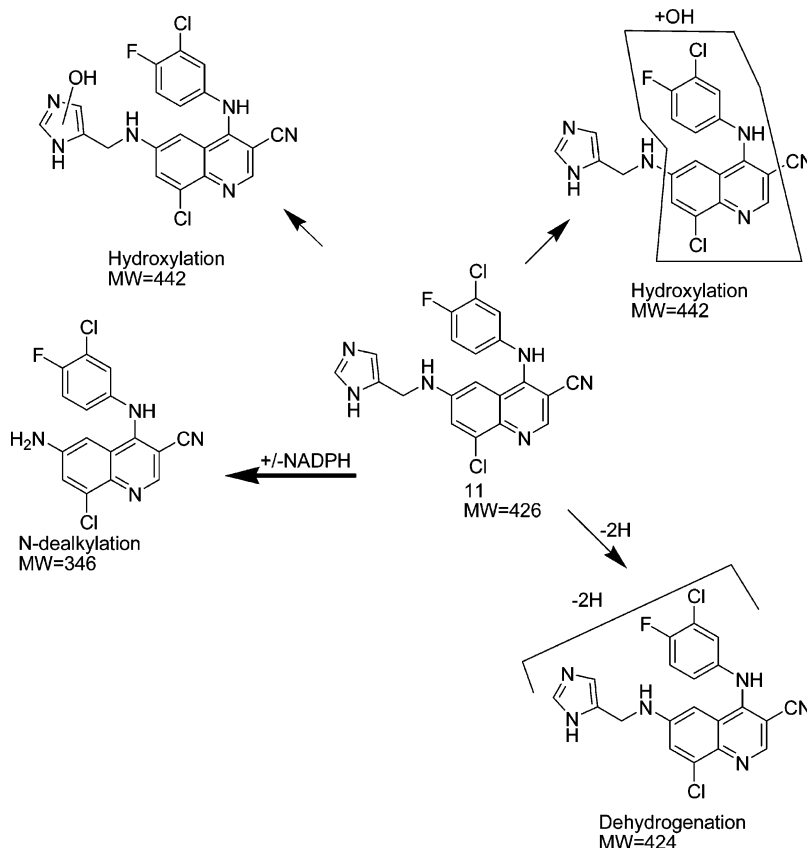


Figure 2. In vitro metabolic pathways of **11**.

Table 4. Selectivity Profiles of Compounds **10**, **11**, **33**, **40**, **44**, **51**, and **55**

kinase	IC ₅₀ (μM) ^a						
	10	11	33	40	44	51	55
p38α	>40	11.2	>40	>40	>40	3.6	>40
CAMKII	>40	>40	>40	>40	>40	>100	5.6
PKCα	>40	23.4	>40	>40	>40	>100	>40
PKA	10.3	40.1	>40	76.1	>100	>100	>40
MK2	>40	40.2	40.1	62.7	87.1	35.8	>40
Src	20	19.9	>40	>40	>40	40.8	>40
S6K	>40	>100	>40	18.8	83.1	>100	>40
Jnk1					31.0		>40
GSKβ					>10		
LYN					>30		
BTK					>10		
PKCθ					>50		
LCK					4.5		
CYP3A4	96% @ 3 μM	9.0	18.0	16.0	15.0	NT ^b	66% @ 3 μM

^a Average of at least two runs performed in duplicate with a standard deviation of 20%. ^b NT: not tested.

Introduction of functionality at the C-8 position resulted in reduced inhibition of other kinases including EGFR kinase. Further SAR investigations provided several quinoline-3-carbonitriles with improved potency in human blood (**11**, **33**, **40**, **44**, **51**, and **55**), one of which (**44**) had acceptable pharmacokinetic properties, including oral bioavailability. Compound **44** inhibited the LPS-induced production of TNF-α in the rat model. These results suggest that compounds in this series may have potential as therapeutic agents for the treatment of rheumatoid arthritis and other inflammatory diseases. The findings reported here provided insights into further lead optimization and in vivo studies, which will be reported in due course.

Table 5. Mean Pharmacokinetic Parameters of Compounds **11**, **33**, **40**, **44**, **51**, and **55** after iv or po Dosing in Female Sprague-Dawley Rats^a

parameters	11	33	40	44	51	55
iv Cl _p (mL min ⁻¹ kg ⁻¹)	19	38	>100	50	29	NT ^b
iv V _{ss} (L kg ⁻¹)	2.4	1.6	12.2	14.1	2.7	NT ^b
iv t _{1/2} (h)	4.4	0.8	1.2	5.0	3.2	NT ^b
po AUC _{0-inf} dose ⁻¹ (ng h kg mg ⁻¹ mL ⁻¹)	24	< 10	NT ^b	142	< 10	< 10
po C _{max}	61	< 10	NT ^b	511	34	< 10
po t _{1/2} (h)	8.1	4.2	NT ^b	4.0	3.9	2.6
F (%)	2	< 1	NT ^b	42	2	NT ^b

^a n = 3 animals per study. For iv: dosed 5 mg/kg in 50% DMSO, 50% PEG200 solution. For po: dosed 25 mg/kg in 2% Tween, 0.5% methylcellulose, 0.06% lactic acid in water. ^b NT: not tested.

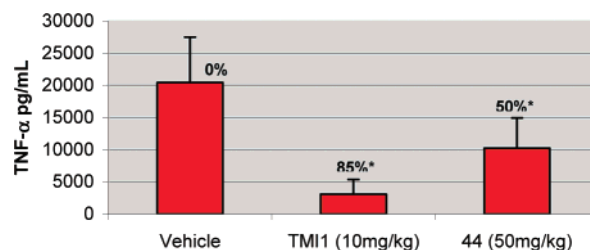


Figure 3. Compounds **44** and TMI-1 tested in LPS-induced TNF-α production in female Sprague-Dawley rats. Animals (average, 200 g) were pretreated (po) with **44** at 50 mg/kg and TMI-1 at 10 mg/kg. After 1 h the rats were challenged with LPS (iv, 1 mg/kg). Postchallenge (10 and 90 min) blood was drawn to determine compound plasma levels. TNF-α levels in plasma were measured by CBA: (*) *p* < 0.0015 by Student *T*-test.

Experimental Section

Chemistry. Reactions were run using commercially available starting materials and solvents without further purification. Proton NMR spectra were recorded at 400 MHz on a Bruker AV-400 spectrometer using TMS (δ 0.0) as a reference. Combustion

analyses were obtained using a Perkin-Elmer series II 2400 CHNS/O analyzer. CHN analyses were carried out by Robertson-Microlit. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ± 0.4 of the theoretical values. Low-resolution mass spectra were obtained using a Micromass Platform electrospray ionization quadrupole mass spectrometer. High-resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 T superconducting magnet (Magnex Scientific Ltd., U.K.) and an external Bruker APOLLO electrospray ionization (ESI) source. The microwave procedures were carried out with a Biotage microwave. Preparative HPLC was run using a Waters reverse-phase preparative HPLC with Xterra C18 5 μ M, 30 mm $\times$ 100 mm column. The flow rate was 40 mL/min. Mobile phase A was water; mobile phase B was CH₃CN. Triethylamine was used as a modifier. Purity in the two solvent systems [H₂O–CH₃CN (HPLC method 1) and H₂O–MeOH (HPLC method 2)] was determined using an Agilent 1100 HPLC instrument.

The literature procedure for the synthesis of 4-anilinoquinoline-3-carbonitriles from nitroanilines (ref 21) was used to synthesize intermediates **8b–k** from substituted nitroanilines **4a–g**. See below for additional modifications and details.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (12). In a microwave vial, **7d** (4.00 g, 12.8 mmol) and 3-chloro-4-fluoroaniline (2.05 g, 14.1 mmol) were dissolved in 20 mL of EtOH. The vial was sealed and heated in a microwave reactor at 140 °C for 10 min. The cap was then removed, tin chloride dihydrate (16.00 g, 71 mmol) was added, and the vial was resealed and heated at 110 °C for 5 min. The contents of the vial were rinsed with EtOH into 300 mL of ice–water and neutralized with aqueous saturated NaHCO₃. The orange suspension was extracted with EtOAc (4 $\times$ 300 mL). The combined organic layers were dried over anhydrous MgSO₄, filtered, and evaporated, and the crude product was purified by flash chromatography over silica gel (3–5% MeOH in CH₂Cl₂) to give **9d** (2.50 g, 50% yield) as a brown solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 6.05 (s, 2 H), 7.29–7.34 (m, 2 H), 7.49–7.55 (m, 2 H), 7.77 (d, *J* = 2.3 Hz, 1 H), 8.53 (s, 1 H), 9.60 (s, 1 H); HRMS (ESI⁺) calcd for C₁₆H₁₀BrClFN₄ (MH⁺) 390.9756, found 390.9754. In a 1 L flask, **9d** (2.50 g, 6.38 mmol) and 4(5)-imidazolecarboxaldehyde (0.65 g, 6.38 mmol) were dissolved in 80 mL of THF and 35 mL of MeOH and stirred overnight. The solution was then acidified to pH 4 with HOAc, NaCNBH₃ (0.58 g, 9.3 mmol) was added, and the mixture was allowed to stir overnight again. Solvent was then removed under reduced pressure, and the crude product was purified by flash chromatography over silica gel (6–9% MeOH in CH₂Cl₂) and trituration with 165 mL of boiling EtOH to give **12** as a bright-yellow powder (1.35 g, 45% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.26 (d, *J* = 5.1 Hz, 2 H), 6.67 (t, *J* = 5.2 Hz, 1 H), 7.05 (s, 1 H), 7.25 (d, *J* = 2.3 Hz, 1 H), 7.26–7.32 (m, 1 H), 7.45 (t, *J* = 9.0 Hz, 1 H), 7.53 (dd, *J* = 6.6, 2.5 Hz, 1 H), 7.62 (s, 1 H), 7.79 (d, *J* = 2.0 Hz, 1 H), 8.38 (s, 1 H), 9.47 (s, 1 H), 11.96 (br s, 1 H); HRMS (ESI⁺) calcd for C₂₀H₁₄BrClFN₆ (MH⁺) 471.0131, found 471.0140. HPLC method 1: 98.6%, 4.15 min. HPLC method 2: 98.8%, 4.81 min.

By use of this same general procedure, all the compounds in this study were synthesized from intermediates **8b–k** unless otherwise noted.

4-[(3-Chloro-4-fluorophenyl)amino]-8-fluoro-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (10). Intermediate **7b** (0.60 g, 2.38 mmol) was dissolved with 3-chloro-4-fluoroaniline (0.42 g, 2.86 mmol) in 10 mL of EtOH and heated at 65 °C for 4 h. After workup, **8b** (0.7 g, 82% yield) was obtained as a yellow solid: ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.51–7.58 (m, 1 H), 7.62 (t, *J* = 8.97 Hz, 1 H), 7.82 (dd, *J* = 6.44, 2.15 Hz, 1 H), 8.59 (dd, *J* = 10.11, 2.02 Hz, 1 H), 8.91 (s, 1 H), 9.52 (s, 1 H), 10.73 (s, 1 H); HRMS (ESI⁺) calcd for C₁₆H₇ClF₂N₄O₂ (MH⁺) 361.029 83, found 361.0294. Reduction of **8b** (0.7 g, 2.15 mmol) with tin chloride dihydrate (2.4 g, 10.7 mmol) in 30 mL of EtOH afforded **9b** (710 mg, 100% yield) as a light-brown solid: ¹H NMR

(400 MHz, DMSO-*d*₆) δ ppm 5.94 (s, 2 H), 6.98–7.10 (m, 2 H), 7.15–7.29 (m, 1 H), 7.35–7.48 (m, 2 H), 8.26–8.39 (m, 1 H), 9.45 (br, s, 1 H); HRMS (ESI⁺) calcd for C₁₆H₉ClF₂N₄ (MH⁺) 331.0556, found 331.0562. The procedure described above for the synthesis of **12** was followed, reacting **9b** (70 mg, 0.21 mmol) with 4(5)-imidazolecarboxaldehyde (28 mg, 0.3 mmol) and NaCNBH₃ (9 mg, 0.1 mmol) in 2 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give the product as a bright-yellow solid (26 mg, 31%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.19 (d, *J* = 5.05 Hz, 2 H), 6.61 (t, *J* = 5.05 Hz, 1 H), 6.95–7.07 (m, 2 H), 7.15 (d, *J* = 12.88, 2.02 Hz, 1 H), 7.19–7.28 (m, 1 H), 7.38 (t, *J* = 9.09 Hz, 1 H), 7.48 (dd, *J* = 6.57, 2.53 Hz, 1 H), 7.56 (d, *J* = 1.01 Hz, 1 H), 8.11 (s, 1 H), 8.23 (s, 1 H), 9.40 (s, 1 H); HRMS (ESI⁺) calcd for C₂₀H₁₃ClF₂N₆ (MH⁺) 411.093 10, found 411.0925. HPLC method 1: 95.9%, 3.88 min. HPLC method 2: 96.5%, 3.42 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (11). The procedure described above for the synthesis of **12** was followed, reacting **9c** (89 mg, 0.26 mmol) with 4(5)-imidazolecarboxaldehyde (28 mg, 0.29 mmol) and NaCNBH₃ (22 mg, 0.35 mmol) in 6 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **11** as a tan solid (65 mg, 60%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.26 (d, *J* = 5.05 Hz, 2 H), 6.67 (t, *J* = 5.43 Hz, 1 H), 7.05 (s, 1 H), 7.22 (d, *J* = 2.02 Hz, 1 H), 7.27–7.32 (m, 1 H), 7.45 (t, *J* = 8.97 Hz, 1 H), 7.53 (dd, *J* = 6.57, 2.78 Hz, 1 H), 7.57–7.63 (m, 2 H), 8.16 (s, 1 H), 8.38 (s, 1 H), 9.47 (s, 1 H); HRMS (ESI⁺) calcd for C₂₀H₁₃Cl₂FN₆ (MH⁺) 427.063 55, found 427.062. HPLC method 1: 99.3%, 4.01 min. HPLC method 2: 97.0%, 5.31 min. Anal. (C₂₀H₁₃Cl₂FN₆·HCO₂H) C, H, N: 53.29, 3.19, 17.76. Found: 52.84, 3.17, 17.59.

4-[(3-Chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]-8-(trifluoromethyl)quinoline-3-carbonitrile (13). In a 100 mL round-bottomed flask equipped with a condenser, **7e** (2.44 g, 8.1 mmol) was dissolved in 35 mL of EtOH, and 3-chloro-4-fluoroaniline (1.41 g, 9.7 mmol) was added in one portion. The reaction mixture was heated at reflux for 1 h and then stirred at room temperature overnight. The EtOH was removed under reduced pressure, the residue was then partitioned between 50 mL of ether and 25 mL pg aqueous saturated NaHCO₃ and stirred for 15 min. Partial concentration of the biphasic mixture gave a white solid that was filtered. The resulting solid was dried under vacuum overnight to give **8e** as a dark-yellow solid (3.3 g, 99%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.37–7.48 (m, 1 H), 7.53 (t, *J* = 8.97 Hz, 1 H), 7.71 (dd, *J* = 6.44, 2.40 Hz, 1 H), 8.79 (d, *J* = 2.27 Hz, 1 H), 8.87 (s, 1 H), 9.80 (d, *J* = 2.27 Hz, 1 H), 10.77 (s, 1 H); HRMS (ESI⁺) calcd for C₁₇H₇ClF₄N₄O₂ (MH⁺) 411.026 64, found 411.026. In a 100 mL round-bottomed flask equipped with a condenser, **8e** (1.65 g, 4.0 mmol) was dissolved in 50 mL of EtOH, and tin chloride dihydrate (4.53 g, 20.1 mmol) was added. The reaction mixture was heated at reflux for 1 h, then cooled to room temperature and poured into ice–water. The orange suspension was neutralized with aqueous saturated NaHCO₃ and extracted with CHCl₃ (3 $\times$ 150 mL) and EtOAc (2 $\times$ 150 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, filtered, and evaporated to give **9e** as a yellow solid (1.5 g, 98% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.92–4.12 (m, 1 H), 6.09 (s, 2 H), 7.15–7.31 (m, 1 H), 7.33–7.53 (m, 2 H), 7.70 (d, *J* = 2.27 Hz, 1 H), 8.44 (s, 1 H), 9.57 (s, 1 H); HRMS (ESI⁺) calcd for C₁₇H_{2d}ClF₄N₄ (MH⁺) 381.052 46, found 381.053. The procedure described above for the synthesis of **12** was followed, reacting **9e** (150 mg, 0.39 mmol) with 4(5)-imidazolecarboxaldehyde (75 mg, 0.79 mmol) and NaCNBH₃ (37 mg, 0.72 mmol) in 5 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **13** as a yellow solid (137 mg, 76%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.31 (d, *J* = 5.31 Hz, 2 H), 6.82–6.91 (m, *J* = 5.31, 5.31 Hz, 1 H), 7.08 (s, 1 H), 7.28–7.37 (m, 1 H), 7.41–7.50 (m, 2 H), 7.57 (dd, *J* = 6.69, 2.65 Hz, 1 H), 7.66 (d, *J* = 1.01 Hz, 1 H), 7.86 (d, *J* = 2.27 Hz, 1 H), 8.41 (s, 1 H), 9.55 (s, 1 H), 12.18 (s, 1 H); HRMS (ESI⁺) calcd for

C₂₁H₁₃ClF₄N₆ (MH⁺) 461.089 91, found 461.0903. HPLC method 1: 95.5%, 4.41 min. HPLC method 2: 95.7%, 5.34 min.

4-(3-Chloro-4-fluorophenylamino)-6-[(3H-imidazol-4-ylmethyl)amino]-8-methoxyquinoline-3-carbonitrile (15). The procedure for the synthesis of **12** was followed, reacting **9f** (190 mg, 0.55 mmol) with 4(5)-imidazolecarboxaldehyde (53 mg, 0.55 mmol) and NaCNBH₃ (24 mg, 0.39 mmol) in 8 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give the product as a solid (33 mg, 14%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.85 (s, 3 H), 4.22–4.27 (m, *J* = 4.29 Hz, 2 H), 6.34–6.41 (m, 1 H), 6.77 (d, *J* = 1.77 Hz, 1 H), 6.92 (s, 1 H), 7.05 (s, 1 H), 7.18–7.24 (m, 1 H), 7.38–7.45 (m, 2 H), 7.61 (d, *J* = 1.01 Hz, 1 H), 8.24 (s, 1 H), 9.21 (s, 1 H). HPLC method 1: 100.0%, 3.52 min. HPLC method 2: 100.0%, 2.85 min.

4-(3-Chloro-4-fluorophenylamino)-8-hydroxy-6-nitroquinoline-3-carbonitrile (8k). Intermediate **8f** (323 mg, 0.87 mmol), pyridine hydrochloride (130 mg, 1.12 mmol), and DMF (6 mL) were heated to 200 °C for 35 min in a microwave reactor. The crude product was purified by preparative HPLC and lyophilized to give **8k** as a solid (222 mg, 71%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.43 (dd, *J* = 6.82, 2.27 Hz, 1 H), 7.51 (t, *J* = 8.84 Hz, 1 H), 7.69 (d, *J* = 4.80 Hz, 1 H), 7.83 (s, 1 H), 7.83 (s, 1 H), 8.98 (s, 1 H), 10.40 (s, 1 H), 10.89 (s, 1 H); HRMS (ESI⁺) calcd for C₁₆H₈ClFN₄O₃ (MH⁺) 359.034 17, found 359.034.

6-[(1H-Imidazol-5-yl)methylamino]-4-(3-chloro-4-fluorophenylamino)-8-hydroxyquinoline-3-carbonitrile (14). The procedure for the synthesis of **12** was followed, reacting **8k** (176 mg, 0.49 mmol) with SnCl₂·2H₂O (547 mg, 2.42 mmol) in EtOH (5 mL) to give **9k** (160 mg, 99%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 5.64 (s, 2 H), 6.59–6.67 (m, 2 H), 7.09–7.17 (m, 1 H), 7.32–7.43 (m, 2 H), 8.26 (s, 1 H), 9.24 (s, 1 H), 9.56 (s, 1 H); HRMS (ESI⁺) calcd for C₁₆H₁₀ClFN₄O (MH⁺) 329.059 99, found 329.0601. Intermediate **9k** (122 mg, 0.37 mmol) was reacted with 4(5)-imidazolecarboxaldehyde (54 mg, 0.56 mmol) and NaCNBH₃ (30 mg, 0.48 mmol) in 10 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **14** as a tan solid (59 mg, 39%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.16 (d, *J* = 4.80 Hz, 2 H), 6.25–6.33 (m, 1 H), 6.65–6.71 (m, 1 H), 6.94 (s, 1 H), 7.13–7.22 (m, 1 H), 7.33–7.42 (m, 2 H), 7.53 (d, *J* = 1.26 Hz, 1 H), 8.11 (s, 1 H), 8.18 (s, 1 H), 9.20 (s, 1 H), 9.42 (s, 1 H); HRMS (ESI⁺) calcd for C₂₀H₁₄ClFN₆O (MH⁺) 409.097 44, found 409.0975. HPLC method 1: 91.4%, 3.53 min (broad). HPLC method 2: 100.0%, 2.96 min.

4-[(3-Chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]-8-(methylsulfinyl)quinoline-3-carbonitrile (16). In a 50 mL round-bottomed flask, **6b** (0.50 g, 2.14 mmol) was dissolved in 9 mL of DMPU. MeSNa (0.57 g, 8.1 mmol) was added, and the reaction mixture was stirred at room temperature overnight and poured into ice–water. An amount of 1 N HCl was added slowly until a copious precipitate formed. The mixture was filtered and the solid was washed with water to give a black solid. The crude product was purified by preparative HPLC to give **6h** as a yellow solid (0.21 g, 38%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.72 (s, 3 H), 8.39 (d, *J* = 2.27 Hz, 1 H), 8.54–8.76 (m, 2 H), 12.45 (s, 1 H). In a 100 mL round-bottomed flask equipped with a condenser, **6h** (0.19 g, 0.71 mmol) was dissolved in 6 mL of POCl₃ and heated at reflux for 5 h. The reaction mixture was then stirred at room temperature overnight, and then POCl₃ was removed under reduced pressure. Ice chips were added to the residue, and then aqueous saturated NaHCO₃ solution was added carefully. The mixture was filtered and dried under high vacuum overnight to give **7h** as a brown solid (0.18 g, 90% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.68 (s, 3 H), 8.21 (s, 1 H), 8.71 (s, 1 H), 9.40 (s, 1 H). The procedure described above for the synthesis of **12** was followed convert **7h** to **8h**, isolated as a yellow solid (0.18 g, 72% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.60 (s, 3 H), 7.41–7.48 (m, 1 H), 7.53 (t, *J* = 8.97 Hz, 1 H), 7.71 (dd, *J* = 6.69, 2.40 Hz, 1 H), 8.11 (d, *J* = 2.02 Hz, 1 H), 8.78 (s, 1 H), 9.26 (d, *J* = 2.27 Hz, 1 H), 10.52 (s, 1 H). To a solution of **8h** (50.0 mg, 0.13 mmol) in 2 mL of CH₂Cl₂ was added a solution of *m*-CPBA (28.8 mg, 0.13 mmol) in 2 mL of CH₂Cl₂ slowly

through an addition funnel at –5 °C. The reaction mixture was then stirred at –5 to 0 °C for 1.5 h, until TLC analysis showed complete disappearance of starting material. Aqueous saturated NaHCO₃ solution (5 mL) was added to the reaction mixture at 0 °C. The organic layer was washed with aqueous saturated NaHCO₃ solution and then washed with brine and concentrated to give **8i** as a yellow solid (56 mg, 100% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.95 (s, 3 H), 7.38 (s, 1 H), 7.46–7.57 (m, 1 H), 7.65 (s, 1 H), 8.73 (d, *J* = 2.27 Hz, 2 H), 9.65 (d, *J* = 3.03 Hz, 1 H), 10.82 (s, 1 H). Reduction of **8i** using the procedure above for **12** gave **9i** as a dark-yellow solid (45 mg, 91% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.42 (s, 3 H), 5.80 (s, 2 H), 6.88 (d, *J* = 2.02 Hz, 1 H), 7.01 (d, *J* = 2.02 Hz, 1 H), 7.13–7.16 (m, 1 H), 7.34–7.40 (m, 2 H), 8.33 (s, 1 H), 9.34 (s, 1 H). Reductive alkylation according to the procedure for **12**, reacting **9i** with 4(5)-imidazolecarboxaldehyde (21 mg, 0.22 mmol) and NaCNBH₃ (5 mg, 0.08 mmol) in 3 mL of EtOH, gave the product **16** as a tan solid (28 mg, 53%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.39 (s, 3 H), 4.25 (d, *J* = 5.05 Hz, 2 H), 6.48 (d, 1 H), 6.95 (d, *J* = 1.77 Hz, 1 H), 7.05 (s, 1 H), 7.18 (d, *J* = 2.02 Hz, 1 H), 7.22–7.28 (m, 1 H), 7.38–7.51 (m, 2 H), 7.62 (d, *J* = 1.01 Hz, 1 H), 8.19 (s, 1 H), 8.30 (s, 1 H), 9.34 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₆ClFN₆S (MH⁺) 439.090 24, found 439.0898. HPLC method 1: 99.7%, 4.04 min. HPLC method 2: 98.5%, 4.69 min.

4-[(3-Chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]-8-(methylsulfonyl)quinoline-3-carbonitrile (17). To a solution of **8h** (70 mg, 0.18 mmol) in 2 mL of THF was added a solution of *m*-CPBA (100 mg, 0.45 mmol) in 3 mL of THF slowly through an addition funnel at 0 °C. The reaction mixture was then stirred at 0 °C for 30 min, then at room temperature for 2 days. Aqueous saturated NaHCO₃ solution (5 mL) was added to the reaction mixture at 0 °C, and then 15 mL of EtOAc was added. The organic layer was washed with aqueous saturated NaHCO₃ solution and then washed with brine and concentrated to give **8j** as a light-brown solid (70 mg, 93% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.47–3.61 (m, 3 H), 7.45–7.53 (m, 2 H), 7.56–7.67 (m, 2 H), 7.86–7.89 (m, 3 H). Reduction of **8j** using the procedure above for **12** gave **9j** isolated as a dark-yellow solid (50 mg, 77% yield). Reductive alkylation according to the procedure for **12**, reacting **9j** with 4(5)-imidazolecarboxaldehyde (20 mg, 0.22 mmol) and NaCNBH₃ (5 mg, 0.08 mmol) in 3 mL of EtOH, gave **17** as a yellow solid (9 mg, 15%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.52 (s, 3 H), 4.32 (d, *J* = 5.05 Hz, 2 H), 7.04–7.08 (m, 1 H), 7.11 (t, *J* = 5.81 Hz, 1 H), 7.31–7.37 (m, 1 H), 7.47 (t, *J* = 8.97 Hz, 1 H), 7.51 (d, *J* = 2.27 Hz, 1 H), 7.58 (dd, *J* = 6.57, 2.53 Hz, 1 H), 7.61 (d, *J* = 1.01 Hz, 1 H), 8.12 (d, *J* = 2.27 Hz, 1 H), 8.28 (s, 1 H), 8.45 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₆ClFN₆O₂S (MH⁺) 471.080 07, found 471.0796. HPLC method 1: 95.0%, 4.02 min. HPLC method 2: 96.0%, 4.04 min.

4-[(3-Chloro-4-fluorophenyl)amino]-3-cyano-6-[(1H-imidazol-5-ylmethyl)amino]-N,N-dimethylquinoline-8-carboxamide (18). In a 2 L flask, 5-nitroanthranilic acid (100 g, 0.55 mol) and dimethylamine hydrochloride (50 g, 0.60 mol) were dissolved in 500 mL of DMF. Once both reagents had dissolved, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (268 g, 0.604 mol) was added, followed by 4-methylmorpholine (134 mL, 122 g, 1.21 mol). The mixture was stirred at room temperature overnight and then poured into 5 L of water and stirred vigorously until the suspension was evenly mixed. The precipitate was collected by suction filtration, washed with water, and dried under vacuum to give pure product 2-amino-N,N-dimethyl-5-nitrobenzamide **4g** as a yellow powder (103 g, 89% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.93 (br s, 6 H), 6.69 (s, 2 H), 6.75 (d, *J* = 9.4 Hz, 1 H), 7.88 (d, *J* = 2.8 Hz, 1 H), 7.98 (dd, *J* = 9.1, 2.8 Hz, 1 H). In a 2 L flask, **4g** (103 g, 0.55 mol) and ethyl (ethoxymethylene)-cyanoacetate (188 g, 1.1 mol) were dissolved in 580 mL of DMF, and Cs₂CO₃ (362 g, 1.1 mol) was added. The mixture was heated to 45 °C for 2 h and then cooled to room temperature, stirred overnight, and poured into 5 L of water. The yellow precipitate was collected by suction filtration, washed generously three times with water, and dried under vacuum for 2 days to give pure ethyl

2-cyano-3-(2-(dimethylcarbamoyl)-4-nitrophenylamino)acrylate **5g** (174 g, 94% yield): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 1.28 (t, $J = 7.1$ Hz, 3 H), 2.94 (s, 3 H), 3.06 (s, 3 H), 4.26 (q, $J = 7.2$ Hz, 2 H), 8.00 (d, $J = 9.4$ Hz, 1 H), 8.27 (d, $J = 2.5$ Hz, 1 H), 8.30–8.37 (m, 1 H), 8.72 (d, $J = 12.9$ Hz, 1 H), 11.34 (d, $J = 13.4$ Hz, 1 H). In a 2 L three-necked round-bottomed flask fitted with inert gas inlets/outlets and an internal device to monitor reaction temperature, **5g** (26.9 g, 80.9 mmol) was suspended in 1 L of Dowtherm A. Nitrogen gas was bubbled through each suspension for 40 min. The suspension was heated to 259 °C overnight, with inert gas gently flowing over the reaction mixture. After the mixture was allowed to cool to 50 °C, the contents were poured into 2.4 L of hexanes, stirred vigorously, and filtered, and the brown precipitate was washed twice with hexanes and once with EtOH and dried under vacuum overnight to give **6g** as a brown powder of sufficient purity to be used in the next step (28 g, 61% yield): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.86 (s, 3 H), 3.09 (s, 3 H), 8.48 (d, $J = 2.5$ Hz, 1 H), 8.70 (s, 1 H), 8.85 (d, $J = 2.5$ Hz, 1 H), 12.61 (br s, 1 H). In a 1 L flask fitted with an addition funnel, **6g** (28 g, 98 mmol) was suspended in 200 mL of dichloroethane, and 1 mL of DMF was added. Oxalyl chloride (17 mL, 25 g, 0.20 mol) was then added dropwise via the addition funnel. After the addition was complete, the addition funnel was replaced with a reflux condenser, and the mixture was refluxed for 2 h. It was then allowed to cool to room temperature, and the solvent and excess oxalyl chloride were removed under reduced pressure. The residue was dissolved in CHCl_3 and passed over a short column of silica gel in a Büchner funnel, eluting with additional CHCl_3 . Evaporation of the solvent gave **7g** as a brown powder (13.8 g, 46% yield): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.69 (s, 3 H), 3.11 (s, 3 H), 8.64 (d, $J = 2.3$ Hz, 1 H), 9.06 (d, $J = 2.5$ Hz, 1 H), 9.43 (s, 1 H). The procedure described above for **12** gave **9g** (0.30 g, 0.78 mmol), which was reacted with 4(5)-imidazolecarboxaldehyde (75 mg, 0.78 mmol) and NaCNBH_3 (33 mg, 0.52 mmol). The crude product was purified by preparative HPLC and lyophilized to give **18** as a fluffy, bright-yellow solid (96 mg, 26% yield): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.67 (s, 3 H), 3.03 (s, 3 H), 4.28 (d, $J = 5.1$ Hz, 2 H), 6.62 (t, $J = 5.3$ Hz, 1 H), 7.05 (s, 1 H), 7.19–7.33 (m, 3 H), 7.44 (t, $J = 9.1$ Hz, 1 H), 7.53 (dd, $J = 6.6, 2.8$ Hz, 1 H), 7.62 (s, 1 H), 8.31 (s, 1 H), 9.47 (br s, 1 H); HRMS (ESI $^+$) calcd for $\text{C}_{23}\text{H}_{20}\text{ClFN}_7\text{O}$ (MH^+) 464.1397, found 464.1401. HPLC method 1: 98.6%, 3.86 min. HPLC method 2: 98.8%, 3.35 min. Anal. ($\text{C}_{23}\text{H}_{20}\text{ClFN}_7\text{O}$) C, H, N.

8-Acetyl-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (19). The iodide **9n** was prepared in a similar fashion to bromide **12** as shown in Scheme 1. To a microwave vial was added **9n** (50 mg, 0.10 mmol) tributyl-(1-ethoxyvinyl)stannane (52.2 mg, 0.14 mmol), and $\text{PdCl}_2(\text{PPh}_3)_2$ (6.8 mg, 0.01 mmol) in 2 mL of DMF. The vial was heated in a microwave reactor at 180 °C for 30 min. When the sample was cooled, 1 N HCl (1 mL) was added to the vial and the reaction mixture was stirred for 3 h at room temperature. The mixture was filtered through a pad of Celite and concentrated. The residue was purified via preparative HPLC to give the product as a yellow solid (5.8 mg, 14% overall yield): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.69 (s, 3 H), 4.28 (d, $J = 5.05$ Hz, 2 H), 6.73 (t, 1 H), 7.04 (s, 1 H), 7.27–7.35 (m, 2 H), 7.38–7.48 (m, 2 H), 7.52–7.55 (m, 1 H), 7.61 (s, 1 H), 8.17 (s, 1 H), 8.36 (s, 1 H), 9.49 (s, 1 H), HRMS: calcd for $\text{C}_{22}\text{H}_{16}\text{ClFN}_6\text{O} + \text{H}^+$, 435.113 09; found (ESI-FTMS, $[\text{M} + \text{H}]^{1+}$), 435.114. HPLC method 1: 98.0%, 4.55 min. HPLC method 2: 99.0%, 3.92 min.

4-[(3-Chloro-4-fluorophenyl)amino]-8-(2,3-dihydroxypropyl)-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (20). In a 20 mL microwave vial were added **9d** (500 mg, 1.3 mmol), DMF (15 mL), allyltributylstannane (630 mg, 1.9 mmol), and $\text{PdCl}_2(\text{PPh}_3)_2$ (100 mg, 0.13 mmol). The reaction mixture was heated in a microwave reactor at 180 °C for 30 min. The reaction mixture was diluted with water, and the aqueous layer was extracted three times with EtOAc. The combined EtOAc extracts were dried over MgSO_4 and concentrated. The residue was purified via preparative

HPLC and lyophilized to give **9m** as a yellow solid (214 mg, 48%). In a 15 mL round-bottom flask were added **9m** (214 mg, 0.6 mmol), dichloromethane (3 mL), pyridine (119 mg, 1.5 mmol), and trifluoroacetic anhydride (265 mg, 1.3 mmol) in that order. The mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with water, and the aqueous layer was washed with EtOAc (3 $\times$). The combined EtOAc extracts were dried over MgSO_4 and concentrated. The crude acetamide (258 mg, 0.48 mmol) was dissolved in a 1:1 mixture of acetone and water (2 mL). To this were added OsO_4 (2.5 wt % in *t*-BuOH; 293 mg, 0.03 mmol) and NMO (112 mg, 0.96 mmol). The reaction mixture was stirred at room temperature for 4 h. To this was added 1 mL of 5 M LiOH solution, and the mixture was stirred for another 3 h. The heterogeneous mixture was filtered through a pad of Celite and washed with acetone and water. The filtrate was diluted with EtOAc, and the two layers were separated. The aqueous reaction mixture was extracted with EtOAc (2 $\times$). The combined EtOAc extracts were dried over MgSO_4 and concentrated to give crude **9n**. The residue (186 mg, 0.48 mmol) was dissolved in EtOH (2 mL), and 1H-imidazole-5-carbaldehyde (51 mg, 0.53 mmol) was added, followed by HOAc to bring the pH of the solution to 4. The mixture was stirred for 15 min. $\text{Na}(\text{OAc})_3\text{BH}$ (204 mg, 0.96 mmol) was then added, and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated and the residue was purified via preparative HPLC and lyophilized to give **20** as a yellow solid (30 mg, 13%): ^1H NMR (400 MHz, MeOD) δ ppm 2.02–2.04 (m, 1 H), 2.66 (s, 1 H), 3.14 (dd, $J = 13.77, 7.71$ Hz, 1 H), 3.35 (s, 1 H), 3.52 (d, $J = 5.31$ Hz, 2 H), 3.94–4.02 (m, 1 H), 4.58 (s, 2 H), 7.13 (s, 1 H), 7.21–7.35 (m, 3 H), 7.38–7.47 (m, 2 H), 8.41 (s, 1 H), 8.81 (s, 1 H). HPLC method 1: 96.9%, 3.53 min. HPLC method 2: 97.7%, 3.01 min.

4-[(3-Chloro-4-fluorophenyl)amino]-8-(2,3-dihydroxypropoxy)-6-nitroquinoline-3-carbonitrile (8l). To a mixture of **8k** (274 mg, 0.77 mmol) and potassium carbonate (218 mg, 1.58 mmol) in DMF (7 mL) was added allyl bromide (0.073 mL, 0.84 mmol). After being stirred for 12 h at room temperature, the mixture was filtered, concentrated, and purified via preparative HPLC to give 4-[(3-chloro-4-fluorophenyl)amino]-8-allyloxy-6-nitroquinoline-3-carbonitrile as a tan solid (229 mg, 75%): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 4.92 (d, $J = 5.31$ Hz, 2 H), 5.36 (dd, $J = 10.48, 1.64$ Hz, 1 H), 5.49–5.57 (m, 1 H), 6.11–6.23 (m, 1 H), 7.36 (s, 1 H), 7.49 (t, $J = 8.97$ Hz, 1 H), 7.62 (s, 1 H), 7.95 (d, $J = 2.27$ Hz, 1 H), 8.68 (s, 1 H), 9.08 (s, 1 H), 10.45 (s, 1 H). To a mixture of this intermediate in acetone (16 mL) and water (6 mL) was added 4-methylmorpholine *N*-oxide (235 mg, 2.01 mmol) followed by OsO_4 (0.5 mL, 2.5% in *t*-BuOH). After being stirred for 12 h at room temperature, the mixture was diluted with EtOAc, washed with water, dried over Na_2SO_4 , and concentrated. The crude product was purified via preparative HPLC to give **8l** as a tan solid (71 mg, 47%): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.07 (s, 1 H), 4.33 (d, $J = 5.56$ Hz, 2 H), 5.03 (s, 2 H), 6.60 (s, 1 H), 6.83 (s, 1 H), 7.06 (s, 1 H), 7.19–7.26 (m, 2 H), 7.33–7.50 (m, 2 H), 7.70 (d, $J = 9.09$ Hz, 1 H), 8.33 (s, 1 H), 9.29 (s, 1 H); HRMS (ESI $^+$) calcd for $\text{C}_{19}\text{H}_{14}\text{ClFN}_4\text{O}_5$ (MH^+) 433.070 95, found 433.0705. HPLC method 1: 97.1%, 4.54 min. HPLC method 2: 99.6%, 5.69 min.

4-[(3-Chloro-4-fluorophenyl)amino]-8-(2,3-dihydroxypropoxy)-6-[(1H-imidazol-5-ylmethyl)amino]quinoline-3-carbonitrile (21). Reduction and reductive alkylation of **8l** using the method for the synthesis of **12** gave **21** as an orange solid (10 mg, 25%): ^1H NMR (400 MHz, DMSO- d_6) δ ppm 2.65–2.69 (m, 1 H), 3.49–3.55 (m, 2 H), 3.98 (dd, $J = 9.09, 6.32$ Hz, 2 H), 4.08–4.13 (m, 1 H), 4.22–4.27 (m, 2 H), 4.72–4.77 (m, 1 H), 6.32–6.42 (m, 1 H), 6.82 (s, 1 H), 6.97 (s, 1 H), 7.03 (s, 1 H), 7.22 (d, $J = 9.09$ Hz, 1 H), 7.41 (d, $J = 9.09$ Hz, 2 H), 7.61 (s, 1 H), 8.25–8.34 (m, 2 H), 9.25 (s, 1 H); HRMS (ESI $^+$) calcd for $\text{C}_{23}\text{H}_{20}\text{ClFN}_6\text{O}_3$ (MH^+) 483.134 22, found 483.1328. HPLC method 1: 98.9%, 3.24 min. HPLC method 2: 98.3%, 2.25 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[2-(1H-imidazol-5-yl)ethyl]amino]quinoline-3-carbonitrile (22). In a mi-

crowave vial, methoxymethyltriphenylphosphonium bromide (300 mg, 0.77 mmol) was dissolved in 4 mL of THF, and NaH (46 mg, 0.46 mmol) was added. The vial was sealed and heated in a microwave reactor at 80 °C for 5 min. Then tritylimidazolealdehyde (104 mg, 0.31 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The solvent was removed and the crude product was purified by flash chromatography over silica gel (5% MeOH in CH₂Cl₂) to give 5-(2-methoxyvinyl)-1-trityl-1H-imidazole as a white solid (90 mg, 79% yield). In a 50 mL round-bottomed flask equipped with a condenser, 5-(2-methoxyvinyl)-1-trityl-1H-imidazole (90 mg, 0.25 mmol) was dissolved in 5 mL of 1 N HCl and 3 mL of THF and heated at 60 °C for 2 h. The reaction mixture was then allowed to cool to room temperature, and the solvent was removed to give (3H-imidazol-4-yl)acetaldehyde as a white solid of sufficient purity to be used in the next step (26 mg, 96% yield). The procedure described above for the synthesis of **12** was followed reacting **9c** (85 mg, 0.24 mmol) with (3H-imidazol-4-yl)acetaldehyde (26 mg, 0.24 mmol) and NaCNBH₃ (13 mg, 0.22 mmol) in 5 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give a yellow solid (15 mg, 14%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.83 (t, *J* = 7.07 Hz, 2 H), 3.31–3.44 (m, 2 H), 6.56 (t, *J* = 5.68 Hz, 1 H), 6.87 (s, 1 H), 7.12 (d, *J* = 2.27 Hz, 1 H), 7.24–7.35 (m, 1 H), 7.45 (t, *J* = 9.09 Hz, 1 H), 7.49 (d, *J* = 2.27 Hz, 1 H), 7.51–7.60 (m, 2 H), 8.20 (s, 1 H), 8.37 (s, 1 H), 9.53 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₅Cl₂FN₆ (MH⁺) 439.064 65, found 439.0661. HPLC method 1: 99.0%, 4.11 min. HPLC method 2: 99.0%, 4.55 min.

N-(8-Bromo-4-(3-chloro-4-fluorophenylamino)-3-cyanoquinolin-6-yl)-2-(1H-imidazol-5-yl)acetamide (23). A solution of **9d** (500 mg, 1.28 mmol), imidazoleacetic acid hydrochloride (228 mg, 1.40 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (619 mg, 1.40 mmol), and 4-methylmorpholine (0.31 mL, 0.29 g, 2.8 mmol) in 10 mL of DMF was heated at 60 °C overnight. Additional imidazoleacetic acid hydrochloride (847 mg), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (2.48 g), and 4-methylmorpholine (2.1 mL) were added, and stirring continued at room temperature for 3 days. The reaction mixture was poured into 100 mL of water, and the dark-brown precipitate was collected by suction filtration, washed with water, and dried under vacuum. This crude product was then purified twice by preparative HPLC and lyophilized to give **23** as a pale-yellow powder (76 mg, 12% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.64 (s, 2 H), 6.98 (s, 1 H), 7.28 (ddd, *J* = 8.8, 4.2, 2.9 Hz, 1 H), 7.44 (t, *J* = 9.0 Hz, 1 H), 7.52 (dd, *J* = 6.6, 2.8 Hz, 1 H), 7.60 (s, 1 H), 8.38 (d, *J* = 2.0 Hz, 1 H), 8.61–8.70 (m, 2 H), 9.95 (s, 1 H), 10.54 (s, 1 H), 12.21 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₄BrClFN₆O (MH⁺) 499.0080, found 499.0071. HPLC method 1: 99.0%, 4.12 min. HPLC method 2: 99.0%, 4.80 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1-methyl-1H-pyrazol-4-yl)methyl]aminoquinoline-3-carbonitrile (24). In a test tube were added **9c** (50 mg), dichloroethane (2 mL), and 1-methyl-1H-pyrazole-4-carbaldehyde (1.2 equiv). The mixture was stirred for 15 min at room temperature. Na(OAc)₃BH (2 equiv) was then added, and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated and the residue was purified to give **24** (29 mg): ¹H NMR (400 MHz, MeOD) δ ppm 4.44–4.53 (m, 2 H), 7.31 (s, 1 H), 7.40–7.54 (m, 2 H), 7.59–7.65 (m, 1 H), 7.69 (s, 1 H), 7.79 (s, 1 H), 8.53 (s, 1 H), 8.77 (s, 1 H). HPLC method 1: 100.0%, 5.24 min. HPLC method 2: 99.6%, 6.46 min.

8-Bromo-4-(3-chloro-4-fluorophenylamino)-6-[(2-methyl-2H-pyrazol-3-ylmethyl)amino]quinoline-3-carbonitrile (25). The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.20 mmol), 2-methyl-2H-pyrazole-3-carbaldehyde (20 mg, 0.24 mmol), and NaCNBH₃ (17 mg, 0.27 mmol) in 3 mL of EtOH and 5 drops of HOAc. Concentration of the mixture gave a brown semisolid, which was dissolved in 2 mL of DMSO. Purification by HPLC afforded **25** (5 mg, 5%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.81 (s, 3 H), 4.42

(d, *J* = 5.56 Hz, 2 H), 6.26 (d, *J* = 2.02 Hz, 1 H), 6.87 (t, *J* = 5.56 Hz, 1 H), 7.21–7.31 (m, 3 H), 7.32 (d, *J* = 2.02 Hz, 1 H), 7.39–7.49 (m, 1 H), 7.48–7.55 (m, 1 H), 7.75 (s, 1 H), 8.40 (s, 1 H), 8.43 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₅BrClFN₆ (MH⁺) 485.028 69, found (ESI-FTMS, [M + H]¹⁺), 485.0277. HPLC method 1: 99.0%, 5.45 min. HPLC method 2: 99.0%, 6.70 min.

8-Bromo-4-(3-chloro-4-fluorophenylamino)-6-[(2-phenyl-2H-pyrazol-3-ylmethyl)amino]quinoline-3-carbonitrile (26). The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.20 mmol), 1-phenyl-2H-pyrazole-5-carbaldehyde (49 mg, 0.28 mmol), and NaCNBH₃ (17 mg, 0.27 mmol) in 3 mL of EtOH and 5 drops of HOAc. Concentration of the mixture gave a brown semisolid, which was dissolved in 2 mL of DMSO. Purification by HPLC afforded **26** (16 mg, 11%) as a yellow solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.48 (s, 2 H), 6.59 (d, *J* = 1.77 Hz, 1 H), 7.24 (d, *J* = 1.77 Hz, 1 H), 7.27–7.37 (m, 1 H), 7.44–7.52 (m, 2 H), 7.52–7.60 (m, 4 H), 7.61–7.67 (m, 2 H), 7.73 (d, *J* = 1.77 Hz, 1 H), 7.81 (d, *J* = 2.27 Hz, 1 H), 8.48 (s, 1 H), 9.46 (s, 1 H); HRMS (ESI⁺) calcd for C₂₆H₁₇BrClFN₆ (MH⁺) 547.044 34, found (ESI-FTMS, [M + H]¹⁺), 547.0437. HPLC method 1: 97.0%, 6.12 min. HPLC method 2: 97.0%, 7.18 min.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(imidazo[1,2-*a*]pyridin-3-ylmethyl)amino]quinoline-3-carbonitrile (27). The procedure described above for the synthesis of **12** was followed, reacting **9c** (40 mg, 0.10 mmol) with 1H-imidazo[1,2-*a*]pyridine-3-carbaldehyde (18 mg, 0.12 mmol) and NaCNBH₃ (7 mg, 0.12 mmol) in 10 mL of EtOH. The reaction mixture was concentrated and the residue was purified via preparative HPLC and lyophilized to give **27** as a yellow solid (35 mg, 65%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.73 (d, *J* = 5.05 Hz, 2 H), 6.53 (s, 1 H), 6.93–7.01 (m, 2 H), 7.25–7.34 (m, 2 H), 7.46 (t, *J* = 9.09 Hz, 1 H), 7.55 (dd, *J* = 6.57, 2.78 Hz, 1 H), 7.60 (d, *J* = 9.09 Hz, 1 H), 7.68 (s, 1 H), 7.71 (d, *J* = 2.27 Hz, 1 H), 8.39 (d, *J* = 6.82 Hz, 1 H), 8.41 (s, 1 H), 9.52 (s, 1 H); HRMS (ESI⁺) calcd for C₂₄H₁₅BrClFN₆ (MH⁺) 521.028 69, found 521.0302. HPLC method 1: 95.5%, 4.38 min. HPLC method 2: 95.8%, 5.25 min.

8-Chloro-4-(3-chloro-4-fluorophenylamino)-6-(pyridin-3-ylmethylamino)quinoline-3-carbonitrile (28). The procedure described above for the synthesis of **12** was followed, reacting **9c** (87 mg, 0.25 mmol) with nicotinaldehyde (0.02 mL, 0.28 mmol) and NaCNBH₃ (22 mg, 0.35 mmol) in 9 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **28** as a tan solid (52 mg, 47%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.44 (d, *J* = 5.81 Hz, 2 H), 6.51 (d, 1 H), 7.01 (t, *J* = 5.94 Hz, 1 H), 7.21 (d, *J* = 2.27 Hz, 1 H), 7.24–7.29 (m, 1 H), 7.37 (dd, *J* = 8.21, 5.18 Hz, 1 H), 7.43 (t, *J* = 9.09 Hz, 1 H), 7.51 (dd, *J* = 6.57, 2.78 Hz, 1 H), 7.55 (d, *J* = 2.27 Hz, 1 H), 7.77–7.80 (m, 1 H), 8.13 (s, 1 H), 8.40 (s, 1 H), 8.48 (dd, *J* = 4.67, 1.64 Hz, 1 H), 8.62 (d, *J* = 1.77 Hz, 1 H), 9.44 (s, 1 H); HRMS (ESI⁺) calcd for C₂₂H₁₄Cl₂FN₆ (MH⁺) 438.068 30, found 438.0675. HPLC method 1: 99.1%, 4.41 min. HPLC method 2: 99.0%, 5.51 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(5-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]pyridin-3-yl)methyl]aminoquinoline-3-carbonitrile (29). To a mixture of 3,5-dibromopyridine (1.09 g, 4.60 mmol) in dry THF (20 mL) under a nitrogen atmosphere was added isopropylmagnesium chloride (2.4 mL, 2.0 M in THF, 4.8 mmol) dropwise. After 2 h, 1,1,1,3,3,3-hexafluoropropan-2-one was introduced into the reaction mixture slowly. After another hour, water (2 mL) was added to the reaction mixture. The mixture was diluted with CHCl₃ (500 mL), washed with H₂O (200 mL), and dried over MgSO₄, and the organic layer was concentrated. Purification (SiO₂, 8–25% EtOAc/hexane) gave 2-(5-bromopyridin-3-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (1.06 g, 71%) as a thick oil. To a mixture of 2-(5-bromopyridin-3-yl)-1,1,1,3,3,3-hexafluoropropan-2-ol (347 mg, 1.07 mmol) in dry THF (15 mL) under a nitrogen atmosphere was added isopropylmagnesium chloride (1.2 mL, 2.0 M in THF, 2.4 mmol) dropwise. After 1 h, DMF (150 μL, 1.94 mmol) was added. After another 2 h, HOAc

(400 μ L) was added. The solvent was removed to give crude 5-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)nicotinaldehyde, which was used in the next step without further purification. The procedure described above for the synthesis of **12** was followed, reacting **9c** (78 mg, 0.23 mmol) with this crude aldehyde, HOAc (100 μ L), and NaCNBH₃ (25 mg, 0.40 mmol) in 1 mL of EtOH and 6 mL of DMF. After 12 h, the solvent was removed to minimum volume and the mixture was filtered. Purification using preparative HPLC gave **29** as a yellow solid (31 mg, 22%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.55 (d, *J* = 6.06 Hz, 2H), 7.13 (t, *J* = 5.94 Hz, 1H), 7.21–7.28 (m, 2H), 7.40–7.50 (m, 2H), 7.54 (d, *J* = 2.27 Hz, 1H), 8.09 (s, 1H), 8.39 (s, 1H), 8.74 (d, *J* = 2.02 Hz, 1H), 8.78 (d, *J* = 2.02 Hz, 1H), 9.06 (s, 1H), 9.42 (s, 1H); HRMS calcd for C₂₅H₁₄Cl₂F₇N₅O + H⁺, 604.053 64, found (ESI-FTMS, [M + H]⁺), 604.0533. HPLC method 1: 97.7%, 5.80 min. HPLC method 2: 100.0%, 6.97 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(5-(4-hydroxy-1-isopropylpiperidin-4-yl)pyridin-3-yl)methyl]amino}quinoline-3-carbonitrile (30). To a mixture of 3,5-dibromopyridine (1.4 g, 6.16 mmol) in dry THF (12 mL) under a nitrogen atmosphere was added isopropylmagnesium chloride (3.7 mL, 2.0 M in THF, 7.4 mmol) dropwise. After 2 h, *tert*-butyl 4-oxopiperidine-1-carboxylate (1.35 g, 6.78 mmol) in THF (6 mL) was slowly added. After 12 h, the mixture was diluted with EtOAc (500 mL), washed with H₂O (200 mL), and dried over MgSO₄. The organic layer was concentrated. Purification (SiO₂, 1–8% MeOH/CH₂Cl₂) gave *tert*-butyl 4-(5-bromopyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (415 mg, 19%). To a mixture of *tert*-butyl 4-(5-bromopyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (415 mg, 1.17 mmol) in dry THF (12 mL) under a nitrogen atmosphere was added isopropylmagnesium chloride (1.3 mL, 2.0 M in THF, 2.6 mmol) dropwise. After 12 h, DMF (0.25 mL, 3.23 mmol) was added. After another 2 h, HOAc (400 μ L) was added. The solvent was removed to give crude *tert*-butyl 4-(5-formylpyridin-3-yl)-4-hydroxypiperidine-1-carboxylate, which was used in the next step without further purification. The procedure described above for the synthesis of **12** was followed, reacting **9c** (204 mg, 0.56 mmol) with the above aldehyde, HOAc (150 μ L), and NaCNBH₃ (50 mg, 0.80 mmol) in 6 mL of DMF. After 12 h, purification using preparative HPLC gave the BOC-protected precursor as a yellow solid (177 mg, 50%). MS (*m/z*): M⁺, 637.2; M[–], 635.6. To a mixture of this product (125 mg) in CH₂Cl₂ (3 mL) was added TFA (3 mL). After 1 h, the solvent was removed. Purification using HPLC gave the deprotected amine in quantitative yield. MS (*m/z*): M⁺, 537.1; M[–], 535.5. To this amine (31 mg, 0.06 mmol) in acetone (4 mL) was added Na(OAc)₃BH (30 mg, 0.14 mmol). After 2 h, purification using preparative HPLC gave **30** as an off-white solid (5 mg). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.00 (d, *J* = 6.57 Hz, 6H), 1.57 (d, *J* = 12.13 Hz, 2H), 1.83–1.95 (m, 2H), 2.53–2.62 (m, 4H), 2.70–2.80 (m, 1H), 4.44 (d, *J* = 5.81 Hz, 2H), 5.00 (s, 1H), 7.11 (t, *J* = 6.19 Hz, 1H), 7.20 (d, *J* = 2.02 Hz, 1H), 7.24–7.31 (m, 1H), 7.45 (t, *J* = 9.09 Hz, 1H), 7.50–7.55 (m, 2H), 7.95 (t, *J* = 2.15 Hz, 1H), 8.38 (s, 1H), 8.48 (d, *J* = 2.02 Hz, 1H), 8.57 (t, *J* = 2.15 Hz, 1H), 9.47 (s, 1H). MS (*m/z*) M⁺, 565.1; M[–], 563.6. HPLC method 1: 99.5%, 3.98 min. HPLC method 2: 98.4%, 3.74 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(6-methylpyridin-2-yl)methyl]amino}quinoline-3-carbonitrile (31). The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.23 mmol) with 6-methylpicolinaldehyde (120 mg, 0.99 mmol) and NaCNBH₃ (24 mg, 0.38 mmol) in 6 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **31** as a light-yellow solid (62 mg, 59%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.46 (s, 3H), 4.49 (d, *J* = 6.06 Hz, 2H), 7.06 (t, *J* = 5.94 Hz, 1H), 7.14 (dd, *J* = 9.85, 7.83 Hz, 2H), 7.19 (d, *J* = 2.27 Hz, 1H), 7.21–7.26 (m, 1H), 7.41 (t, *J* = 8.97 Hz, 1H), 7.47 (dd, *J* = 6.57, 2.53 Hz, 1H), 7.60–7.67 (m, 2H), 8.39 (s, 1H), 9.45 (s, 1H); HRMS (ESI⁺) calcd for C₂₃H₁₆Cl₂FN₅ (MH⁺) 452.083 95, found 452.0834. HPLC method 1: 99.0%, 4.72 min. HPLC method 2: 99.2%, 6.17 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(6-morpholin-4-ylpyridin-2-yl)methyl]amino}quinoline-3-carbonitrile (32). The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.23 mmol) with 6-morpholin-4-ylpyridine-2-carbaldehyde (49 mg, 0.25 mmol) and NaCNBH₃ (10 mg, 0.16 mmol) in 4 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **32** as a yellow solid (55 mg, 46%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.37–3.51 (m, 4H), 3.61–3.73 (m, 4H), 4.36 (d, *J* = 6.06 Hz, 2H), 6.68 (t, *J* = 7.83 Hz, 2H), 6.92 (t, *J* = 6.06 Hz, 1H), 7.17–7.31 (m, 2H), 7.42 (t, *J* = 8.97, 1H), 7.45–7.55 (m, 2H), 7.65 (d, *J* = 2.27 Hz, 1H), 8.39 (s, 1H), 9.46 (s, 1H); HRMS (ESI⁺) calcd for C₂₆H₂₁Cl₂FN₆O (MH⁺) 523.121 07, found 523.1207. HPLC method 1: 96.3%, 5.78 min. HPLC method 2: 96.5%, 7.07 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1-oxido-pyridin-2-yl)methyl]amino}quinoline-3-carbonitrile (33). The procedure described above for the synthesis of **12** was followed, reacting **9c** (97 mg, 0.28 mmol) with pyridine-2-carbaldehyde-1-oxide (71 mg, 0.58 mmol) and NaCNBH₃ (35 mg, 0.56 mmol) in 10 mL of EtOH. The crude product was purified by preparative HPLC to **33** as a yellow solid (64 mg, 50%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.52 (d, *J* = 6.06 Hz, 2H), 6.98 (t, *J* = 5.94 Hz, 1H), 7.08 (d, *J* = 2.02 Hz, 1H), 7.14–7.37 (m, 5H), 7.42 (d, *J* = 5.56 Hz, 1H), 7.55 (s, 1H), 8.24–8.27 (m, 1H), 8.30 (s, 1H), 9.41 (s, 1H); HRMS (ESI⁺) calcd for C₂₂H₁₄Cl₂FN₅O (MH⁺) 454.063 22, found 454.0628. HPLC method 1: 100.0%, 4.82 min. HPLC method 2: 95.8%, 6.21 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(2,3-dihydroxypropyl)amino]quinoline-3-carbonitrile (34). The procedure described above for the synthesis of **12** was followed, reacting **9c** (148 mg, 0.43 mmol) with 2,3-dihydroxypropanal (55 mg, 0.61 mmol), HOAc (150 μ L), and NaCNBH₃ (40 mg, 0.64 mmol) in 10 mL of EtOH. After 12 h, the reaction mixture was concentrated. Purification using HPLC gave **29** as a yellow solid (140 mg, 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.04–3.12 (m, 1H), 3.23–3.31 (m, 1H), 3.40–3.46 (m, 2H), 3.68–3.77 (m, 1H), 4.67 (t, *J* = 5.43 Hz, 1H), 4.90 (d, *J* = 5.05 Hz, 1H), 6.36 (t, *J* = 5.31 Hz, 1H), 7.08 (d, *J* = 2.27 Hz, 1H), 7.26–7.31 (m, 1H), 7.45 (t, *J* = 8.97 Hz, 1H), 7.52 (dd, *J* = 6.57, 2.78 Hz, 1H), 7.61 (d, *J* = 2.53 Hz, 1H), 8.36 (s, 1H), 9.48 (s, 1H). HPLC method 1: 97.1%, 4.54 min. HPLC method 2: 99.6%, 5.69 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1H-tetrazol-5-ylmethyl)amino]quinoline-3-carbonitrile (35). In a 100 mL round-bottomed flask, diethoxy acetonitrile (1.0 g, 7.7 mmol) was dissolved in 35 mL of dichloroethane. Azidotributyltin (3.34 g, 10.1 mmol) was added, and the reaction mixture was heated at reflux temperature overnight. The reaction mixture was then concentrated to a black residue, dissolved in 20 mL of 1.25 M HCl in MeOH, and heated at 65 °C for 3 h, then allowed to cool to room temperature. Evaporation of the solvent gave 1H-tetrazole-5-carbaldehyde as a black oil, which was used directly in the next step without purification. The procedure described above for the synthesis of **12** was followed, reacting **9c** (200 mg, 0.58 mmol) with 1H-tetrazole-5-carbaldehyde (141.2 mg, 1.44 mmol) and NaCNBH₃ (25.3 mg, 0.40 mmol) in 5 mL of EtOH. The crude product was purified by preparative HPLC and lyophilized to give **35** as a yellow solid (17 mg, 7%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.45 (d, *J* = 5.05 Hz, 2H), 6.74 (t, *J* = 4.93 Hz, 1H), 7.26 (d, *J* = 2.27 Hz, 1H), 7.28–7.34 (m, 1H), 7.45 (t, *J* = 9.09 Hz, 1H), 7.54 (dd, *J* = 6.57, 2.78 Hz, 1H), 7.68 (d, *J* = 2.27 Hz, 1H), 8.37 (s, 1H), 9.54 (s, 1H); HRMS (ESI⁺) calcd for C₁₈H₁₁Cl₂FN₈ (MH⁺) 429.054 05, found 429.0539. HPLC method 1: 99.2%, 4.75 min. HPLC method 2: 99.8%, 4.90 min.

2-(8-Chloro-4-(3-chloro-4-fluorophenylamino)-3-cyanoquinolin-6-ylamino) Acetic Acid (36). The procedure described above for the synthesis of **12** was followed, reacting **9c** (301 mg, 0.77 mmol) with 2-oxoacetic acid (100 mg, 1.09 mmol), HOAc (150 μ L), and NaCNBH₃ (50 mg, 0.80 mmol) in 10 mL of EtOH. Purification using HPLC gave **36** as a yellow solid (140 mg, 41%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.98 (d, *J* = 4.04 Hz, 2

H), 6.67 (s, 1 H), 7.16 (d, $J = 2.27$ Hz, 1 H), 7.28–7.34 (m, 1 H), 7.46 (t, $J = 8.97$ Hz, 1 H), 7.56 (dd, $J = 6.69, 2.65$ Hz, 1 H), 7.83 (d, $J = 2.02$ Hz, 1 H), 8.38 (s, 1 H), 9.47–9.51 (m, 1 H), 12.80 (s, 1 H). HPLC method 1: 100.0%, 4.85 min. HPLC method 2: 95.5%, 6.17 min.

***N*²-[8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-3-cyanoquinolin-6-yl]-*N*-pyridin-3-ylglycinamide (37).** To a mixture of **36** (233 mg, 0.58 mmol), 3-aminopyridine (70 μ L, 0.70 mmol), and diisopropylethylamine (402 μ L, 2.31 mmol) in DMF (5 mL) was added BOP (301 mg, 0.68 mmol). After 2 h, the mixture was purified by HPLC to give **37** as a tan solid (129 mg, 54%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.15 (s, 2 H), 6.88 (s, 1 H), 7.17 (d, $J = 2.27$ Hz, 1 H), 7.24–7.30 (m, 1 H), 7.43 (t, $J = 8.97$ Hz, 1 H), 7.51 (dd, $J = 6.57, 2.53$ Hz, 1 H), 7.61–7.69 (m, 2 H), 8.22–8.28 (m, 1 H), 8.41–8.46 (m, 2 H), 8.97 (s, 1 H), 9.55 (s, 1 H), 10.56 (s, 1 H); HRMS calcd for C₂₃H₁₅Cl₂FN₆O + H⁺, 481.074 12, found (ESI-FTMS, [M + H]¹⁺), 481.0754. HPLC method 1: 97.4%, 4.37 min. HPLC method 2: 100.0%, 5.24 min.

General Procedure for the Synthesis of Imidazolecarbaldehydes 52–54. 4-Isopropyl-1*H*-imidazole-5-carbaldehyde (52). Sulfuryl chloride (5.3 mL, 9.0 g, 66 mmol) was added dropwise to ethyl isobutyrylacetate (10.2 mL, 10.0 g, 63.2 mmol) in 50 mL of CHCl₃ at 0 °C. The mixture was heated at reflux for 2 h and concentrated to give ethyl 2-chloro-4-methyl-3-oxopentanoate of sufficient purity to be used directly in the next step (12.2 g, 94% yield): ¹H NMR (400 MHz, CDCl₃) δ ppm 1.17 (dd, $J = 9.1, 6.8$ Hz, 6 H), 1.30 (t, $J = 6.8$ Hz, 3 H), 2.99–3.16 (m, 1 H), 4.24–4.33 (m, 2 H), 4.92 (s, 1 H). Ethyl 2-chloro-4-methyl-3-oxopentanoate (12.2 g, 63.3 mmol) was reacted with formamide (25 mL, 29 g, 0.63 mol) and water (2.3 mL, 2.3 g, 0.13 mol). The crude product was purified by flash chromatography over silica gel (4–6% MeOH in CH₂Cl₂) to give ethyl 4-isopropyl-1*H*-imidazole-5-carboxylate of sufficient purity to be used in the next step (0.558 g, 4.8% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.20 (d, $J = 7.1$ Hz, 6 H), 1.23–1.33 (m, 3 H), 3.44–3.57 (m, 0.35 H), 3.65–3.79 (m, 0.65 H), 4.12–4.33 (m, 2 H), 7.58 (s, 0.65 H), 7.66 (s, 0.35 H), 12.39 (br s, 0.65 H), 12.67 (br s, 0.35 H). Ethyl 4-isopropyl-1*H*-imidazole-5-carboxylate (0.55 g, 3.0 mmol) in 20 mL of THF was reduced with a 1.0 M THF solution of LiAlH₄ (3.1 mL, 3.1 mmol). Workup gave (4-isopropyl-1*H*-imidazol-5-yl)methanol of sufficient purity to be used directly in the next step (0.39 g, 92% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.15 (d, $J = 6.8$ Hz, 6 H), 2.78–3.12 (m, 1 H), 4.33 (s, 2 H), 4.66 (br s, 1 H), 7.38 (s, 1 H), 11.66 (br s, 1 H). (4-Isopropyl-1*H*-imidazol-5-yl)methanol (0.21 g, 1.55 mmol) was oxidized with activated manganese dioxide (0.40 g, 4.64 mmol) in 5 mL of acetone. The crude product was purified by flash chromatography over silica gel (gradient elution, 5–100% EtOAc in CH₂Cl₂) to give pure **52** as a pale-pink solid (0.27 g, 37% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.23 (d, $J = 6.8$ Hz, 6 H), 3.41 (br s, 0.35 H), 3.53–3.68 (m, 0.65 H), 7.71 (s, 0.65 H), 7.85 (s, 0.35 H), 9.82 (s, 1 H), 12.66 (br s, 0.65 H), 12.91 (br s, 0.35 H).

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(4-isopropyl-1*H*-imidazol-5-yl)methyl]amino}quinoline-3-carbonitrile (38). The procedure described above for the synthesis of **12** was followed, reacting **9d** (300 mg, 0.76 mmol) with **52** (106 mg, 0.76 mmol) and NaCNBH₃ (32 mg, 0.51 mmol) in 9 mL of THF and 3 mL of MeOH. The crude product was purified by HPLC and lyophilized to give **38** as a yellow solid (162 mg, 41% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.16 (d, $J = 6.8$ Hz, 6 H), 3.09 (br s, 1 H), 4.13 (s, 2 H), 6.49 (s, 1 H), 7.21 (s, 1 H), 7.24–7.33 (m, 1 H), 7.44 (t, $J = 9.0$ Hz, 1 H), 7.48–7.55 (m, 2 H), 7.83 (s, 1 H), 8.39 (s, 1 H), 9.45 (s, 1 H), 11.82 (s, 1 H); HRMS (ESI⁺) calcd for C₂₃H₂₀BrClFN₆ (MH⁺) 513.0600, found 513.0594. HPLC method 1: 99.3%, 4.46 min. HPLC method 2: 97.4%, 5.64 min. Anal. (C₂₃H₂₀BrClFN₆·H₂O): C, 51.94; H, 3.98; N, 15.80. Found: C, 51.92; H, 3.66; N, 15.35.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(5-isopropyl-1*H*-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (39). The procedure described above for the synthesis of **12** was followed, reacting **9c** (76 mg, 0.22 mmol) with **52** (36 mg, 0.26 mmol) and

NaCNBH₃ (22 mg, 0.35 mmol) in 9 mL of EtOH. The crude product was purified by HPLC and lyophilized to give **39** as a yellow solid (74 mg, 72%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.16 (d, $J = 7.07$ Hz, 6 H), 4.16 (d, $J = 4.55$ Hz, 2 H), 6.54 (d, $J = 4.42$ Hz, 1 H), 7.19 (d, $J = 2.02$ Hz, 1 H), 7.26–7.34 (m, 1 H), 7.45 (t, $J = 8.97$ Hz, 1 H), 7.49–7.56 (m, 2 H), 7.61 (d, $J = 2.27$ Hz, 1 H), 8.16 (s, 1 H), 8.39 (s, 1 H), 9.47 (s, 1 H); HRMS (ESI⁺) calcd for C₂₃H₁₂Cl₂FN₆ (MH⁺) 469.110 50, found 469.1096. HPLC method 1: 98.8%, 4.37 min. HPLC method 2: 99.5%, 5.51 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(5-propyl-1*H*-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (40). The imidazolecarbaldehyde **53** was prepared according to the general procedure described above for **52**. The procedure described above for the synthesis of **12** was then followed, reacting **9c** (80 mg, 0.23 mmol) with **53** (38 mg, 0.28 mmol) and NaCNBH₃ (17 mg, 0.28 mmol) in 9 mL of EtOH. The crude product was purified by HPLC and lyophilized to give **40** as a yellow solid (61 mg, 56%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.83 (t, $J = 7.45$ Hz, 3 H), 1.53 (q, 2 H), 4.15 (d, $J = 4.80$ Hz, 2 H), 6.27 (s, 1 H), 6.51 (d, 2 H), 7.18 (s, 1 H), 7.25–7.32 (m, 1 H), 7.45 (t, $J = 8.97$ Hz, 1 H), 7.49–7.54 (m, 2 H), 7.61 (s, 1 H), 8.16–8.20 (m, 1 H), 8.39 (s, 1 H), 9.46 (s, 1 H). HPLC method 1: 99.1%, 4.47 min. HPLC method 2: 99.8%, 5.70 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(5-ethyl-1*H*-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (41). The imidazolecarbaldehyde **54** was prepared according to the general procedure described above for **42a**. The procedure described above for the synthesis of **12** was then followed, reacting **9c** (43 mg, 0.12 mmol) with **54** (15 mg, 0.12 mmol) and NaCNBH₃ (9 mg, 0.14 mmol) in 4 mL of EtOH. The reaction was concentrated and the residue was purified by HPLC to give **41** as a yellow solid (16 mg, 30%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.12 (t, $J = 7.58$ Hz, 3 H), 2.51–2.61 (m, 2 H), 4.16 (d, $J = 4.04$ Hz, 2 H), 6.54 (s, 2 H), 7.20 (s, 1 H), 7.26–7.33 (m, 1 H), 7.45 (t, $J = 9.22$ Hz, 1 H), 7.50–7.63 (m, 2 H), 8.14 (s, 1 H), 8.39 (s, 1 H), 9.48 (s, 1 H); HRMS calcd for C₂₂H₁₇Cl₂FN₆ + H⁺, 455.094 85, found (ESI-FTMS, [M + H]¹⁺), 455.0945. HPLC method 1: 96.9%, 4.28 min. HPLC method 2: 97.0%, 5.31 min.

Methyl 5-[(8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-3-cyanoquinolin-6-yl)amino)methyl]-1*H*-imidazole-4-carboxylate (42). In a microwave vial, methyl 5-hydroxymethyl-1*H*-imidazole-4-carboxylate (0.20 g, 1.28 mmol) was dissolved in 2.5 mL each of CH₂Cl₂ and 1,4-dioxane, and activated MnO₂ (0.95 g, 11 mmol) was added. The vial was heated in a microwave reactor at 140 °C for 5 min. The contents of the vial were then rinsed into a 1 L flask and stirred with 400 mL of MeOH for 30 min. The suspension was then filtered to remove the deactivated MnO₂, and the filtrate was dried over anhydrous Na₂SO₄, filtered a second time, and evaporated to give methyl 5-formyl-1*H*-imidazole-4-carboxylate of sufficient purity to be used in the next step (163 mg, 83% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.88 (s, 3 H), 8.06 (s, 1 H), 10.22 (s, 1 H), 13.76 (s, 1 H); HRMS (ESI⁺) calcd for C₆H₇N₂O₃ (MH⁺) 155.0451, found 155.0450. The procedure described above for the synthesis of **12** was followed, reacting **9d** (400 mg, 1.02 mmol) with methyl 5-formyl-1*H*-imidazole-4-carboxylate (157 mg, 1.02 mmol) and NaCNBH₃ (43 mg, 0.68 mmol) in 12 mL of THF and 4 mL of MeOH. The crude product was purified by HPLC and lyophilized to give **42** as a bright-yellow powder (0.18 g, 33% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.76 (br s, 3 H), 4.50 (br s, 1 H), 4.62 (br s, 1 H), 6.65 (br s, 0.5 H), 6.74 (br s, 0.5 H), 7.20–7.31 (m, 2 H), 7.43 (t, $J = 9.0$ Hz, 1 H), 7.50 (dd, $J = 6.4, 2.7$ Hz, 1 H), 7.63–7.88 (m, 2 H), 8.39 (s, 1 H), 9.45 (br s, 1 H), 12.70 (br s, 0.5 H), 13.09 (br s, 0.5 H); HRMS (ESI⁺) calcd for C₂₂H₁₆BrClFN₆O₂ (MH⁺) 529.0185, found 529.0186. HPLC method 1: 99.1%, 4.98 min. HPLC method 2: 98.5%, 6.63 min.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(4-[3-(dimethylamino)propyl]-1*H*-imidazol-5-yl)methyl]amino}quinoline-3-carbonitrile (43). In a 50 mL round-bottomed flask were added **56** (355 mg, 1.04 mmol), *N,N*-dimethylprop-2-yn-1-amine (178 mg, 2.08 mmol), dichlorobis(triphenylphosphine)palladium (85 mg, 0.12

mmol), and triethylamine (800 mg, 7.89 mmol) in 5 mL of DMF. The mixture was heated to 90 °C for 2.5 h. 1-(Benzyloxymethyl)-4-(3-(dimethylamino)prop-1-ynyl)-1H-imidazole-5-carbaldehyde was obtained upon a standard EtOAc aqueous workup and was sufficiently pure for the next step. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.25 (s, 6 H), 3.54 (s, 2 H), 4.54 (s, 2 H), 5.72 (s, 2 H), 7.23–7.37 (m, 5 H), 8.28 (s, 1 H), 9.86 (s, 1 H). Crude **57** was dissolved in THF (15 mL), and approximately 10 mg of 5% Pd on carbon was added. The mixture was hydrogenated in a Parr shaker apparatus at 50 psi for 3 h. After filtration through Celite, the solvent was removed under reduced pressure. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.73–1.79 (m, 2 H), 2.16 (s, 6 H), 2.24–2.30 (m, 2 H), 2.78–2.84 (m, 2 H), 4.51 (s, 2 H), 5.71 (s, 2 H), 7.21–7.38 (m, 5 H), 8.13 (s, 1 H), 9.86 (s, 1 H). The crude material from the previous step was stirred in 10 mL of 6 N HCl and 10 mL of MeOH to give 4-(3-(dimethylamino)propyl)-1H-imidazole-5-carbaldehyde (**57**), which was immediately used as a solution in the next step. The procedure described above for the synthesis of **12** was followed, reacting **9c** (100 mg, 0.29 mmol) with a solution of **57** and NaCNBH₃ (21.0 mg, 0.33 mmol) at room temperature for 5 h. The mixture was concentrated and purified via preparative HPLC to give **43** as a yellow solid (98 mg, 66%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.64–1.74 (m, 2 H), 2.19 (s, 6 H), 2.29–2.36 (m, 2 H), 2.53–2.58 (m, 2 H), 4.17 (d, *J* = 4.04 Hz, 2 H), 6.57 (t, *J* = 4.42 Hz, 1 H), 7.21 (d, *J* = 2.27 Hz, 1 H), 7.25–7.33 (m, 1 H), 7.45 (t, *J* = 8.97 Hz, 1 H), 7.53 (q, *J* = 2.95 Hz, 1 H), 7.59 (d, *J* = 2.27 Hz, 1 H), 8.19 (s, 2 H), 8.39 (s, 1 H), 9.48–9.56 (m, 1 H); HRMS calcd for C₂₅H₂₄Cl₂FN₇ + H⁺, 512.152 70, found (ESI-FTMS, [M + H]⁺), 512.1522. HPLC method 1: 95.2%, 3.53 min. HPLC method 2: 95.1%, 3.01 min.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1-methyl-1H-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (44**).** (4-Ethyl-1H-imidazol-5-yl)methanol (1.00 g, 7.14 mmol) was dissolved in 20 mL of THF and cooled to 0 °C. A 1.0 M solution of LiAlH₄ in THF (7.1 mL, 7.1 mmol) was then added slowly via syringe. The reaction mixture allowed to warm to room temperature over 30 min and then cooled to 0 °C and quenched by addition of 10 mL of saturated Na₂SO₄ followed by addition of enough 1 M HCl to bring the solution to pH 8. The white precipitate was then filtered off and washed with EtOAc. The filtrate was dried, filtered, and evaporated to give (1-methyl-1H-imidazol-4-yl)methanol as a waxy yellow solid (0.80 g, 100% yield) of sufficient purity to be used in the next step. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.60 (s, 3 H), 4.30 (s, 2 H), 4.79 (br s, 1 H), 6.92 (s, 1 H), 7.45 (s, 1 H). Crude (1-methyl-1H-imidazol-4-yl)methanol (0.81 g, 7.19 mmol) was stirred overnight at room temperature with activated manganese dioxide (1.87 g, 21.6 mmol) in 15 mL of acetone. The crude product was filtered through a pad of Celite and purified by flash chromatography over silica gel (10–100% EtOAc in CH₂Cl₂) to give pure 1-methyl-1H-imidazole-4-carbaldehyde as a waxy yellow solid (0.23 g, 30% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.73 (s, 3 H), 7.81 (s, 1 H), 8.00 (s, 1 H), 9.70 (s, 1 H). The procedure described above for the synthesis of **12** was followed, reacting **9d** (200 mg, 0.51 mmol) with 1-methyl-1H-imidazole-4-carbaldehyde (56 mg, 0.51 mmol) and NaCNBH₃ (22 mg, 0.34 mmol) in 6 mL of THF and 2 mL of MeOH. The yellow precipitate that appeared was collected by suction filtration, washed with MeOH, and dried under vacuum to give **44** as a yellow powder (155 mg, 63% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.60 (s, 3 H), 4.21 (d, *J* = 5.3 Hz, 2 H), 6.67 (t, *J* = 5.2 Hz, 1 H), 7.05 (s, 1 H), 7.23 (d, *J* = 2.3 Hz, 1 H), 7.28 (ddd, *J* = 8.7, 4.2, 2.8 Hz, 1 H), 7.45 (t, *J* = 9.0 Hz, 1 H), 7.52 (dd, *J* = 6.7, 2.7 Hz, 1 H), 7.54 (s, 1 H), 7.79 (d, *J* = 2.3 Hz, 1 H), 8.38 (s, 1 H), 9.47 (s, 1 H); HRMS (ESI⁺) calcd for C₂₁H₁₆BrClFN₆ (MH⁺) 485.0287, found 485.0278. HPLC method 1: 98.0%, 4.20 min. HPLC method 2: 96.6%, 4.90 min. Anal. (C₂₁H₁₆BrClFN₆·H₂O) C, H, N.

8-Chloro-4-(3-chloro-4-fluorophenylamino)-6-((2-ethyl-5-methyl-1H-imidazol-4-yl)methylamino)quinoline-3-carbonitrile (45**).** The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.23 mmol) with 2-ethyl-5-methyl-1H-imidazole-4-carbaldehyde (67 mg, 0.49 mmol) and NaCNBH₃ (24

mg, 0.38 mmol) in 6 mL of EtOH. The crude product was purified by HPLC and lyophilized to give **45** as a tan solid (52 mg, 48%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 1.17 (t, *J* = 7.58 Hz, 3 H), 2.06–2.17 (m, 3 H), 2.51–2.60 (m, 2 H), 4.09 (d, *J* = 4.55 Hz, 2 H), 6.53 (t, *J* = 4.80 Hz, 1 H), 7.18 (t, *J* = 2.27 Hz, 1 H), 7.25–7.32 (m, 1 H), 7.45 (t, *J* = 8.97 Hz, 1 H), 7.52 (dd, *J* = 6.57, 2.78 Hz, 1 H), 7.60 (d, *J* = 2.27 Hz, 1 H), 8.16 (s, 1 H), 8.39 (s, 1 H), 9.47 (s, 1 H); HRMS (ESI⁺) calcd for C₂₃H₁₂Cl₂FN₆ (MH⁺) 469.110 50, found 469.1102. HPLC method 1: 97.1%, 4.28 min. HPLC method 2: 98.5%, 5.16 min. Anal. (C₂₃H₁₂Cl₂FN₆·H₂O·HCO₂H) C, H, N.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(2,5-dimethyl-1H-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (46**).** The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.23 mmol) with 2,4-dimethyl-1H-imidazole-5-carbaldehyde (34 mg, 0.28 mmol) and NaCNBH₃ (17 mg, 0.28 mmol) in 9 mL of EtOH. The product precipitated out of solution and was washed with EtOH to give **46** as a yellow solid (49 mg, 46%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.10 (s, 3 H), 2.19 (s, 3 H), 4.07 (m, 2 H), 6.51 (s, 1 H), 7.17 (s, 1 H), 7.24–7.30 (m, 1 H), 7.45 (t, *J* = 9.09 Hz, 1 H), 7.53 (d, *J* = 3.03 Hz, 1 H), 7.59 (s, 1 H), 8.14 (s, 1 H), 8.39 (s, 1 H), 9.45 (s, 1 H); HRMS, calcd for C₂₂H₁₇Cl₂FN₆ + H⁺, 455.094 85, found (ESI-FTMS, [M + H]⁺), 455.0947. HPLC method 1: 97.2%, 3.96 min. HPLC method 2: 95.3%, 3.68 min. Anal. (C₂₂H₁₇Cl₂FN₆·H₂O·HCO₂H) C, H, N.

8-Chloro-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1-methyl-1H-imidazol-5-yl)methyl]amino}quinoline-3-carbonitrile (47**).** The procedure described above for the synthesis of **12** was followed, reacting **9c** (80 mg, 0.23 mmol) with 1-methyl-1H-imidazole-5-carbaldehyde (38 mg, 0.25 mmol) and NaCNBH₃ (17 mg, 0.28 mmol) in 9 mL of EtOH. The mixture was concentrated and the residue was purified via preparative HPLC and lyophilized to give **47** as a yellow solid (65 mg, 78%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 3.69 (s, 3 H), 4.39 (d, *J* = 5.05 Hz, 2 H), 6.78–6.88 (m, 1 H), 7.14 (s, 1 H), 7.25 (d, *J* = 2.02 Hz, 1 H), 7.27–7.34 (m, 1 H), 7.46 (t, *J* = 8.97 Hz, 1 H), 7.51–7.58 (m, 1 H), 8.00 (s, 1 H), 8.13 (s, 1 H), 8.42 (s, 1 H), 9.47 (s, 1 H); HRMS calcd for C₂₁H₁₅Cl₂FN₆ + H⁺, 441.079 20, found (ESI-FTMS, [M + H]⁺), 441.078 60. HPLC method 1: 99.4%, 4.15 min. HPLC method 2: 99.4%, 4.87 min.

8-Bromo-4-(3-chloro-4-fluorophenylamino)-6-((2-phenyl-1H-imidazol-5-yl)methylamino)quinoline-3-carbonitrile (48**).** The procedure described above for the synthesis of **12** was followed, reacting **9d** (300 mg, 0.76 mmol) with 2-phenyl-1H-imidazole-5-carbaldehyde (132 mg, 0.76 mmol) and NaCNBH₃ (32 mg, 0.51 mmol) in 9 mL of THF and 3 mL of MeOH. The crude product was purified by trituration with boiling EtOH and a second crop of crystals were collected from the filtrate to give **48** as a yellow solid (206 mg, 49% yield): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 4.30 (d, *J* = 5.8 Hz, 2 H), 6.77 (t, *J* = 5.3 Hz, 1 H), 7.20 (s, 1 H), 7.26–7.35 (m, 3 H), 7.37–7.47 (m, 3 H), 7.53 (dd, *J* = 6.6, 2.8 Hz, 1 H), 7.81 (s, 1 H), 7.90 (d, *J* = 7.3 Hz, 2 H), 8.37 (s, 1 H), 9.49 (s, 1 H), 11.94 (s, 0.5 H), 12.40 (s, 0.5 H); HRMS (ESI⁺) calcd for C₂₆H₁₈BrClFN₆ (MH⁺) 547.0444, found 547.0457. HPLC method 1: 100.0%, 4.34 min. HPLC method 2: 98.3%, 5.14 min.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-([1-(2-morpholin-4-ylethyl)-1H-imidazol-4-yl)methyl]amino}quinoline-3-carbonitrile (49**).** In a sealable pressure tube was added 1H-imidazole-4-carbaldehyde (750 mg, 7.8 mmol), sodium carbonate (827 mg, 7.8 mmol), sodium iodide (585 mg, 3.9 mmol), 4-(2-chloroethyl)morpholine hydrochloride (726 mg, 3.9 mmol), and 10 mL of DMF under a stream of nitrogen gas. The tube was then sealed and heated to 100 °C overnight. The mixture was allowed to cool to room temperature, filtered, and diluted with EtOAc. The EtOAc layer was extracted twice with brine, and the aqueous phase was back-extracted with EtOAc. The organic layers were combined, dried over MgSO₄, and filtered and concentrated under reduced pressure. The substituted aldehyde was used without further purification.

The procedure described above for the synthesis of **12** was followed using **9d** (150 mg, 0.38 mmol) with crude 1-(2-

morpholinoethyl)-1H-imidazole-4-carbaldehyde and NaCNBH₃ (35 mg, 0.56 mmol) in 10 mL of EtOH. The mixture was concentrated and the residue was purified by HPLC and lyophilized to give **49** as a yellow solid (40 mg, 18%): ¹H NMR (400 MHz, MeOD) δ ppm 2.36–2.45 (m, 4 H), 2.64 (t, 2 H), 3.52–3.59 (m, 4 H), 4.08 (t, *J* = 6.19 Hz, 2 H), 4.33 (s, 2 H), 7.11 (d, *J* = 1.01 Hz, 1 H), 7.14 (d, *J* = 1.26 Hz, 1 H), 7.17–7.25 (m, 1 H), 7.28 (t, *J* = 8.84 Hz, 1 H), 7.40 (dd, *J* = 6.82, 2.02 Hz, 1 H), 7.67 (d, *J* = 1.01 Hz, 2 H), 8.29 (s, 1 H), 8.39 (s, 1 H); HRMS (ESI⁺) calcd for C₂₆H₂₄BrClFN₇O (MH⁺) 584.09710, found 584.097. HPLC method 1: 98.4%, 3.54 min. HPLC method 2: 100.0%, 3.62 min.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(1-[2-(methylsulfonyl)ethyl]-1H-imidazol-4-yl)methyl]amino]quinoline-3-carbonitrile (50). In a microwave tube was added methyl vinyl sulfone (264 mg, 5.0 mmol), 1H-imidazole-5-carbaldehyde (160 mg, 1.67 mmol), and 4 mL of DMF. The mixture was reacted in a microwave reactor at 150 °C for 1 h. The reaction mixture was concentrated to give 1-(2-(methylsulfonyl)ethyl)-1H-imidazole-4-carbaldehyde. The procedure described above for the synthesis of **12** was followed, reacting **9d** (340 mg, 0.87 mmol) with 1-(2-(methylsulfonyl)ethyl)-1H-imidazole-4-carbaldehyde and NaCNBH₃ (30 mg, 0.48 mmol) in 10 mL of EtOH. The reaction mixture was concentrated and the residue was purified by HPLC and lyophilized to give **50** as a yellow solid (90 mg, 18%): ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 2.84 (s, 3 H), 3.64 (t, *J* = 6.95 Hz, 2 H), 4.23 (d, *J* = 4.80 Hz, 2 H), 4.36 (t, *J* = 6.95 Hz, 2 H), 6.70 (t, *J* = 5.43 Hz, 1 H), 7.20 (s, 1 H), 7.24 (d, *J* = 2.02 Hz, 1 H), 7.25–7.32 (m, 1 H), 7.45 (t, *J* = 9.09 Hz, 1 H), 7.52 (dd, *J* = 6.57, 2.78 Hz, 1 H), 7.68 (d, *J* = 1.26 Hz, 1 H), 7.79 (d, *J* = 2.27 Hz, 1 H), 8.14 (s, 1 H), 8.38 (s, 1 H), 9.47 (s, 1 H); HRMS (ESI⁺) calcd for C₂₃H₁₉BrClFN₆O₂S (MH⁺) 577.02189, found 577.0225. HPLC method 1: 98.7%, 4.25 min. HPLC method 2: 100.0%, 4.96 min.

8-Bromo-4-[(3-chloro-4-fluorophenyl)amino]-6-[(2H-1,2,3-triazol-4-yl)methyl]amino]quinoline-3-carbonitrile (51). In a microwave vial were added 3,3-diethoxyprop-1-yne (1.0 g, 7.5 mmol), 1,2-diethoxyethane (15 mL), and tributyltin azide (3.2 g, 9.76 mmol). The sealed vial was heated in a microwave reactor at 180 °C for 3 h. The solvent was evaporated, and the residue was treated with 2 M HCl in MeOH for 16 h. The solvent was evaporated, and the residue was dissolved in methanol (10 mL) and dichloroethane (10 mL). To this was added **9d** (500 mg, 1.3 mmol). Acetic acid was added to bring the pH of the solution to 4, and the mixture was stirred for 15 min. Sodium triacetoxyborohydride (542 mg, 2.55 mmol) was then added, and the mixture was stirred at room temperature overnight. The reaction mixture was evaporated to dryness and the residue was purified via preparative HPLC and lyophilized to give **51** as a yellow solid (151 mg, 24%). ¹H NMR (400 MHz, MeOD) δ ppm 4.54 (s, 2 H), 7.18 (d, *J* = 2.53 Hz, 1 H), 7.21–7.31 (m, 2 H), 7.41 (dd, *J* = 6.44, 2.40 Hz, 1 H), 7.69 (d, *J* = 2.27 Hz, 2 H), 8.30 (s, 1 H); HRMS (ESI⁺) calcd for C₁₉H₁₂BrClFN₇ (MH⁺) 472.0090, found 472.0030. HPLC method 1: 100.0%, 5.01 min. HPLC method 2: 98.9%, 6.26 min.

8-Bromo-4-(3-chloro-4-fluorophenylamino)-6-[(1-(3-(dimethylamino)propyl)-1H-1,2,3-triazol-4-yl)methylamino]quinoline-3-carbonitrile (55). In a 15 mL round-bottomed flask were added **9d** (40 mg, 0.10 mmol), dichloroethane (2 mL), and 1-(3-(dimethylamino)propyl)-1H-1,2,3-triazole-4-carbaldehyde (24 mg, 0.12 mmol). The mixture was stirred for 15 min. Sodium triacetoxyborohydride (55 mg, 0.27 mmol) was then added, and the mixture was stirred at room temperature for 5 h. The reaction mixture was evaporated to dryness and the residue was purified via preparative HPLC and lyophilized to give the product as a yellow solid (42 mg, 75%). ¹H NMR (400 MHz, MeOD) δ ppm 2.36–2.54 (m, 2 H), 2.94 (s, 6 H), 3.18–3.29 (m, 2 H), 4.60–4.69 (m, 4 H), 7.33 (d, *J* = 2.27 Hz, 1 H), 7.35–7.46 (m, 2 H), 7.56 (dd, *J* = 6.44, 2.40 Hz, 1 H), 7.81 (d, *J* = 2.27 Hz, 1 H), 8.11 (s, 1 H), 8.43 (s, 1 H), 8.50 (s, 1 H). HPLC method 1: 94.2%, 4.31 min. HPLC method 2: 96.7%, 5.27 min.

Biology. For a complete description of the in vitro Tpl2 and TNF-α assays, see ref 18.

In Vitro Kinase Selectivity Assays. Scintiplate (³³P) assays were used to measure the activities of PKA (Panvera), PKC (Panvera), S6K (Upstate), JNK1 (Upstate), and MK2 41-400 (in house). The kinase reactions were performed at room temperature in streptavidin-coated Scintiplate (Perkin-Elmer) with a 100 μL/well reaction mixture, which contains the compounds in 1% DMSO, 20 mM HEPES, pH 7.5, 10 mM MgCl₂, 4.5 mM DTT, 0.01% Triton X100, 3 μM ATP, 1.3 nM [γ-³³P]ATP, and 200 nM substrate peptide. All assays were initiated by adding an ATP/peptide mixture. The reaction time was 10 min for PKA, 30 min for PKC, 80 min for S6K, 90 min for Jnk1, and 20 min for MK2 41-400. Reactions were terminated with 50 μL of 0.5 M EDTA. Plates were washed with PBS/0.05% Tween-20 six times and read on a MicroBeta TriLux reader (Perkin-Elmer).

The peptide substrates used were biotin-GRTGRRNSI for PKA/PKCα, biotin-RRRLSSLRA for S6K, biotin-ATF2 (19-96) (Upstate) for JNK1, and biotin-RTPKLARQASIELPSM for MK2. ELISA was used to measure the activities of p38 (in house), Src (Upstate), CAMKII (Upstate), and IKKβ (Upstate). The kinase reactions were performed at room temperature on streptavidin-coated plates (Sigma) or Anti-GST (Amersham) coated Maxisorp plates (Nunc) with 100 μL/well reaction mixture (same as for the Scintiplate assays but without [γ-³³P]ATP). All assays were initiated with the ATP/substrate mixture. The reaction time was 60 min. Reactions were terminated with 50 μL of 0.5 M EDTA. Plates were washed with PBS/0.05% Tween-20 six times. Detection antibodies in antibody dilution buffer (10 mM MOPS 7.5, 150 mM NaCl, 0.05% Tween-20, 0.1% gelatin, 0.02% NaN₃, 1% BSA) were added into plates and incubated for 60 min at ambient temperature, and then plates were washed again. Enhancement buffer (Perkin-Elmer) (100 μL/well) was added to the plates, and the samples were incubated for 10 min. Read Plates were read using a VictorII plate reader (Perkin-Elmer).

The peptide substrates were biotin-QSTKVPQTPLHTSRVL for p38, biotin-KKEGPWLEEEEEAYGWMDF for Src, and biotin-RTPKLARQASIELPSM for CAMKII/IKKβ. Detection antibodies were p38, anti-P-MK2 (in house), 1:4000, and DELFIA Eu-N1 goat anti-rabbit-IgG, 1:2000; Src, P-Tyr-100 (Cell Signaling), 1:1000, and DELFIA Eu-N1 rabbit antimouse-IgG (Perkin-Elmer), 1:1000; CAMKII and IKKβ, anti-P-LSP1 (in house), 1:10000, and DELFIA Eu-N1 goat antirabbit-IgG, 1:5000.

Pharmacokinetic Studies. The animals used in the pharmacokinetic (PK) studies were male adult Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). All PK studies were performed at Wyeth Research Laboratories (Andover, MA) under the supervision of the Institutional Animal Care and Use Committee. The dose formulation for intravenous administration was 50% DMSO/50% polyethylene glycol 200 (PEG 200, v/v, 1 mL/kg). The oral dose formulation was an aqueous suspension containing 2% polysorbate 80 (aka Tween-80), 0.5% methylcellulose, and 0.06% lactic acid. Blood samples of approximately 0.25 mL were collected into K₂EDTA coated sampling tubes at 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 h after dose administration. Plasma samples were harvested and stored at −80 °C until analysis.

In a 0.5 mL 96-well plate, 50 μL of plasma sample was precipitated with 100 μL of acetonitrile containing 500 ng/mL internal standard. The internal standard is a compound with similar chemical structure as the test article. The samples were vortexed and centrifuged at 5700 rpm for 10 min. Supernatants were subjected to LC-MS/MS analysis. HPLC separation was performed on a Perkin-Elmer series 200 HPLC system (Perkin-Elmer, Norwalk, CT) using an XTerra MS C18 column (2.1 mm × 20 mm, 2.5 μm; Waters, Milford, MA). The detection of test articles was performed on a PESCIX API-3000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, L4K4V8, Canada) using Turbolon Spray source. Plasma standard curves were generated by plotting the peak area ratio of test article and internal standard against nominal concentrations.

The pharmacokinetic parameters were determined using WinNonlin (version 4.1, Pharsight, Mountain View, CA). Calculations were performed using the noncompartmental analysis approach. The

estimation of area under the plasma concentration versus time curve (AUC) was based on the log trapezoidal rule. The terminal rate constant (λ) was derived from the slope of the terminal log-linear phase of plasma concentration–time curves. The apparent terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda$. No statistical analysis other than descriptive statistics was conducted.

LPS-Induced Acute TNF- α Production in Rats. Jugular vein cannulated female Sprague-Dawley rats, 200 g, were obtained from Taconic Farms (Germantown, NY). The animals were fed food and water ad libitum, and all procedures were approved by the Institutional Animal Care and Use Committee. The compound was administered orally at 10 and 50 mg/kg. One hour later, the rats were challenged with LPS, administered intravenously (iv) at 1 mg/kg. Blood was drawn 10 and 90 min after the LPS challenge, and plasma was separated and stored at -70°C until testing. The plasma concentration of the compound was determined in samples collected 10 and 90 min after the LPS challenge, while TNF- α concentration was determined in samples collected at 90 min after the challenge, using a mouse cytometric bead assay kit (CBA) obtained from BD Pharmingen (San Jose, CA). Mouse CBA kit detects rat TNF- α because of cross-reactivity of anti-TNF- α antibodies.

In Vitro Microsomal Metabolic Stability and Metabolism. Microsomes purchased from commercial vendors, such as In Vitro Technologies (Baltimore, MD) or Xenotech (Kansas City, KS), or microsomes prepared by differential ultracentrifugation utilizing the method described by Lake³⁵ were used. Microsomal protein and cytochrome P450 content were determined by the methods of Bradford³⁶ and Omura and Sato,³⁷ respectively. For human liver microsomes, the activities of major cytochrome P450 enzymes (i.e., CYP3A4, CYP2D6, and CYP2C9) were provided by the vendor or determined in-house. Microsomes were stored at -80°C in aliquots (e.g., 200 μL) until use. Microsomes were pooled from at least three animals or subjects. The incubation mixture (prior to the addition of NADPH) was preincubated with the test compound for approximately 5 min at 37°C , and the reaction was started by the addition of NADPH. Control incubations were performed in the absence of NADPH. Incubations were conducted for up to 1 h. An aliquot (e.g., 200 μL) of the incubation mixture was removed at zero time and at different time intervals (e.g., 10, 20, 30, and 60 min) to determine the time course of drug disappearance. Microsomal protein was precipitated with an organic solvent such as acetonitrile (2–3 volumes), and the drug and metabolites were extracted. The amount of unchanged drug remaining was determined by HPLC or LC/MS using an appropriate internal standard. An appropriate positive control, such as midazolam for CYP3A4, was included to assess interday and interlaboratory variations. For determination of metabolite profiles, the study was performed similarly to procedures described above with the exception that a higher substrate concentration (e.g., 10 μM or above) and an incubation time of at least 30 min were used to maximize the amounts of metabolites that were produced and characterized. For metabolic stability studies, peak area or peak height ratios (i.e., peak area/height of unchanged drug/peak area/height of internal standard) at each time point were used to determine the amount of unchanged drug, which was converted to the percentage of drug remaining using the peak area/height ratio values at zero time as 100%. The rate of drug disappearance was expressed as $\text{nmol min}^{-1} \text{mg}^{-1}$ or $\text{pmol min}^{-1} \text{mg}^{-1}$ protein based on the first 5 or 10 min of incubation and assuming linearity. The log of the percentage of the drug remaining (y -axis) was plotted against incubation time (x -axis). The slope from the linear regression line equals $-k$, and the in vitro $t_{1/2}$ equals $-0.693/k$.

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Supporting Information Available: Elemental analysis data for compounds **11**, **18**, **33**, **38**, and **44–47**, quantitative pharmacological data for Figure 3 (TNF- α levels and plasma concentrations of **44** in individual animals), and experimental details for solubility and permeability measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JM070436Q

DIRECCIÓN DE NUEVAS CREACIONES

Procedimiento acelerado de exámenes de patente (PPH)

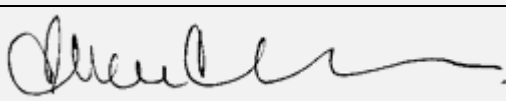
1	NÚMERO DE SOLICITUD ANTE LA SIC			NC2017/0013351		
2	GLOBAL - PPH ☐ PAÍS		3	PCT – PPH ☒ PAÍS Europa		
4	PROSUR ☐ PAÍS		5	Alianza del Pacífico (AdP) ☐ PAÍS		
6	OFICINA DE PRIMERA PRESENTACIÓN (OPP) ☐		7	OFICINA DE EXAMEN ANTERIOR (OEA) ☐		
8	NÚMERO DE SOLICITUD O PUBLICACIÓN DE LA SOLICITUD (OPP) ☒ NÚMERO DE SOLICITUD EN OFICINA DE EXAMEN ANTERIOR (OEA): ☐ NÚMERO DE SOLICITUD PCT: ☐			1. EP 16 738 976.6		
9	☒ REPRESENTANTE LEGAL ☒ APODERADO					
APELLIDOS LLOREDA RICAURTE		NOMBRES ALICIA		IDENTIFICACIÓN C.C .39.690.713 T.P .53.215		
DIRECCIÓN Calle 72 N° 5-83 Piso 5to		No. TELÉFONO 3264270		CORREO ELECTRÓNICO notificacionespatentes@lloredacmacho.com		
CIUDAD Bogotá		No. RADICACIÓN O PROTOCOLO DE PODER GENERAL				
PAÍS Colombia						
10	CONDICIONES PARA ACCEDER A ESTE PROCESO:					
10.1. DATOS DE PRIORIDAD ☒ SI ☐ NO						
(33) PAÍS DE ORIGEN		CÓDIGO PAÍS	(31) NÚMERO	(32) FECHA (AAAA/MM/DD)		
1.ESTADOS UNIDOS DE AMÉRICA		US	62/189,158	2015/07/06		
2.ESTADOS UNIDOS DE AMÉRICA		US	62/269,060	2015/12/17		
10.2. DATOS SOLICITUD INTERNACIONAL PCT ☒ SI ☐ NO						
Solicitud Internacional No.		PCT/US2016/040520		Fecha 2016/06/30		
Publicación Internacional No.		WO/2017/007689		Fecha 2017/01/12		
*El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO						
*El solicitante presenta los resultados de búsqueda de la Oficina de Examen Anterior, junto con una traducción al castellano ☒ SI ☐ NO						
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS						
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:						
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:						
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO						
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO						
(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☒ SI ☐ NO						
(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad. ☒ SI ☐ NO						
11	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL					
Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)						
 Tarjeta Profesional 53.215						

TABLA DE CORRESPONDENCIAS

Reivindicaciones de la presente solicitud (NC 2017/0013351)	Reivindicaciones de la solicitud de patente Europea EP 16 738 976.6	Correspondencia de las reivindicaciones
1	1	Las reivindicaciones son idénticas.
2	2	Las reivindicaciones son idénticas.
3	3	Las reivindicaciones son idénticas.
4	4	Las reivindicaciones son idénticas.
5	5	Las reivindicaciones son idénticas.
6	6	Las reivindicaciones son idénticas.
7	7	Las reivindicaciones son idénticas.
8	8	Las reivindicaciones son idénticas.
9	9	Las reivindicaciones son idénticas.
10	10	Las reivindicaciones son idénticas.
11	11	Las reivindicaciones son idénticas.
12	12	Las reivindicaciones son idénticas.
13	13	Las reivindicaciones son idénticas.
14	14	Las reivindicaciones son idénticas.
15	15	Las reivindicaciones son idénticas.
16	16	Las reivindicaciones son idénticas.
17	17	Las reivindicaciones son idénticas.
18	18	Las reivindicaciones son idénticas.
N.A.	19 a 21	Dichas reivindicaciones de la solicitud de patente europea EP 16 738 976.6 no tienen reivindicaciones correspondientes en el pliego reivindicatorio de la presente solicitud, puesto que la materia definida en las mismas se refiere a usos, considerados materia no patentable de acuerdo a la Decisión Andina 486, por tanto, fueron eliminadas del pliego reivindicatorio de la presente solicitud.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference 1118P2F	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2016/040520	International filing date (<i>day/month/year</i>) 30 June 2016 (30.06.2016)	Priority date (<i>day/month/year</i>) 06 July 2015 (06.07.2015)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GILEAD SCIENCES, INC.		

1.	This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).																								
2.	This REPORT consists of a total of 8 sheets, including this cover sheet.																								
	In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.																								
3.	This report contains indications relating to the following items: <table style="width: 100%;"> <tr> <td style="width: 5%; text-align: center;">☒</td> <td style="width: 25%;">Box No. I</td> <td style="width: 70%;">Basis of the report</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. II</td> <td>Priority</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. III</td> <td>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. IV</td> <td>Lack of unity of invention</td> </tr> <tr> <td style="text-align: center;">☒</td> <td>Box No. V</td> <td>Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td> </tr> <tr> <td style="text-align: center;">☐</td> <td>Box No. VI</td> <td>Certain documents cited</td> </tr> <tr> <td style="text-align: center;">☒</td> <td>Box No. VII</td> <td>Certain defects in the international application</td> </tr> <tr> <td style="text-align: center;">☒</td> <td>Box No. VIII</td> <td>Certain observations on the international application</td> </tr> </table>	☒	Box No. I	Basis of the report	☐	Box No. II	Priority	☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	☐	Box No. IV	Lack of unity of invention	☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	☐	Box No. VI	Certain documents cited	☒	Box No. VII	Certain defects in the international application	☒	Box No. VIII	Certain observations on the international application
☒	Box No. I	Basis of the report																							
☐	Box No. II	Priority																							
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability																							
☐	Box No. IV	Lack of unity of invention																							
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement																							
☐	Box No. VI	Certain documents cited																							
☒	Box No. VII	Certain defects in the international application																							
☒	Box No. VIII	Certain observations on the international application																							
4.	The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis .2).																								

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Date of issuance of this report 09 January 2018 (09.01.2018) Authorized officer Agnès Wittmann-Regis e-mail: pct.team6@wipo.int
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PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/US2016/040520

International filing date (day/month/year)
30.06.2016

Priority date (day/month/year)
06.07.2015

International Patent Classification (IPC) or both national classification and IPC
INV. C07D401/14 C07D405/14 C07D413/14 C07D401/12 C07D409/14 C07D417/14 C07D471/04 C07D495/04
A61K31/4709 A61P29/00 A61P35/00

Applicant
GILEAD SCIENCES, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0
Fax: +49 89 2399 - 4465

Date of completion of
this opinion

see form
PCT/ISA/210

Authorized Officer

Beligny, Samuel

Telephone No. +49 89 2399-0



Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-77</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>19-27, 41-59</u>
	No: Claims	<u>1-18, 28-40, 60-77</u>
Industrial applicability (IA)	Yes: Claims	<u>1-77</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1 WU J ET AL: "Selective inhibitors of tumor progression loci-2 (Tpl2) kinase with potent inhibition of TNF-alpha production in human whole blood",
BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL,
vol. 19, no. 13, 7 May 2009 (2009-05-07), pages 3485-3488, XP026155092,
ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2009.05.009
[retrieved on 2009-05-07]
- D2 KAILA ET AL: "Identification of a novel class of selective Tpl2 kinase inhibitors: 4-Alkylamino-[1,7]naphthyridine-3-carbonitriles",
BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB,
vol. 15, no. 19, 4 July 2007 (2007-07-04), pages 6425-6442, XP022192446,
ISSN: 0968-0896, DOI: 10.1016/J.BMC.2007.06.054
- D3 RAKESH K GOYAL ET AL: "Models for anti-inflammatory activity of 8-substituted-4-anilino-6-aminoquinoline-3-carbonitriles",
MEDICINAL CHEMISTRY RESEARCH, BIRKHÄUSER-VERLAG, BOSTON,
vol. 21, no. 7, 19 March 2011 (2011-03-19), pages 1044-1055, XP035060999,
ISSN: 1554-8120, DOI: 10.1007/S00044-011-9613-5
- D4 NEAL GREEN ET AL: "Inhibitors of Tumor Progression Loci-2 (Tpl2) Kinase and Tumor Necrosis Factor [alpha] (TNF-[alpha]) Production: Selectivity and in Vivo Antiinflammatory Activity of Novel 8-Substituted-4-anilino-6-aminoquinoline-3-carbonitriles",
JOURNAL OF MEDICINAL CHEMISTRY,
vol. 50, no. 19, 2007, pages 4728-4745, XP055294201,
US
ISSN: 0022-2623, DOI: 10.1021/jm070436q

D5 WO 98/43960 A1 (AMERICAN CYANAMID CO [US]) 8 October 1998
(1998-10-08)

Novelty

1. D1-D4 disclose tumor progression locus (Tpl2) inhibitors (see D1, p. 3485-3486, compounds 1-4 and 7-37; D2, p. 6429-6430, compounds 35-43, 50, and 51; D3, p. 1046-1047, compounds 1-44; and D4, p. 4730-4732, compounds 2, 10-55).

The compounds of the present application differ from the compounds of D1-D4 in that the amine at the 6-position of the quinoline core is substituted by a methyl which bear two hetero-rings, when the corresponding amine in the compounds of D1-D4 are substituted by a methyl which bear only one hetero-ring.

D5 discloses quinoline based mitogen-activated protein kinase (MAPK) inhibitors (see D5, claims 1, 22, and 24). The compounds of the present application differ from the compounds of D5 also in that the amine at the 6-position of the quinoline core is substituted by a methyl which bear two hetero-rings, when the corresponding amine in the compounds of D5 cannot have such substituent (see definition of the R₂ substituent in claims 1 and 22, the most similar substituent being benzylamino).

Thus the compounds of the present application are new, and novelty is acknowledged to the subject-matter of claims 1-77 (Article 33(2) PCT).

Inventive Step

2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-18, 28-40, and 60-77 does not involve an inventive step in the sense of Article 33(3) PCT.

Any of D1-D4 could be regarded as the closest prior art.

No further effects appear to be linked to the distinguishing feature of the claimed compounds (*i.e.* the present of a second hetero-ring on the methyl substituting the amino group at the 6-position of the quinoline core). Thus the objective technical problem underlying the present application starting from any of D1-D4 is considered to be the provision of further Tpl2 inhibitors.

The data on p. 537-576 show that the compounds of the present application have the desired activity as they are cancer Osaka thyroid inhibitors, which is an other name for Tpl2.

Nevertheless, the present breadth of meanings claimed cannot be viewed as a reasonable generalisation of the specific embodiments disclosed, which show limited structural variation. Indeed only compounds wherein one of R^3 or R^4 is 1,2,3-triazol-4-yl have been exemplified. The same standards must be used when judging whether an activity can be expected based on the state of the art and whether it is credible that all members of a certain group of chemical compounds solve a particular technical problem.

If the Applicant is implying that minor structural modifications are in themselves non-obvious, then, by the same token, it is not credible that all the compounds falling within the scope of Formula (I) as given in claim 1 have the desired activity.

The technical problem needs therefore to be reformulated and can only be seen as the provision of further compounds. Problem for which no inventive step can be acknowledged.

2.2 On the other hand the subject-matter of claims 19-27, 41-59 is considered to represent a reasonable generalisation of the specific embodiments disclosed.

Moreover, the prior art does not suggest that adding a further hetero-ring on the methyl substituting the amino group at the 6-position of the quinoline core could solve the aforementioned technical problem.

An inventive step is thus acknowledged to the subject-matter of claims 19-27, 41-59 (Article 33(3) PCT).

Industrial Application

3. The subject-matter of claims 1-77 can find industrial applicability in the field of pharmacy, thus meets the requirements of Article 33(4) PCT.

Re Item VII

Certain defects in the international application

4. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in D1-D5 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

5.1 Claims 36-40 and 55-66 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

5.2 It should be noted that in the Regional phase this Authority does not accept references to therapeutic methods being part of the invention in the description, nor references to the prodrugs of the claimed compounds, to the spirit of the invention or the expression "incorporated by reference".

5.3 Claim 33 contains a reference to the description (Table 1). According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.

5.4 Claim 28 refers to claim 29. This inconsistency leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear, Article 6 PCT.

5.5 Claims 4, 5, 6, 19, 41, 43, 45, 47, 49, 51, and 53 appear to comprise all the features of claim 1 and are therefore not appropriately formulated as claims dependent on the latter (Rule 6.4 PCT).

5.6 For the sake of completeness, claim 35 should read "A composition comprising a mixture of stereoisomers of a compound of Formula (I) according to claim 1:"

5.6 The term "deuterated analog" in claims 1, 6, and 19 renders their scope unclear, since it is not known to the skilled reader which structures are intended to be encompassed by this term. Terms like "analog" or "deuterated analog" include an unlimited number of compounds obtainable from another compound by some undetermined chemical modification (including compounds which are structurally remote from the starting material), functional derivatives (such as compounds, wherein hetero atoms are exchanged by alternative atoms), compounds with numerous different types of side groups etc. There is, however, no clear definition to which extent the compounds according to claims 1, 6, and 19 may be modified while still being regarded as "deuterated analogs". This has the effect that the person skilled in the art cannot decide clearly which compounds are to be covered by the claims and which are not. Consequently, claims 1, 6, and 19 do not fulfil the requirements of Article 6 PCT.

TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES

PCT

INFORME DE EXAMEN PRELIMINAR INTERNACIONAL SOBRE LA PATENTABILIDAD

(Capítulo I del Tratado de Cooperación en Materia de Patentes)

(Regla 44bis del PCT)

Referencia de expediente del solicitante o su agente 1118P2F	PARA ACCIÓN ADICIONAL Ver punto 4 más adelante	
Solicitud Internacional No. PCT/US2016/040520	Fecha de presentación internacional (d/m/a) 30 de Junio de 2016 (30.06.2016)	Fecha de prioridad (d/m/a) 6 de Julio de 2015 (06.07.2015)
Clasificación Internacional de Patentes (8a edición a menos que se indique la edición anterior) Ver información relevante en el Formato PCT/ISA/237		
Solicitante GILEAD SCIENCES, INC.		

1. Este informe preliminar internacional acerca de la patentabilidad (Capítulo I) es emitido por la Oficina Internacional como la Autoridad Internacional de Búsqueda de acuerdo con la Regla 44 *bis* 1(a).
2. Este INFORME consiste de un total de 8 páginas, incluyendo esta carátula

En las hojas anexas debe entenderse cualquier referencia a la opinión escrita de la Autoridad Internacional de Búsqueda como una referencia al informe preliminar internacional acerca de la patentabilidad.

3. Este informe contiene indicaciones con respecto a lo siguiente:
 - ☒ Casilla No. I Base del informe
 - ☐ Casilla No. II Prioridad
 - ☐ Casilla No. III No establecimiento de una opinión con respecto a la novedad, el nivel inventivo y la aplicabilidad industrial
 - ☐ Casilla No. IV Falta de unidad de invención

- ☒ Casilla No. V Declaración razonada de acuerdo con el Artículo 35(2) con respecto a la novedad, el nivel inventivo o la aplicación industrial; citas y explicaciones que sustentan dicha declaración
- ☐ Casilla No. VI Ciertos documentos citados
- ☒ Casilla No. VII Ciertos defectos en la solicitud internacional
- ☒ Casilla No. VIII Ciertas observaciones en la solicitud internacional

4. La Oficina Internacional le enviará este informe a las Oficinas designadas de acuerdo con las Reglas 44bis3(c) y 93bis.1 a menos que el solicitante presente una solicitud de acuerdo con el Artículo 23(2) antes de la expiración de 30 meses a partir de la fecha de prioridad (Regla 44bis 2)

Fecha de emisión de este informe Enero 9 de 2018(09.01.2018)	
Oficina internacional de la OMPI 34, chemin des Colombettes 1211 Ginebra 20, Suiza	Oficial autorizado Agnes Wittmann-Regis Email: pct.team6@wipo.int

TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES

PCT

OPINIÓN ESCRITA DE LA AUTORIDAD INTERNACIONAL DE BÚSQUEDA

(Regla 43bis.1 del PCT)

		Fecha de envío (día/mes/año) ver formulario PCT / ISA / 210 (segunda hoja)
Referencia de expediente del solicitante o su agente ver formulario PCT/ISA/220		PARA ACCIÓN FUTURA Ver el párrafo 2 abajo
Solicitud Internacional No. PCT/US2016/040520	Fecha de presentación internacional (día / mes / año) 30.06.2016	Fecha de prioridad (día / mes / año) 06.07.2015
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Solicitante GILEAD SCIENCES, INC.		

1. Esta opinión contiene indicaciones relativas a los siguientes puntos:

- ☒ Casilla No. I Bases de la opinión.
- ☐ Casilla No. II Prioridad
- ☐ Casilla No. III No establecimiento de opinión con respecto a novedad,
nivel inventivo y aplicabilidad industrial
- ☐ Casilla No. IV Falta de unidad de invención.
- ☒ Casilla No. V Declaración razonada según la Regla 43b / s.1 (a) (i) con
respecto a la novedad, actividad inventiva y aplicabilidad industrial; citas y explicaciones
que apoyan tal declaración
- ☐ Casilla No. VI Ciertos documentos citados
- ☒ Casilla No. VII Ciertos defectos en la solicitud internacional
- ☒ Casilla No. VIII Ciertas observaciones sobre la solicitud internacional

2. ACCIONES ADICIONALES

Si se realiza una solicitud de examen preliminar internacional, esta opinión generalmente se considerará como una opinión escrita de la Autoridad de

Examen Preliminar internacional ("IPEA"), excepto que esto no se aplicará cuando el solicitante elija una Autoridad diferente a esta para actuar como la IPEA y la IPEA elegida haya notificado a la Oficina Internacional de conformidad con la Regla 66.1b / s (b) que las opiniones escritas de esta Autoridad de Búsqueda Internacional no se considerarán como tal.

Si se considera que esta opinión, como se indicó anteriormente, es una opinión escrita de la IPEA, se invita al solicitante a enviar una respuesta escrita a la IPEA conjuntamente, cuando corresponda, con modificaciones, antes del vencimiento de 3 meses contados a partir de la fecha de envío del Formulario PCT / ISA / 220 o antes del vencimiento de 22 meses contados desde la fecha de prioridad, lo que ocurra más tarde.

Para más opciones, vea el formulario PCT / ISA / 220.

OPINIÓN ESCRITA DE LA AUTORIDAD INTERNACIONAL DE BÚSQUEDA

Casilla No. I Fundamento de la opinión

1. Con respecto al idioma, esta opinión se ha establecido sobre la base de:
 - ☒ la solicitud internacional en el idioma en que fue presentada.
 - ☐ una traducción de la solicitud internacional a ____, que es el idioma de una traducción proporcionada para los fines de búsqueda internacional (Reglas 12.3 (a) y 23.1 (b)).
2. ☐ Esta opinión se ha establecido teniendo en cuenta la **rectificación de un error evidente** autorizado por o notificado a esta Autoridad según la Regla 91 (Regla 43b / s.1 (a))
3. ☐ Con respecto a cualquier **secuencia de nucleótidos y/o aminoácidos** descrita en la solicitud internacional, esta opinión se ha establecido sobre la base de una lista de secuencia:
 - a. ☐ que forma parte de la solicitud internacional tal como fue presentada:
 - ☐ en la forma de un archivo de texto del Anexo C / ST.25.
 - ☐ en papel o en forma de archivo de imagen segundo.
 - b. ☐ suministrado junto con la solicitud internacional conforme a la Regla 13fer.1 (a) el PCT para fines de búsqueda internacional solo en forma de un archivo de texto del Anexo C / ST.25.
 - c. ☐ suministrado después de la fecha de presentación internacional solo para fines de búsqueda internacional:
 - ☐ en forma de un archivo de texto del Anexo C / ST.25 (Regla 13fer.1 (a)).
 - ☐ en papel o en la forma de un archivo de imagen (Regla 13fer.1 (b) e Instrucciones Administrativas, Sección 713).
4. ☐ Además, en el caso de que se haya presentado o proporcionado más de una versión o copia de una lista de secuencias, las declaraciones requeridas de que la información en las copias subsiguientes o adicionales son idénticas a las que forman parte de la solicitud tal como fueron presentadas o no van más allá de la solicitud tal como fue presentada, según corresponda.
5. Comentarios adicionales:

Casilla No. V Declaración razonada según la Regla 43b / s.1 (a) (i) con respecto a la novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan tal declaración

1. Declaración

Novedad (N)	Sí: Reivindicaciones	<u>1-77</u>
	No: Reivindicaciones	
Nivel Inventivo (IS)	Sí: Reivindicaciones	<u>19-27, 41-59</u>
	No: Reivindicaciones	<u>1-18, 28-40, 60-77</u>
Aplicabilidad industrial (IA)	Sí: Reivindicaciones	<u>1-77</u>
	No: Reivindicaciones	

2. Citas y explicaciones

ver hoja aparte

Casilla No. VII Ciertos defectos en la solicitud internacional.

Se han observado los siguientes defectos en la forma o el contenido de la solicitud internacional:

ver hoja aparte

Casilla No. VIII Ciertas observaciones sobre la solicitud internacional.

Se realizan las siguientes observaciones sobre la claridad de las reivindicaciones, la descripción y los dibujos o sobre la cuestión de si las declaraciones están totalmente respaldadas por la descripción:

ver hoja aparte

Re artículo V

Declaración razonada con respecto a la novedad, nivel inventivo o aplicabilidad industrial; citas y explicaciones que sustentan tal declaración

Se hace referencia a los siguientes documentos:

- D1 WU J ET AL: "Selective inhibitors of tumor progression loci-2 (Tpl2) kinase with potent inhibition of TNF-alpha production in human whole blood", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL,
vol. 19, no. 13, 7 Mayo 2009 (2009-05-07), páginas 3485-3488,
XP026155092,
ISSN: 0960-894X, DOI: 10.1016 / J.BMCL.2009.05.009
[consultado el 2009-05-07]
- D2 KAILA ET AL: "Identification of a novel class of selective Tpl2 kinase inhibitors: 4-Alkylamino-[1,7]naphthyridine-3-carbonitriles", BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB,
vol. 15, no. 19, 4 julio 2007 (2007-07-04), páginas 6425-6442,
XP022192446,
ISSN: 0968-0896, DOI: 10.1016/J.BMC.2007.06.054
- D3 RAKESH K GOYAL ET AL: "Models for anti.inflammatory activity of 8-substitued-4-anilino-6-aminoquinoline-3-carbonitriles",
MEDICINAL CHEMISTRY RESEARCH, BIRKHÄUSER-VERLAG, BOSTON,
vol. 21, no. 7, 19 marzo 2011 (2011-03-19), páginas 1044-1055,
XP035060999,
ISSN: 1554-8120, DOI: 10.1007 / S00044-011-9613-5
- D4 NEAL GREEN ET AL: "Inhibitors of Tumor Progression Loci.2 (Tpl2) Kinase and Tumor Necrosis Factor [alpha] (TNF-[alpha]) Production: Selectivity and in Vivo Antiinflammatory Activity of Novel 8-Substitued-4-anilino-6-aminoquinoline-3-carbonitriles",
JOURNAL OF MEDICINAL CHEMISTRY,
vol. 50, no. 19, 2007, páginas 4728-4745, XP055294201,

US

ISSN: 0022-2623, DOI: 10.1021 / jm070436q

D5 WO 98/43960 A1 (AMERICAN CYANAMID CO [US]) 8 de octubre de 1998 (1998-10-08)

Novedad

1. D1-D4 describe inhibidores de la progresión del locus tumoral (Tpl2) (ver D1, pág. 3485-3486, compuestos 1-4 y 7-37; D2, pág. 6429-6430, compuestos 35-43, 50 y 51; D3, pág. 1046-1047, compuestos 1-44; y D4, pág. 4730-4732, compuestos 2, 10-55).

Los compuestos de la presente solicitud se diferencian de los compuestos de D1-D4 en que la amina en la posición 6 del núcleo de quinolina está sustituida por metilo que lleva dos hetero-anillos, cuando la amina correspondiente en los compuestos de D1-D4 está sustituida por un metilo que lleva solo un hetero- anillo.

D5 describe inhibidores de la proteína quinasa activada por mitógeno a base de quinolina (MAPK) (ver D5, reivindicaciones 1, 22 y 24). Los compuestos de la presente solicitud difieren de los compuestos de D5 también en que la amina en la posición 6 del núcleo de quinolina está sustituida por un metilo que lleva dos hetero-anillos, cuando la amina correspondiente en los compuestos de D5 no puede tener tales sustituyente (véase la definición del sustituyente R₂ en las reivindicaciones 1 y 22, siendo el sustituyente más similar bencilamino).

Por lo tanto, los compuestos de la presente solicitud son nuevos y la novedad se reconoce en la materia de las reivindicaciones 1-77 (Artículo 33 (2) del PCT).

Nivel Inventivo

2.1 La presente solicitud no cumple con los criterios del Artículo 33 (1) PCT, porque el objeto de las reivindicaciones 1-18, 28-40 y 60-77 no implica un nivel inventivo en el sentido del Artículo 33 (3) PCT.

Cualquiera de D1-D4 podría considerarse como el arte previo más cercano.

No parece que otros efectos estén relacionados con la característica distintiva de los compuestos reivindicados (es decir, la presencia de un segundo hetero-anillo en el metilo que sustituye al grupo amino en la posición 6 del núcleo de quinolina). Por lo tanto, el problema técnico objetivo que subyace en la presente solicitud a partir de cualquiera de D1-D4 se considera la provisión de inhibidores de Tpl2 adicionales.

Los datos en p. 537-576 muestran que los compuestos de la presente solicitud tienen la actividad deseada, ya que son inhibidores de la tiroides de Osaka para el cáncer, que es otro nombre para Tpl2.

Sin embargo, la amplitud actual de significados reivindicados no puede considerarse como una generalización razonable de las realizaciones específicas divulgadas, que muestran una variación estructural limitada. De hecho, solo se han ejemplificado los compuestos en los que uno de R_3 o R_4 es 1,2,3-triazol-4-ilo. Se deben utilizar los mismos estándares al juzgar si una actividad puede esperarse según el estado de la técnica y si es creíble que todos los miembros de un determinado grupo de compuestos químicos resuelven un problema técnico particular.

Si el solicitante implica que las modificaciones estructurales menores no son en sí mismas obvias, entonces, de la misma manera, no es creíble que todos los compuestos que están dentro del alcance de la Fórmula (I) como se indica en la reivindicación 1 tengan la actividad deseada.

Por lo tanto, el problema técnico debe reformularse y solo puede verse como la provisión de otros compuestos. Problema para el que no se puede reconocer ningún nivel, inventivo.

2.2 Por otra parte, se considera que el objeto de las reivindicaciones 19-27, 41-59 representa una generalización razonable de las realizaciones específicas descritas.

Además, el arte previo no sugiere que agregar un hetero-anillo adicional en el metilo sustituyendo al grupo amino en la posición 6 del núcleo de quinolina podría resolver el problema técnico mencionado anteriormente.

Por lo tanto, se reconoce un nivel inventivo en el objeto de las reivindicaciones 19-27, 41-59 (Artículo 33 (3) del PCT).

Aplicación industrial

3. El objeto de las reivindicaciones 1-77 puede encontrar la aplicabilidad industrial en el campo de la farmacia, por lo tanto cumple con los requisitos del Artículo 33 (4) del PCT.

Re artículo VII

Ciertos defectos en la solicitud internacional.

4. Contrariamente a los requisitos de la Regla 5.1 (a) (ii) del PCT, el arte previo relevante descrito en D1-D5 no se menciona en la descripción, ni estos documentos están identificados en la misma.

Re Item VIII

Ciertas observaciones sobre la solicitud internacional.

5.1 Las reivindicaciones 36-40 y 55-66 se relacionan con el Objeto que esta Autoridad considera que está cubierto por las disposiciones de la Regla 39.1 (iv) / 67.1 (iv) PCT. La patentabilidad puede depender de la formulación de las reivindicaciones. La EPO, por ejemplo, no reconoce como reivindicaciones patentables el uso de un compuesto en el tratamiento médico, pero puede permitir reivindicaciones a un producto, en sustancias o composiciones en particular para usar en un primer tratamiento médico o uno adicional.

5.2 Cabe señalar que, en la fase regional, esta Autoridad no acepta referencias a métodos terapéuticos que formen parte de la invención en la descripción, ni referencias a los profármacos de los compuestos reivindicados, al espíritu de la invención o la expresión "incorporada por referencia".

5.3 La reivindicación 33 contiene una referencia a la descripción (Tabla 1). De acuerdo con la Regla 6.2 (a) del PCT, las reivindicaciones no deben contener tales referencias, excepto cuando sea absolutamente necesario, lo que no ocurre en este caso.

5.4 La reivindicación 28 se refiere a la reivindicación 29. Esta inconsistencia lleva a dudas sobre el objeto para el que se solicita la protección, lo que hace que las reivindicaciones no sean claras, Artículo 6 del PCT.

5.5 Las reivindicaciones 4, 5, 6, 19, 41, 43, 45, 47, 49, 51 y 53 parecen comprender todas las características de la reivindicación 1 y, por lo tanto, no están formuladas de manera adecuada como una reivindicación dependiente de esta última (Regla 6.4 del PCT).

5.6 En aras de la exhaustividad, la reivindicación 35 debe leer "Una composición que comprende una mezcla de estereoisómeros de un compuesto de Fórmula (I) de acuerdo con la reivindicación 1"

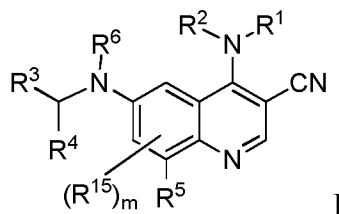
5.6 El término "análogo deuterado" en las reivindicaciones 1, 6 y 19 hace que su alcance no sea claro, ya que el lector experto no sabe qué estructuras deben incluirse

en este término. Los términos como "análogo" o "análogo deuterado" incluyen un número ilimitado de compuestos que se pueden obtener de otro compuesto por alguna modificación química indeterminada (incluyendo los compuestos que están estructuralmente alejados del material de partida), derivados funcionales (como los compuestos, en donde los heteroátomos son intercambiados por átomos alternativos), compuestos con numerosos tipos diferentes de grupos laterales, etc. Sin embargo, no existe una definición clara de hasta qué punto los compuestos según las reivindicaciones 1, 6 y 19 pueden modificarse mientras se consideran como "análogos deuterados". Esto tiene el efecto de que la persona versada en la materia no puede decidir claramente qué compuestos deben ser cubiertos por las reivindicaciones y cuáles no. En consecuencia, las reivindicaciones 1, 6 y 19 no cumplen con los requisitos del Artículo 6 del PCT.

EP 16 738 976.6
 Gilead Sciences, Inc.
 Our Ref.: 411-142 PCTEP

CLAIMS:

1. A compound of Formula I:



- 5 wherein

R^1 is hydrogen, $-O-R^7$, $-N(R^8)(R^9)$, $-C(O)-R^7$, $-S(O)_2-R^7$, $-C_{1-9}$ alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-15} cycloalkyl, heterocyclyl, aryl, or heteroaryl;

wherein each C_{1-9} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-15} cycloalkyl, heterocyclyl, aryl, and heteroaryl may be optionally substituted with one to four Z^1 ;

- 10 R^2 is hydrogen, $-C(O)-R^7$, $-C(O)O-R^7$, $-C(O)N(R^7)_2$, C_{1-9} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-15} cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C_{1-9} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkyl, C_{3-15} cycloalkyl, aryl, heterocyclyl, and heteroaryl may be optionally substituted with one to four Z^2 ;

- 15 or R^1 and R^2 together with the nitrogen to which they are attached form a heterocyclyl or heteroaryl, wherein each heterocyclyl or heteroaryl is optionally substituted with one to four Z^2 ;

R^3 is heterocyclyl or heteroaryl, wherein each heterocyclyl or heteroaryl is optionally substituted with one to four Z^3 ;

- 20 R^4 is heterocyclyl or heteroaryl, wherein each heterocyclyl or heteroaryl is optionally substituted with one to four Z^4 ;

R^5 is hydrogen, halo, $-CN$, $-NO_2$, $-O-R^7$, $-N(R^8)(R^9)$, $-S(O)-R^7$, $-S(O)_2R^7$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-OC(O)-R^7$, $-C(O)O-R^7$, $-OC(O)O-R^7$, $-OC(O)N(R^{10})(R^{11})$, $-C(O)N(R^7)_2$, $-N(R^7)C(O)(R^7)$, C_{1-9} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-9} alkylthio, C_{1-6} haloalkyl, C_{3-15}

- 25 cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₉ alkylthio, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, and heteroaryl may be optionally substituted with one to four Z⁵;

R⁶ is hydrogen, -C(O)-R⁷, -C(O)O-R⁷, -C(O)N(R⁷)₂, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆

5 alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, and heteroaryl may be optionally substituted with one to four Z⁶;

each R⁷ is independently hydrogen, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl,

10 C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, and heteroaryl may be optionally substituted with one to four Z⁷;

R⁸ and R⁹ at each occurrence are independently hydrogen, -S(O)₂R¹⁰, -C(O)-

15 R¹⁰, -C(O)O-R¹⁰, -C(O)N(R¹⁰)(R¹¹), C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl may be optionally substituted with one to four Z⁸;

20 R¹⁰ and R¹¹ at each occurrence are independently hydrogen, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl,

wherein each C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, and heteroaryl optionally is substituted with one to four Z^{1b};

25 each Z¹, Z², Z³, Z⁴, Z⁵, Z⁶, Z⁷, and Z⁸ is independently hydrogen, oxo, halo, -NO₂, -N₃, -CN, thioxo, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₅ cycloalkyl, C₁₋₈ haloalkyl, aryl, heteroaryl, heterocyclyl, -O-R¹², -C(O)-R¹², -C(O)O-R¹², -C(O)-N(R¹³)(R¹⁴), -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -N(R¹²)C(O)-R¹², -N(R¹²)C(O)O-R¹², -N(R¹²)C(O)N(R¹³)(R¹⁴), -N(R¹²)S(O)₂(R¹²), -NR¹²S(O)₂N(R¹³)(R¹⁴), -NR¹²S(O)₂O(R¹²), -OC(O)R¹², -OC(O)-

30 N(R¹³)(R¹⁴), -P(O)(OR¹²)₂, -OP(O)(OR¹²)₂, -CH₂P(O)(OR¹²)₂, -OCH₂P(O)(OR¹²)₂, -C(O)OCH₂P(O)(OR¹²)₂, -P(O)(R¹²)(OR¹²), -OP(O)(R¹²)(OR¹²), -CH₂P(O)(R¹²)(OR¹²),

-OCH₂P(O)(R¹²)(OR¹²), -C(O)OCH₂P(O)(R¹²)(OR¹²), -P(O)(N(R¹²)₂)₂, -OP(O)(N(R¹²)₂)₂,
 -CH₂P(O)(N(R¹²)₂)₂, -OCH₂P(O)(N(R¹²)₂)₂, -C(O)OCH₂P(O)(N(R¹²)₂)₂,
 -P(O)(N(R¹²)₂)(OR¹²), -OP(O)(N(R¹²)₂)(OR¹²), -CH₂P(O)(N(R¹²)₂)(OR¹²),
 -OCH₂P(O)(N(R¹²)₂)(OR¹²), -C(O)OCH₂P(O)(N(R¹²)₂)(OR¹²), -P(O)(R¹²)(N(R¹²)₂),
 5 -OP(O)(R¹²)(N(R¹²)₂), -CH₂P(O)(R¹²)(N(R¹²)₂), -OCH₂P(O)(R¹²)(N(R¹²)₂),
 -C(O)OCH₂P(O)(R¹²)(N(R¹²)₂), -Si(R¹²)₃, -S-R¹², -S(O)R¹², -S(O)(NH)R¹², -S(O)₂R¹²
 or -S(O)₂N(R¹³)(R¹⁴);

wherein any alkyl, alkenyl, alkynyl, cycloalkyl, haloalkyl, aryl, heteroaryl or heterocyclyl is optionally substituted with one to four Z^{1a} groups;

10 each Z^{1a} is independently oxo, halo, thioxo, -NO₂, -CN, -N₃, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₅ cycloalkyl, C₁₋₈ haloalkyl, aryl, heteroaryl, heterocyclyl, -O-R¹², -C(O)R¹²,
 -C(O)O-R¹², -C(O)N(R¹³)(R¹⁴), -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -N(R¹²)-C(O)R¹²,
 -N(R¹²)C(O)O(R¹²), -N(R¹²)C(O)N(R¹³)(R¹⁴), -N(R¹²)S(O)₂(R¹²), -N(R¹²)S(O)₂-
 N(R¹³)(R¹⁴), -N(R¹²)S(O)₂O(R¹²), -OC(O)R¹², -OC(O)OR¹², -OC(O)-N(R¹³)(R¹⁴),
 15 -Si(R¹²)₃, -S-R¹², -S(O)R¹², -S(O)(NH)R¹², -S(O)₂R¹² or -S(O)₂N(R¹³)(R¹⁴);

wherein any alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl or heterocyclyl is optionally substituted with one to four Z^{1b} groups;

each R¹² is independently hydrogen, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₅ cycloalkyl, aryl, heteroaryl or heterocyclyl,

20 wherein any alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl or heterocyclyl is optionally substituted with one to four Z^{1b} groups;

R¹³ and R¹⁴ at each occurrence are each independently hydrogen, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₅ cycloalkyl, aryl, heteroaryl or heterocyclyl;

25 wherein any alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl or heterocyclyl is optionally substituted with one to four Z^{1b} groups, or R¹³ and R¹⁴ together with the nitrogen to which they are attached form a heterocyclyl, wherein said heterocyclyl is optionally substituted with one to four Z^{1b} groups;

each R¹⁵ is independently halo, -CN, -NO₂, -O-R⁷, -N(R⁸)(R⁹), -S(O)-R⁷, -S(O)₂R⁷,
 -S(O)₂N(R⁷)₂, -C(O)R⁷, -OC(O)-R⁷, -C(O)O-R⁷, -OC(O)O-R⁷, -OC(O)N(R¹⁰)(R¹¹),
 30 -C(O)N(R⁷)₂, -N(R⁷)C(O)(R⁷), C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₉ alkylthio, C₁₋₆ haloalkyl, C₃₋₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl; and

each Z^{1b} is independently oxo, thioxo, hydroxy, halo, -NO₂, -N₃, -CN, C₁₋₉ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₃₋₁₅ cycloalkyl, C₁₋₈ haloalkyl, aryl, heteroaryl, heterocyclyl, -O(C₁₋₉ alkyl), -O(C₂₋₆ alkenyl), -O(C₂₋₆ alkynyl), -O(C₃₋₁₅ cycloalkyl), -O(C₁₋₈ haloalkyl), -O(aryl), -O(heteroaryl), -O(heterocyclyl), -NH₂, -NH(C₁₋₉ alkyl), -NH(C₂₋₆ alkenyl), -NH(C₂₋₆ alkynyl), -NH(C₃₋₁₅ cycloalkyl), -NH(C₁₋₈ haloalkyl), -NH(aryl), -NH(heteroaryl), -NH(heterocyclyl), -N(C₁₋₉ alkyl)₂, -N(C₂₋₆ alkenyl)₂, -N(C₂₋₆ alkynyl)₂, -N(C₃₋₁₅ cycloalkyl)₂, -N(C₁₋₈ haloalkyl)₂, -N(aryl)₂, -N(heteroaryl)₂, -N(heterocyclyl)₂, -N(C₁₋₉ alkyl)(C₂₋₆ alkenyl), -N(C₁₋₉ alkyl)(C₂₋₆ alkynyl), -N(C₁₋₉ alkyl)(C₃₋₁₅ cycloalkyl), -N(C₁₋₉ alkyl)(C₁₋₈ haloalkyl), -N(C₁₋₉ alkyl)(aryl), -N(C₁₋₉ alkyl)(heteroaryl), -N(C₁₋₉ alkyl)(heterocyclyl), -C(O)(C₁₋₉ alkyl), -C(O)(C₂₋₆ alkenyl), -C(O)(C₂₋₆ alkynyl), -C(O)(C₃₋₁₅ cycloalkyl), -C(O)(C₁₋₈ haloalkyl), -C(O)(aryl), -C(O)(heteroaryl), -C(O)(heterocyclyl), -C(O)O(C₁₋₉ alkyl), -C(O)O(C₂₋₆ alkenyl), -C(O)O(C₂₋₆ alkynyl), -C(O)O(C₃₋₁₅ cycloalkyl), -C(O)O(C₁₋₈ haloalkyl), -C(O)O(aryl), -C(O)O(heteroaryl), -C(O)O(heterocyclyl), -C(O)NH₂, -C(O)NH(C₁₋₉ alkyl), -C(O)NH(C₂₋₆ alkenyl), -C(O)NH(C₂₋₆ alkynyl), -C(O)NH(C₃₋₁₅ cycloalkyl), -C(O)NH(C₁₋₈ haloalkyl), -C(O)NH(aryl), -C(O)NH(heteroaryl), -C(O)NH(heterocyclyl), -C(O)N(C₁₋₉ alkyl)₂, -C(O)N(C₂₋₆ alkenyl)₂, -C(O)N(C₂₋₆ alkynyl)₂, -C(O)N(C₃₋₁₅ cycloalkyl)₂, -C(O)N(C₁₋₈ haloalkyl)₂, -C(O)N(aryl)₂, -C(O)N(heteroaryl)₂, -C(O)N(heterocyclyl)₂, -NHC(O)(C₁₋₉ alkyl), -NHC(O)(C₂₋₆ alkenyl), -NHC(O)(C₂₋₆ alkynyl), -NHC(O)(C₃₋₁₅ cycloalkyl), -NHC(O)(C₁₋₈ haloalkyl), -NHC(O)(aryl), -NHC(O)(heteroaryl), -NHC(O)(heterocyclyl), -NHC(O)O(C₁₋₉ alkyl), -NHC(O)O(C₂₋₆ alkenyl), -NHC(O)O(C₂₋₆ alkynyl), -NHC(O)O(C₃₋₁₅ cycloalkyl), -NHC(O)O(C₁₋₈ haloalkyl), -NHC(O)O(aryl), -NHC(O)O(heteroaryl), -NHC(O)O(heterocyclyl), -NHC(O)NH(C₁₋₉ alkyl), -NHC(O)NH(C₂₋₆ alkenyl), -NHC(O)NH(C₂₋₆ alkynyl), -NHC(O)NH(C₃₋₁₅ cycloalkyl), -NHC(O)NH(C₁₋₈ haloalkyl), -NHC(O)NH(aryl), -NHC(O)NH(heteroaryl), -NHC(O)NH(heterocyclyl), -SH, -S(C₁₋₉ alkyl), -S(C₂₋₆ alkenyl), -S(C₂₋₆ alkynyl), -S(C₃₋₁₅ cycloalkyl), -S(C₁₋₈ haloalkyl), -S(aryl), -S(heteroaryl), -S(heterocyclyl), -NHS(O)(C₁₋₉ alkyl), -N(C₁₋₉ alkyl)S(O)(C₁₋₉ alkyl), -S(O)N(C₁₋₉ alkyl)₂, -S(O)(C₁₋₉ alkyl), -S(O)(NH)(C₁₋₉ alkyl), -S(O)(C₂₋₆ alkenyl), -S(O)(C₂₋₆ alkynyl), -S(O)(C₃₋₁₅ cycloalkyl), -S(O)(C₁₋₈ haloalkyl), -S(O)(aryl), -S(O)(heteroaryl), -S(O)(heterocyclyl), -S(O)₂(C₁₋₉ alkyl), -S(O)₂(C₂₋₆ alkenyl), -S(O)₂(C₂₋₆ alkynyl), -S(O)₂(C₃₋₁₅ cycloalkyl), -S(O)₂(C₁₋₈ haloalkyl), -S(O)₂(aryl), -S(O)₂(heteroaryl), -S(O)₂(heterocyclyl), -S(O)₂NH(C₁₋₉ alkyl), or -S(O)₂N(C₁₋₉ alkyl)₂;

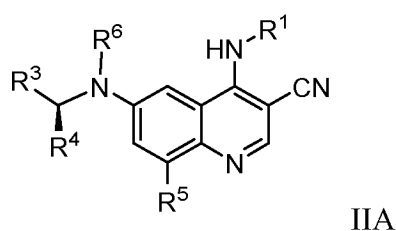
wherein any alkyl, cycloalkyl, aryl, heteroaryl, or heterocyclyl is optionally substituted with one to four halo, C₁₋₉ alkyl, C₁₋₈ haloalkyl, -OH, -NH₂, -NH(C₁₋₉ alkyl), -NH(C₃₋₁₅ cycloalkyl), -NH(C₁₋₈ haloalkyl), -NH(aryl), -NH(heteroaryl), -NH(heterocyclyl), -N(C₁₋₉ alkyl)₂, -N(C₃₋₁₅ cycloalkyl)₂,
 5 -NHC(O)(C₃₋₁₅ cycloalkyl), -NHC(O)(C₁₋₈ haloalkyl), -NHC(O)(aryl), -NHC(O)(heteroaryl), -NHC(O)(heterocyclyl), -NHC(O)O(C₁₋₉ alkyl), -NHC(O)O(C₂₋₆ alkynyl), -NHC(O)O(C₃₋₁₅ cycloalkyl), -NHC(O)O(C₁₋₈ haloalkyl), -NHC(O)O(aryl), -NHC(O)O(heteroaryl), -NHC(O)O(heterocyclyl), -NHC(O)NH(C₁₋₉ alkyl), -S(O)(NH)(C₁₋₉ alkyl), S(O)₂(C₁₋₉ alkyl), -S(O)₂(C₃₋₁₅ cycloalkyl), -S(O)₂(C₁₋₈ haloalkyl), -S(O)₂(aryl), -S(O)₂(heteroaryl),
 10 -S(O)₂(heterocyclyl), -S(O)₂NH(C₁₋₉ alkyl), -S(O)₂N(C₁₋₉ alkyl)₂, -O(C₃₋₁₅ cycloalkyl), -O(C₁₋₈ haloalkyl), -O(aryl), -O(heteroaryl), -O(heterocyclyl), or -O(C₁₋₉ alkyl);

m is 0, 1, or 2;

15 or a pharmaceutically acceptable salt, stereoisomer, mixture of stereoisomers, or deuterated analog thereof.

2. The compound of claim 1, wherein

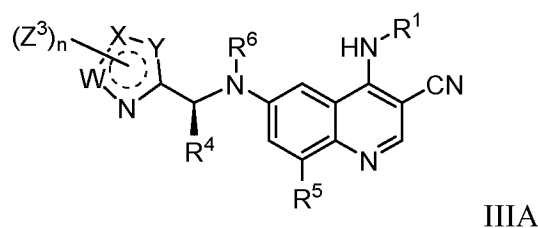
- (a) R² is hydrogen and/or m is 0, or a pharmaceutically acceptable salt thereof;
- (b) the compound has the Formula IIA:



20

wherein R¹, R³, R⁴, R⁵ and R⁶ are as defined in claim 1, or a pharmaceutically acceptable salt thereof; or

(c) the compound has the Formula IIIA:



wherein R¹, R⁴, R⁵, R⁶ and Z³ are as defined in claim 1,

W, X and Y are each independently N or C; and

5 n is 1, 2, or 3;

or a pharmaceutically acceptable salt, stereoisomer, mixture of stereoisomers or deuterated analog thereof.

3. The compound of claim 1 or 2, wherein

10 (i) R⁵ is hydrogen, halo, -CN, O-R⁷, -S(O)-R⁷, -S(O)₂R⁷, -S(O)₂N(R⁷)₂, -C(O)R⁷, -C(O)N(R⁷)₂, C₁-₉ alkyl, C₂-₆ alkenyl, C₂-₆ alkynyl, C₃-₁₅ cycloalkyl, aryl, heterocyclyl, or heteroaryl;

wherein each C₁-₉ alkyl, C₂-₆ alkenyl, C₂-₆ alkynyl, C₃-₁₅ cycloalkyl, aryl, heterocyclyl, and heteroaryl may be optionally substituted with one to four Z⁵, or a pharmaceutically acceptable salt thereof, or

15 R⁵ is hydrogen, halo, -CN, -C(O)R⁷, -O-R⁷, -S(O)₂R⁷ or heteroaryl, or a pharmaceutically acceptable salt thereof; and/or

(ii) R⁶ is hydrogen, or a pharmaceutically acceptable salt thereof; and/or

20 (iii) R¹ is -O-R⁷, C₁-₉ alkyl, C₃-₁₅ cycloalkyl, heterocyclyl, aryl, or heteroaryl; and said C₁-₉ alkyl, C₃-₁₅ cycloalkyl, heterocyclyl, aryl, or heteroaryl may be optionally substituted with one to four substituents independently selected the group consisting of halo, -CN, -O-R¹², -S(O)₂R¹², C₁-₉ alkyl, C₁-₉ haloalkyl, C₃-₁₅ cycloalkyl, heterocyclyl, and aryl, wherein said C₃-₁₅ cycloalkyl may be optionally substituted with one to four substituents independently selected the group consisting of C₁-₉ alkyl, and C₁-₉ haloalkyl, or a pharmaceutically acceptable salt thereof, or

25

R¹ is C₁₋₉ alkyl, optionally substituted with one to three substituents independently selected the group consisting of halo, -CN, -O-R¹², -S(O)₂R¹², C₃₋₁₅ cycloalkyl, heterocyclyl, and aryl, wherein said C₃₋₁₅ cycloalkyl or heterocyclyl may be optionally substituted with one to four substituents independently selected the group consisting of C₁₋₉ alkyl, and C₁₋₉ haloalkyl, or a pharmaceutically acceptable salt thereof, or

R¹ is C₃₋₁₅ cycloalkyl, heterocyclyl or heteroaryl, wherein said C₃₋₁₅ cycloalkyl, heterocyclyl or heteroaryl is optionally substituted with one to three substituents independently selected the group consisting of halo, -CN, -O-R¹², C₁₋₉ alkyl, and aryl, or a pharmaceutically acceptable salt thereof, or

R¹ is heterocyclyl or heteroaryl, wherein said heterocyclyl or heteroaryl is optionally substituted with one to three substituents independently selected the group consisting of halo, and C₁₋₉ alkyl, or a pharmaceutically acceptable salt thereof, or

R¹ is aryl, optionally substituted with one to three substituents independently selected the group consisting of halo, -CN, -O-R⁷, C₁₋₉ alkyl, and aryl, or a pharmaceutically acceptable salt thereof, or

R¹ is aryl, optionally substituted with one to three substituents independently selected the group consisting of halo, -O-R⁷, and C₁₋₉ alkyl, or a pharmaceutically acceptable salt thereof.

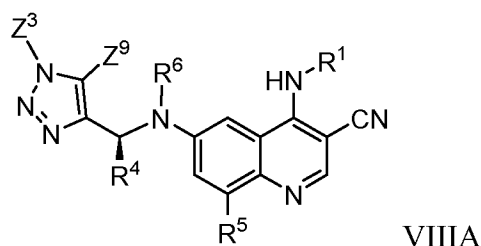
4. The compound of claim 2(c), wherein

(1) W is N, X is N-Z³, and Y is C-Z³, or a pharmaceutically acceptable salt thereof; and/or

(2) Z³ is hydrogen or C₁₋₉ alkyl optionally substituted with one to four substituents independently selected from the group consisting of -CN, halo, -O-R¹², -C(O)O-R¹², -OC(O)-R¹², -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -C(O)N(R¹²)-S(O)₂R¹², C₁₋₉ alkyl, heterocyclyl, aryl, and heteroaryl, or a pharmaceutically acceptable salt thereof, or

Z^3 is C_{3-15} cycloalkyl, heterocyclyl, aryl, or heteroaryl; and said C_{3-15} cycloalkyl, heterocyclyl, aryl, or heteroaryl may be optionally substituted with one to four substituents independently selected from the group consisting of -CN, halo, -O- R^{12} , -C(O)- R^{12} , -C(O)O- R^{12} , -OC(O)- R^{12} , -N(R^{13})(R^{14}), -N(R^{13})₂(R^{14})⁺, C_{1-9} alkyl, C_{1-8} haloalkyl, C_{1-8} hydroxyalkyl, C_{3-15} cycloalkyl, heterocyclyl, and heteroaryl, or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 having the Formula VIIIA:



wherein Z^3 , R^1 , R^4 , R^5 and R^6 are as defined in claim 1, and Z^9 is hydrogen, halo, -CN, or -O- R^{12} ; or a pharmaceutically acceptable salt, stereoisomer, mixture of stereoisomers or deuterated analog thereof.

6. The compound of claim 5, wherein:

Z^3 is hydrogen, C_{1-9} alkyl, C_{3-15} cycloalkyl, heterocyclyl, aryl, or heteroaryl;

wherein said C_{1-9} alkyl, C_{3-15} cycloalkyl, or heterocyclyl may be optionally substituted with one to four substituents independently selected from the group consisting of oxo, -CN, halo, -O- R^{12} , -C(O)- R^{12} , -C(O)O- R^{12} , -OC(O)- R^{12} , -C(O)-N(R^{13})(R^{14}), -N(R^{12})S(O)₂(R^{12}), -N(R^{13})(R^{14}), -N(R^{13})₂(R^{14})⁺, -C(O)N(R^{12})-S(O)₂ R^{12} , C_{1-9} alkyl, C_{1-8} haloalkyl, C_{1-8} hydroxyalkyl, C_{3-15} cycloalkyl, aryl, heterocyclyl, and heteroaryl;

Z^9 is hydrogen;

R^1 is C_{1-9} alkyl, C_{3-15} cycloalkyl, heterocyclyl, aryl, or heteroaryl;

wherein said C_{1-9} alkyl, heterocyclyl, aryl, or heteroaryl may be optionally substituted with one to three substituents independently selected the group consisting of halo, -CN, -O- R^{12} , -S(O)₂ R^{12} , C_{1-9} alkyl, C_{1-9} haloalkyl, heterocyclyl, and aryl, wherein said C_{3-15} cycloalkyl may be optionally

substituted with one to four substituents independently selected the group consisting of C₁₋₉ alkyl, and C₁₋₉ haloalkyl;

R⁴ is heterocyclyl or heteroaryl;

wherein said heterocyclyl or heteroaryl is optionally substituted with one to three substituents independently selected from the group consisting of -CN, halo, -O-R¹², -C(O)-R¹², -N(R¹³)(R¹⁴), C₁₋₉ alkyl, C₁₋₉ haloalkyl, and heterocyclyl;

R⁵ is -CN, halo, -O-R⁷ or -S(O)₂R⁷;

R⁶ is hydrogen;

each R⁷ is independently hydrogen or C₁₋₉ alkyl;

wherein said C₁₋₉ alkyl may be optionally substituted with one to three substituents independently selected from the group consisting of hydroxyl, halo, -O(C₁₋₉ alkyl) and aryl;

each R¹² is independently hydrogen, C₁₋₉ alkyl or heterocyclyl;

wherein said C₁₋₉ alkyl may be optionally substituted with one to three substituents independently selected from the group consisting of hydroxyl, halo, -O(C₁₋₉ alkyl) and aryl; and

each R¹³ and R¹⁴ is independently hydrogen or C₁₋₉ alkyl;

wherein said C₁₋₉ alkyl may be optionally substituted with one to three substituents independently selected from the group consisting of hydroxyl, halo, -O(C₁₋₉ alkyl) and aryl;

or a pharmaceutically acceptable salt, stereoisomer, mixture of stereoisomers thereof.

7. The compound of claim 6, wherein Z³ is C₃₋₁₅ cycloalkyl optionally substituted with one to four substituents independently selected from the group consisting of -CN, halo, -C(O)-R¹², -OC(O)-R¹², -C(O)N(R¹³)(R¹⁴), C₁₋₉ alkyl, C₁₋₈ haloalkyl, C₁₋₈ hydroxyalkyl, C₃₋₁₅ cycloalkyl, and heteroaryl, or a pharmaceutically acceptable salt thereof, or

Z³ is heterocyclyl optionally substituted with one to four substituents independently selected from the group consisting of -O-R¹², -C(O)O-R¹², C₁₋₉

alkyl, C₁₋₈ haloalkyl, C₁₋₈ hydroxyalkyl, and heterocyclyl, or a pharmaceutically acceptable salt thereof.

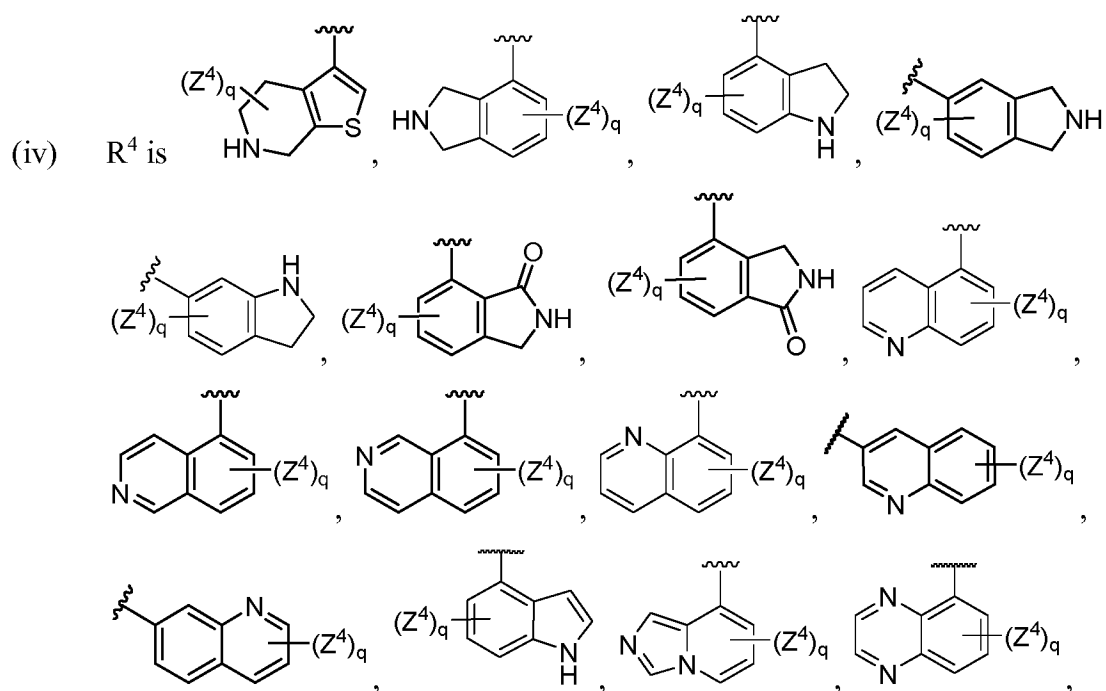
8. The compound of claim 5 or 6, wherein R⁵ is cyano or halo, or a pharmaceutically acceptable salt thereof; or R⁶ is hydrogen, or a pharmaceutically acceptable salt thereof.

9. The compound of any preceding claim, wherein

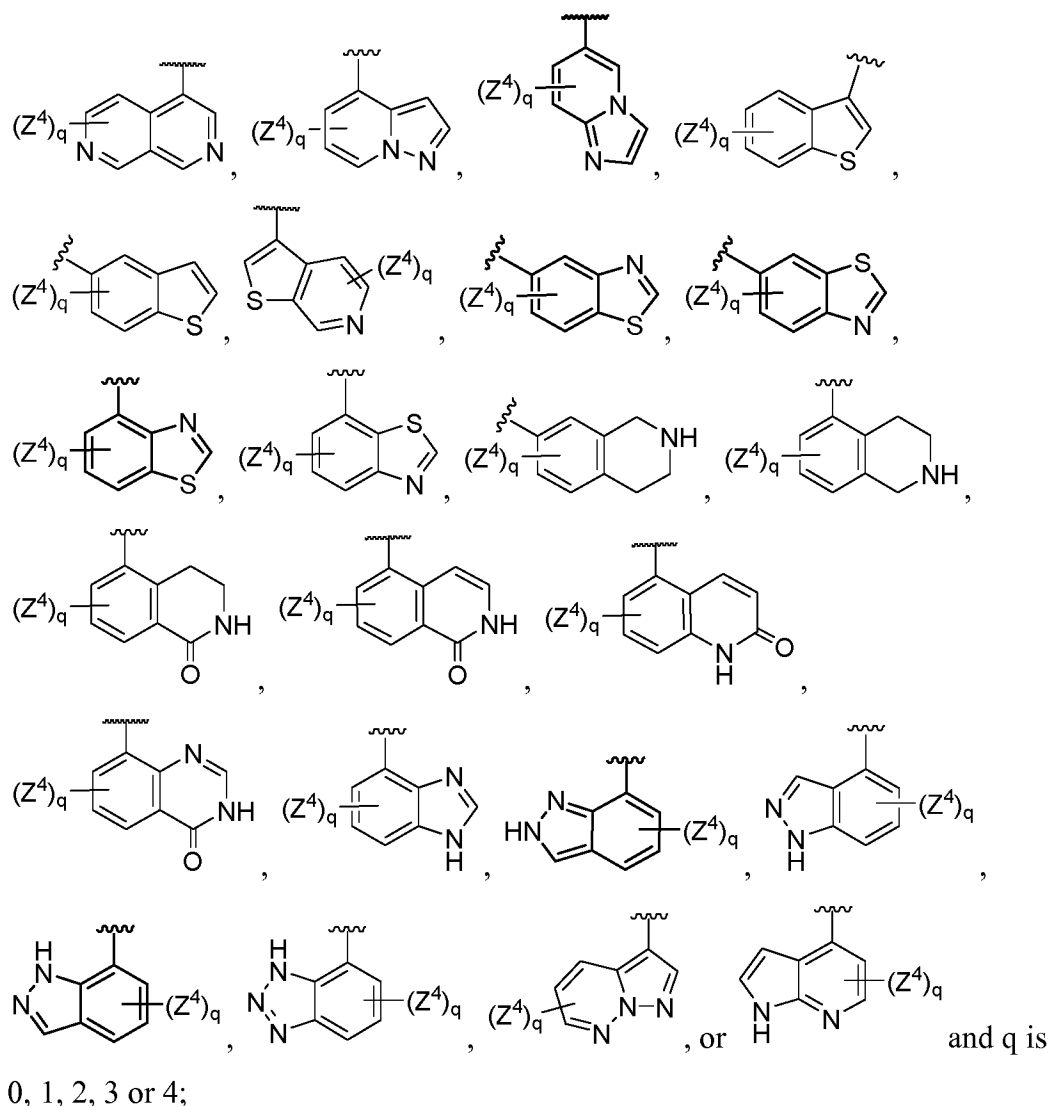
(i) R⁴ is heterocyclyl or heteroaryl; and said heterocyclyl or heteroaryl is optionally substituted with one to three substituents independently selected from the group consisting of -CN, halo, -O-R¹², -C(O)-R¹², -N(R¹³)(R¹⁴), C₁₋₉ alkyl, C₁₋₉ haloalkyl, and heterocyclyl, or a pharmaceutically acceptable salt thereof;

(ii) R⁴ is heteroaryl optionally substituted with one to three substituents independently selected from the group consisting of -CN, halo, -O-R¹², -C(O)-R¹², -N(R¹³)(R¹⁴), C₁₋₉ alkyl, C₁₋₉ haloalkyl, and heterocyclyl, or a pharmaceutically acceptable salt thereof;

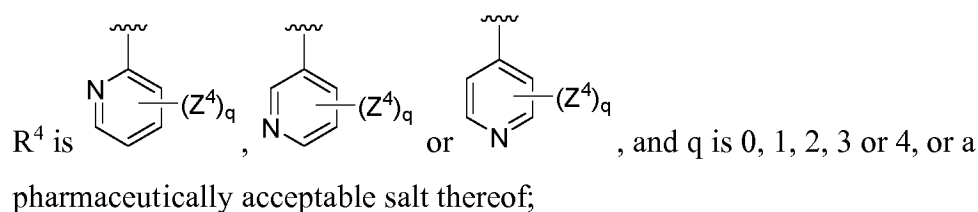
(iii) R⁴ is heterocyclyl optionally substituted with one to three substituents independently selected from the group consisting of -CN, halo, -O-R¹², -C(O)-R¹², -N(R¹³)(R¹⁴), C₁₋₉ alkyl, C₁₋₉ haloalkyl, and heterocyclyl, or a pharmaceutically acceptable salt thereof;



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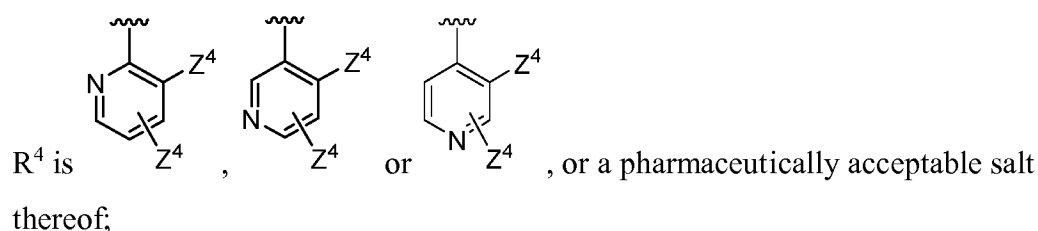


(v)

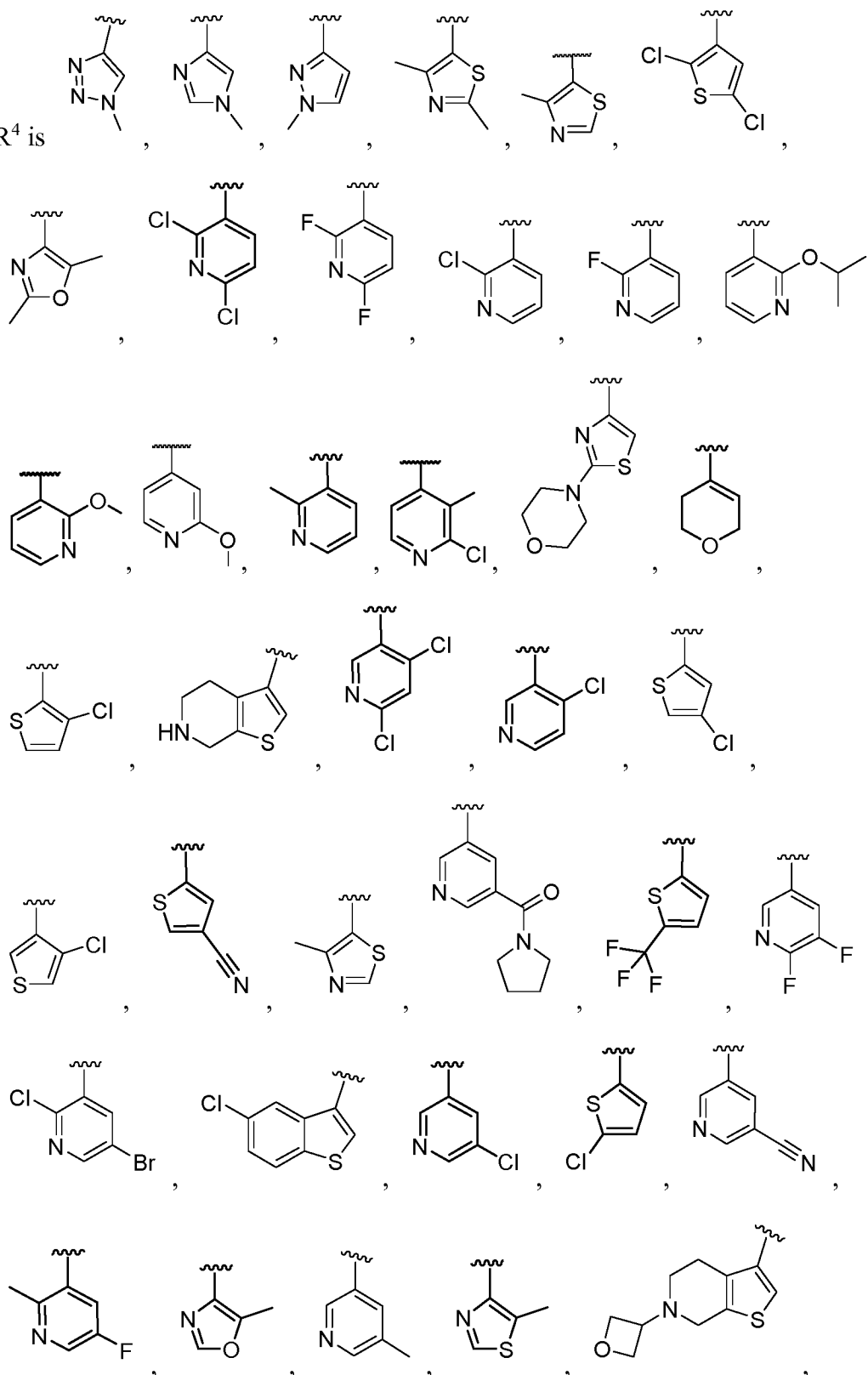


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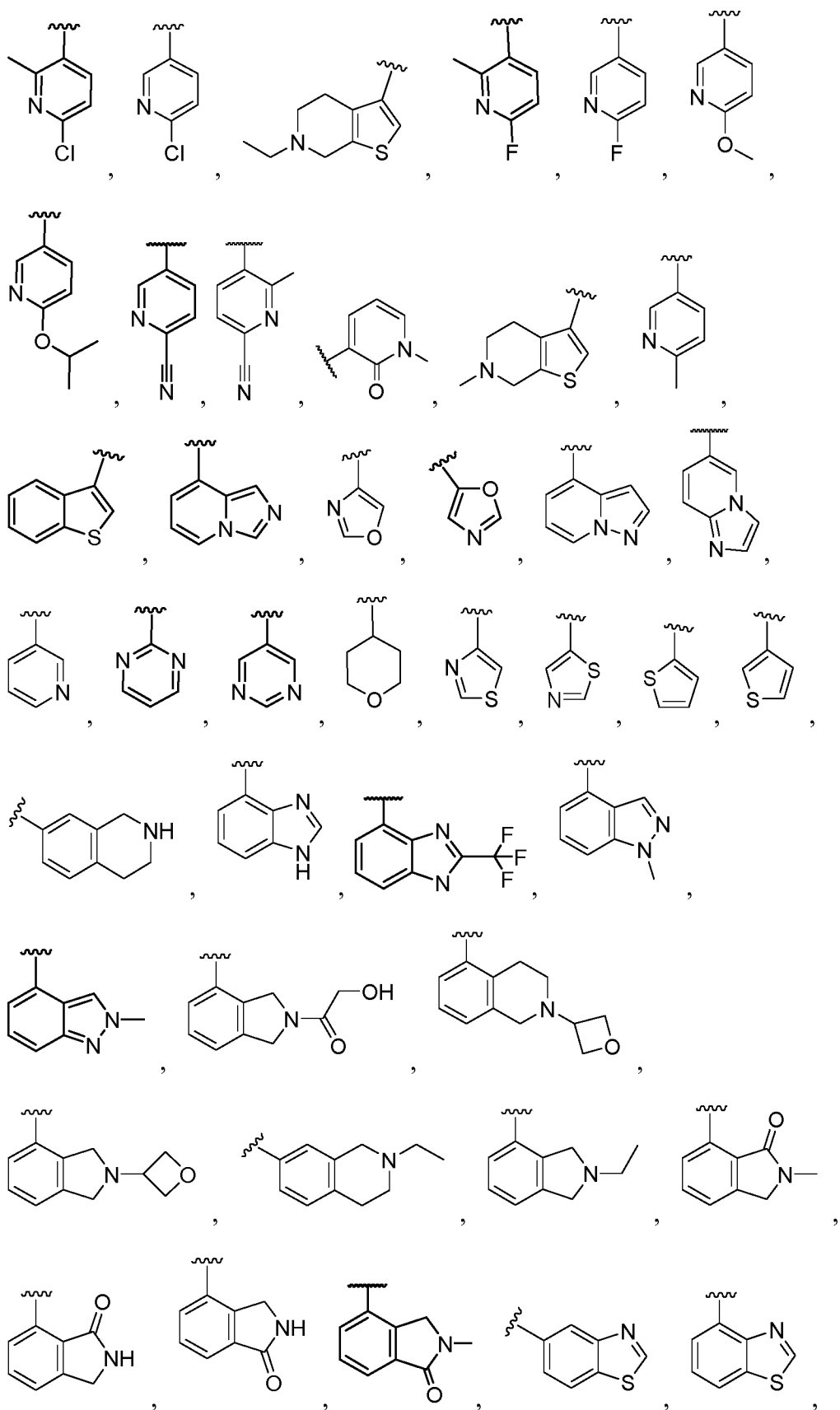
(vi)

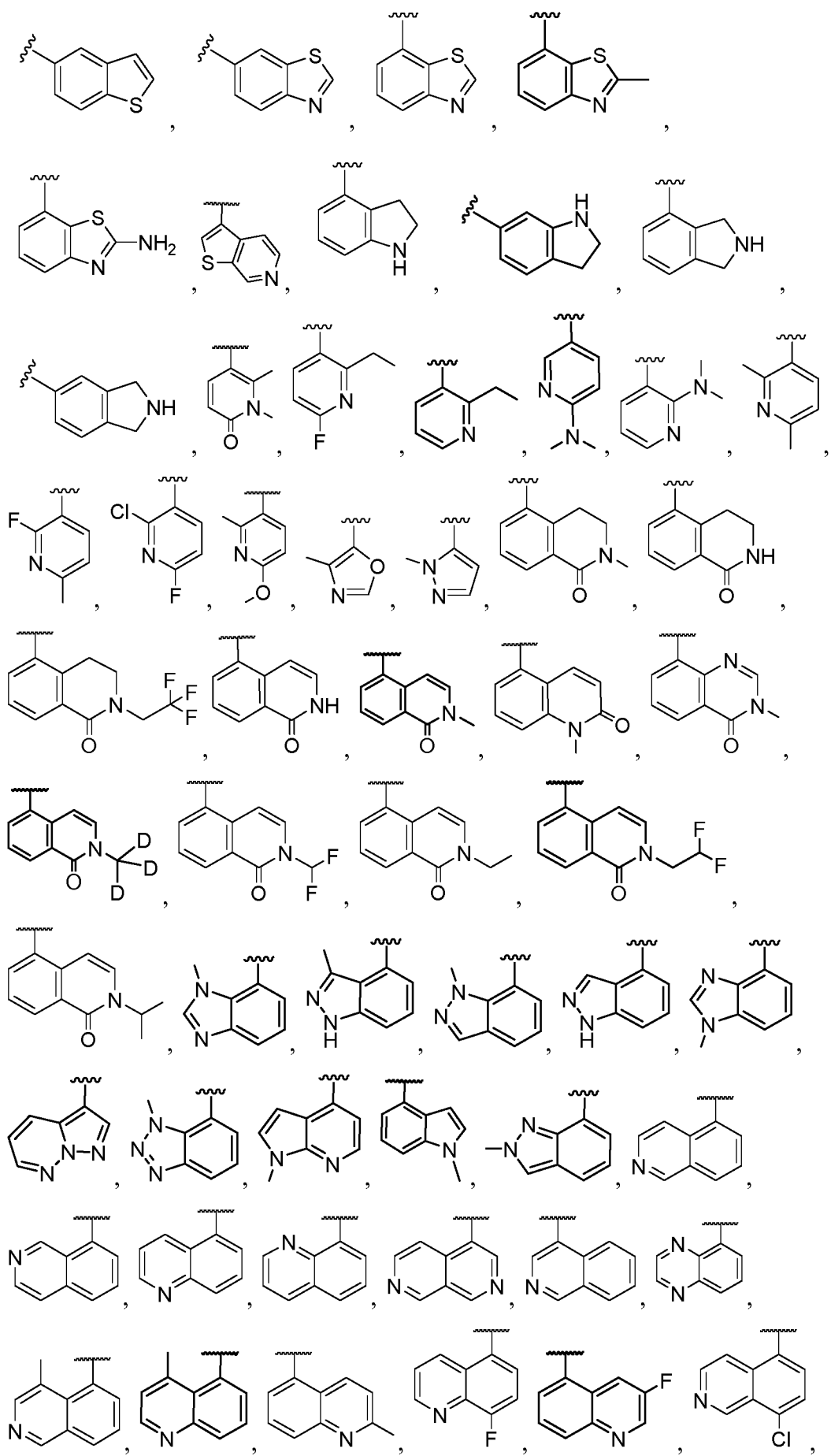


wherein each Z^4 of (iv) to (vi) may be independently selected from the group consisting of -CN, halo, -O- R^{12} , -C(O)- R^{12} , -N(R^{13})(R^{14}), C_{1-9} alkyl, C_{1-9} haloalkyl, and heterocyclyl, or a pharmaceutically acceptable salt thereof;

(vii) R⁴ is

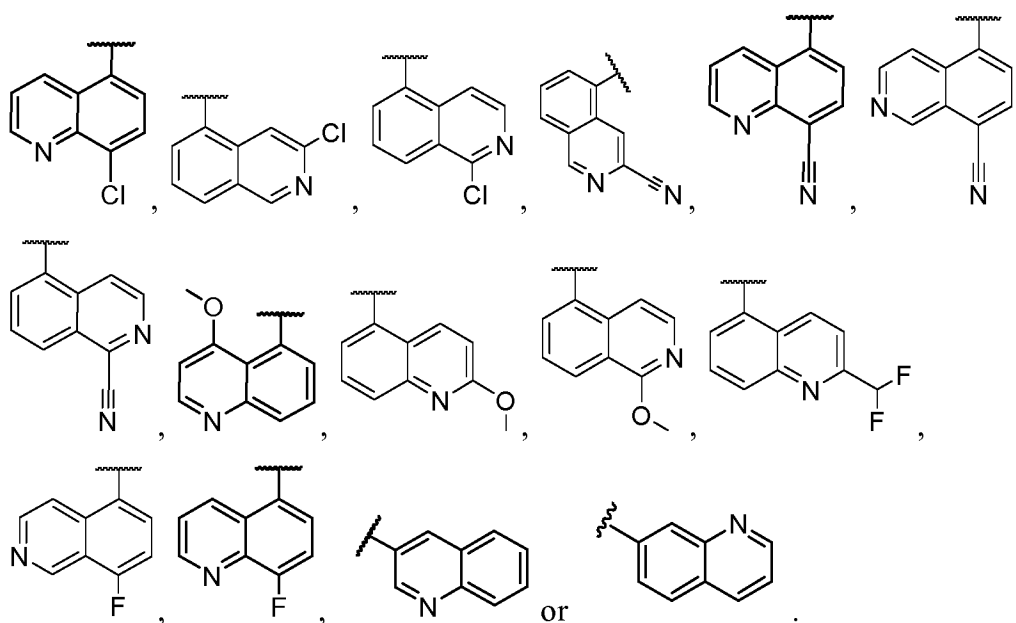
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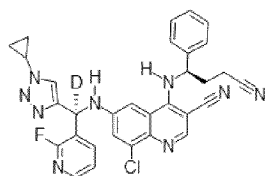


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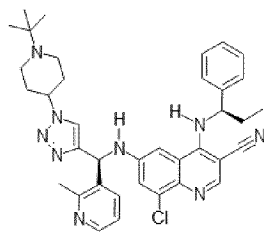
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10. The compound of claim 1 selected from **Table 1**, or a pharmaceutically acceptable salt thereof.
11. A composition comprising a compound of any one of claims 1-10 and a pharmaceutically acceptable carrier.
12. The compound of claim 1 or the composition of claim 11, wherein the compound has the formula



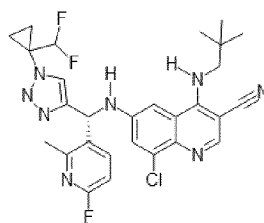
- or a pharmaceutically acceptable salt thereof.
13. The compound of claim 1 or the composition of claim 11, wherein the compound has the formula



,

or a pharmaceutically acceptable salt thereof.

14. The compound of claim 1 or the composition of claim 11, wherein the compound has the formula

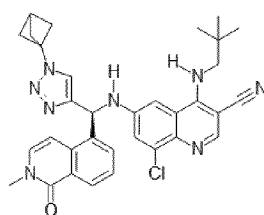


5

,

or a pharmaceutically acceptable salt thereof.

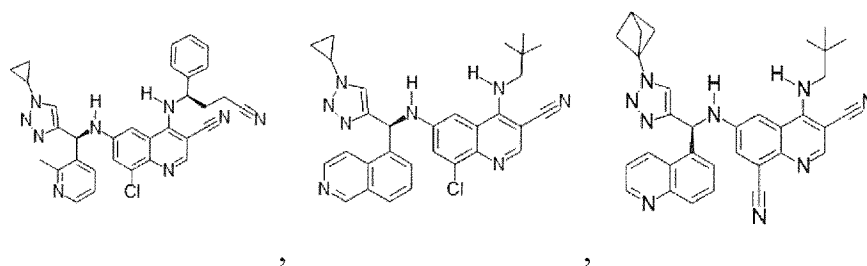
15. The compound of claim 1 or the composition of claim 11, wherein the compound has the formula



,

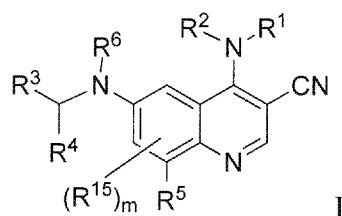
- 10 or a pharmaceutically acceptable salt thereof.

16. The compound of claim 1 or the composition of claim 11, wherein the compound has one of the following formulas:



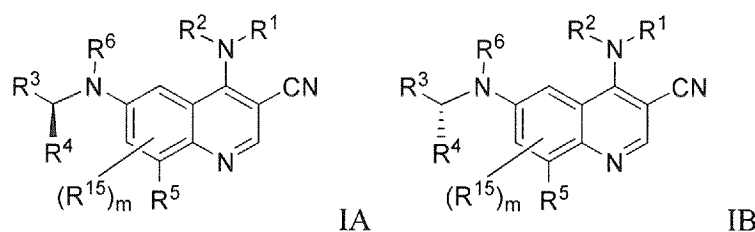
or a pharmaceutically acceptable salt thereof.

17. A composition comprising a mixture of stereoisomers of a compound of Formula I according to claim 1:



5

wherein the mixture comprises compounds of Formula IA and IB in a ratio of at least about 3:1:



wherein m , R^1 , R^2 , R^3 , R^4 , R^5 , R^6 and R^{15} are as defined in claim 1.

- 10 18. A composition comprising a compound or composition according to any one of claims 1-17 and an additional anti-inflammatory agent, wherein said additional anti-inflammatory agent preferably is an $\alpha 4\beta 7$ inhibitor, a steroid, a MMP-9 antibody, a S1P1 agonist, a TNF biologic, or any combination thereof.
19. The compound according to any one of claims 1-10 and 12-16 for use in therapy.
- 15 20. The compound or composition according to any one of claims 1-17 for use in a method for treating a disease or condition mediated by cancer Osaka thyroid

(Cot) in a human patient in need thereof, wherein the disease or condition is preferably selected from the group consisting of:

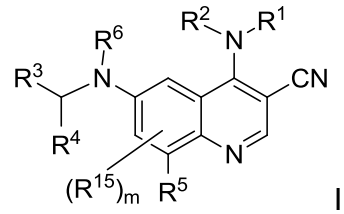
- (A) cancer;
- (B) diabetes;
- 5 (C) an inflammatory disease such as inflammatory bowel disease (IBD);
- (D) a solid tumor selected from pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors (*e.g.*,
10 glioma, anaplastic oligodendroglioma, adult glioblastoma multiforme, and adult anaplastic astrocytoma), bone cancer, and soft tissue sarcoma;
- (E) type 1 and type 2 diabetes, gestational diabetes, prediabetes, insulin resistance, metabolic syndrome, impaired fasting glycaemia and impaired glucose tolerance;
- (F) systemic lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis
15 (RA), acute disseminated encephalomyelitis, idiopathic thrombocytopenic purpura, multiple sclerosis (MS), inflammatory bowel disease (IBD), sepsis, psoriasis, Sjogren's syndrome, autoimmune hemolytic anemia, asthma, or chronic obstructive pulmonary disease (COPD), ankylosing spondylitis, reactive arthritis, monoarticular arthritis, osteoarthritis, gouty arthritis, juvenile arthritis,
20 juvenile onset rheumatoid arthritis, juvenile rheumatoid arthritis, and psoriatic arthritis;
- (G) diversion colitis, ischemic colitis, infectious colitis, chemical colitis, microscopic colitis (including collagenous colitis and lymphocytic colitis), atypical colitis, pseudomembranous colitis, fulminant colitis, autistic enterocolitis, indeterminate
25 colitis, Behçet's disease, gastroduodenal CD, jejunoileitis, ileitis, ileocolitis, Crohn's (granulomatous) colitis, irritable bowel syndrome, mucositis, radiation induced enteritis, short bowel syndrome, celiac disease, stomach ulcers, diverticulitis, pouchitis, proctitis, and chronic diarrhea;
- (H) alcoholic hepatitis;

- (I) systemic lupus erythematosus (SLE), lupus nephritis, lupus-related, and autoimmune disorders or a symptom of SLE, wherein said symptom of SLE is preferably selected from joint pain, joint swelling, arthritis, fatigue, hair loss, mouth sores, swollen lymph nodes, sensitivity to sunlight, skin rash, headaches, numbness, tingling, seizures, vision problems, personality changes, abdominal pain, nausea, vomiting, abnormal heart rhythms, coughing up blood and difficulty breathing, patchy skin color and Raynaud's phenomenon.
- 5
21. The use of a compound or composition according to any one of claims 1-17 in the manufacture of a medicament.

10

REIVINDICACIONES

1. Un compuesto de Formula I:



en donde

R¹ es hidrógeno, -O-R⁷, -N(R⁸)(R⁹), -C(O)-R⁷, -S(O)₂-R⁷, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, cicloalquilo C₃₋₁₅, heterociclilo, arilo, o heteroarilo;

en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, cicloalquilo C₃₋₁₅, heterociclilo, arilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z¹;

R² es hidrógeno, -C(O)-R⁷, -C(O)O-R⁷, -C(O)N(R⁷)₂, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo; en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z²;

o R¹ y R² junto con el nitrógeno al que están unidos forman un heterociclilo o heteroarilo, en donde cada heterociclilo o heteroarilo está opcionalmente sustituido con uno a cuatro Z²;

R³ es heterociclilo o heteroarilo, en donde cada heterociclilo o heteroarilo está opcionalmente sustituido con uno a cuatro Z³;

R⁴ es heterociclilo o heteroarilo, en donde cada heterociclilo o heteroarilo está opcionalmente sustituido con uno a cuatro Z⁴;

R⁵ es hidrógeno, halo, -CN, -NO₂, -O-R⁷, -N(R⁸)(R⁹), -S(O)-R⁷, -S(O)₂R⁷, -S(O)₂N(R⁷)₂, -C(O)R⁷, -OC(O)-R⁷, -C(O)O-R⁷, -OC(O)O-R⁷, -OC(O)N(R¹⁰)(R¹¹), -C(O)N(R⁷)₂, -N(R⁷)C(O)(R⁷), alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, alquiltio C₁₋₉, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo; en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, alquiltio C₁₋₉, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z⁵;

R⁶ es hidrógeno, -C(O)-R⁷, -C(O)O-R⁷, -C(O)N(R⁷)₂, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo; en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z⁶;

cada R⁷ es independientemente hidrógeno, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo;

en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z⁷;

R⁸ y R⁹ en cada aparición son independientemente hidrógeno, -S(O)₂R¹⁰, -C(O)-R¹⁰, -C(O)O-R¹⁰, -C(O)N(R¹⁰)(R¹¹), alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo;

en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z⁸;

R¹⁰ y R¹¹ en cada aparición son independientemente hidrógeno, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, o heteroarilo,

en donde cada alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, haloalquilo C₁₋₆, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo opcionalmente está sustituido con uno a cuatro Z^{1b};

cada Z¹, Z², Z³, Z⁴, Z⁵, Z⁶, Z⁷, y Z⁸ es independientemente hidrógeno, oxo, halo, -NO₂, -N₃, -CN, tioxo, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, cicloalquilo C₃₋₁₅, haloalquilo C₁₋₈, arilo, heteroarilo, heterociclilo, -O-R¹², -C(O)-R¹², -C(O)O-R¹², -C(O)-N(R¹³)(R¹⁴), -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -N(R¹²)C(O)-R¹², -N(R¹²)C(O)O-R¹², -N(R¹²)C(O)N(R¹³)(R¹⁴), -N(R¹²)S(O)₂(R¹²), -NR¹²S(O)₂N(R¹³)(R¹⁴), -NR¹²S(O)₂O(R¹²), -OC(O)R¹², -OC(O)-N(R¹³)(R¹⁴), -P(O)(OR¹²)₂, -OP(O)(OR¹²)₂, -CH₂P(O)(OR¹²)₂, -OCH₂P(O)(OR¹²)₂, -C(O)OCH₂P(O)(OR¹²)₂, -P(O)(R¹²)(OR¹²), -OP(O)(R¹²)(OR¹²), -CH₂P(O)(R¹²)(OR¹²), -OCH₂P(O)(R¹²)(OR¹²), -C(O)OCH₂P(O)(R¹²)(OR¹²), -P(O)(N(R¹²)₂)₂, -OP(O)(N(R¹²)₂)₂, -CH₂P(O)(N(R¹²)₂)₂, -OCH₂P(O)(N(R¹²)₂)₂, -C(O)OCH₂P(O)(N(R¹²)₂)₂, -P(O)(N(R¹²)₂)(OR¹²), -OP(O)(N(R¹²)₂)(OR¹²), -CH₂P(O)(N(R¹²)₂)(OR¹²), -OCH₂P(O)(N(R¹²)₂)(OR¹²), -C(O)OCH₂P(O)(N(R¹²)₂)(OR¹²), -P(O)(R¹²)(N(R¹²)₂), -OP(O)(R¹²)(N(R¹²)₂), -CH₂P(O)(R¹²)(N(R¹²)₂), -OCH₂P(O)(R¹²)(N(R¹²)₂), -C(O)OCH₂P(O)(R¹²)(N(R¹²)₂), -Si(R¹²)₃, -S-R¹², -S(O)R¹², -S(O)(NH)R¹², -S(O)₂R¹² o -S(O)₂N(R¹³)(R¹⁴);

en donde cualquier alquilo, alquenilo, alquinilo, cicloalquilo, haloalquilo, arilo, heteroarilo o heterociclilo está opcionalmente sustituido con uno a cuatro grupos Z^{1a};

cada Z^{1a} es independientemente oxo, halo, tioxo, -NO₂, -CN, -N₃, alquilo C₁₋₉, alquenilo C₂₋₆, alquinilo C₂₋₆, cicloalquilo C₃₋₁₅, haloalquilo C₁₋₈, arilo, heteroarilo, heterociclilo, -O-R¹², -C(O)R¹², -C(O)O-R¹², -C(O)N(R¹³)(R¹⁴), -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -N(R¹²)-C(O)R¹², -N(R¹²)C(O)O(R¹²), -N(R¹²)C(O)N(R¹³)(R¹⁴), -N(R¹²)S(O)₂(R¹²), -N(R¹²)S(O)₂-N(R¹³)(R¹⁴), -N(R¹²)S(O)₂O(R¹²), -OC(O)R¹², -OC(O)OR¹², -OC(O)-N(R¹³)(R¹⁴), -Si(R¹²)₃, -S-R¹², -S(O)R¹², -S(O)(NH)R¹², -S(O)₂R¹² o -S(O)₂N(R¹³)(R¹⁴);

en donde cualquier alquilo, alqueno, alquino, cicloalquilo, arilo, heteroarilo o heterociclilo está opcionalmente sustituido con uno a cuatro grupos Z^{1b} ; cada R^{12} es independientemente hidrógeno, alquilo C_{1-9} , alqueno C_{2-6} , alquino C_{2-6} , cicloalquilo C_{3-15} , arilo, heteroarilo o heterociclilo, en donde cualquier alquilo, alqueno, alquino, cicloalquilo, arilo, heteroarilo o heterociclilo está opcionalmente sustituido con uno a cuatro grupos Z^{1b} ; R^{13} y R^{14} en cada aparición son cada uno independientemente hidrógeno, alquilo C_{1-9} , alqueno C_{2-6} , alquino C_{2-6} , cicloalquilo C_{3-15} , arilo, heteroarilo o heterociclilo; en donde cualquier alquilo, alqueno, alquino, cicloalquilo, arilo, heteroarilo o heterociclilo está opcionalmente sustituido con uno a cuatro grupos Z^{1b} , o R^{13} y R^{14} junto con el nitrógeno al que están unidos forman un heterociclilo, en donde dicho heterociclilo está opcionalmente sustituido con uno a cuatro grupos Z^{1b} ; cada R^{15} es independientemente halo, -CN, -NO₂, -O-R⁷, -N(R⁸)(R⁹), -S(O)-R⁷, -S(O)₂R⁷, -S(O)₂N(R⁷)₂, -C(O)R⁷, -OC(O)-R⁷, -C(O)O-R⁷, -OC(O)O-R⁷, -OC(O)N(R¹⁰)(R¹¹), -C(O)N(R⁷)₂, -N(R⁷)C(O)(R⁷), alquilo C_{1-9} , alqueno C_{2-6} , alquino C_{2-6} , alquilo C_{1-9} , haloalquilo C_{1-6} , cicloalquilo C_{3-15} , arilo, heterociclilo, o heteroarilo; y cada Z^{1b} es independientemente oxo, tioxo, hidroxilo, halo, -NO₂, -N₃, -CN, alquilo C_{1-9} , alqueno C_{2-6} , alquino C_{2-6} , cicloalquilo C_{3-15} , haloalquilo C_{1-8} , arilo, heteroarilo, heterociclilo, -O(alquilo C_{1-9}), -O(alqueno C_{2-6}), -O(alquino C_{2-6}), -O(cicloalquilo C_{3-15}), -O(haloalquilo C_{1-8}), -O(arilo), -O(heteroarilo), -O(heterociclilo), -NH₂, -NH(alquilo C_{1-9}), -NH(alqueno C_{2-6}), -NH(alquino C_{2-6}), -NH(cicloalquilo C_{3-15}), -NH(haloalquilo C_{1-8}), -NH(arilo), -NH(heteroarilo), -NH(heterociclilo), -N(alquilo C_{1-9})₂, -N(alqueno C_{2-6})₂, -N(alquino C_{2-6})₂, -N(cicloalquilo C_{3-15})₂, -N(haloalquilo C_{1-8})₂, -N(arilo)₂, -N(heteroarilo)₂, -N(heterociclilo)₂, -N(alquilo C_{1-9})(alqueno C_{2-6}), -N(alquilo C_{1-9})(alquino C_{2-6}), -N(alquilo C_{1-9})(cicloalquilo C_{3-15}), -N(alquilo C_{1-9})(haloalquilo C_{1-8}), -N(alquilo C_{1-9})(arilo), -N(alquilo C_{1-9})(heteroarilo), -N(alquilo C_{1-9})(heterociclilo), -C(O)(alquilo C_{1-9}), -C(O)(alqueno C_{2-6}), -C(O)(alquino C_{2-6}), -C(O)(cicloalquilo C_{3-15}), -C(O)(haloalquilo C_{1-8}), -C(O)(arilo), -C(O)(heteroarilo), -C(O)(heterociclilo), -C(O)O(alquilo C_{1-9}), -C(O)O(alqueno C_{2-6}), -C(O)O(alquino C_{2-6}), -C(O)O(cicloalquilo C_{3-15}), -C(O)O(haloalquilo C_{1-8}), -C(O)O(arilo), -C(O)O(heteroarilo), -C(O)O(heterociclilo), -C(O)NH₂, -C(O)NH(alquilo C_{1-9}), -C(O)NH(alqueno C_{2-6}), -C(O)NH(alquino C_{2-6}), -C(O)NH(cicloalquilo C_{3-15}), -C(O)NH(haloalquilo C_{1-8}), -C(O)NH(arilo), -C(O)NH(heteroarilo), -C(O)NH(heterociclilo), -C(O)N(alquilo C_{1-9})₂, -C(O)N(alqueno C_{2-6})₂, -C(O)N(alquino C_{2-6})₂, -C(O)N(cicloalquilo C_{3-15})₂, -C(O)N(haloalquilo C_{1-8})₂, -C(O)N(arilo)₂, -C(O)N(heteroarilo)₂, -C(O)N(heterociclilo)₂, -NHC(O)(alquilo C_{1-9}), -NHC(O)(alqueno C_{2-6}),

6), -NHC(O)(alquinilo C₂₋₆), -NHC(O)(cicloalquilo C₃₋₁₅), -NHC(O)(haloalquilo C₁₋₈), -NHC(O)(arilo), -NHC(O)(heteroarilo), -NHC(O)(heterociclilo), -NHC(O)O(alquilo C₁₋₉), -NHC(O)O(alquenilo C₂₋₆), -NHC(O)O(alquinilo C₂₋₆), -NHC(O)O(cicloalquilo C₃₋₁₅), -NHC(O)O(haloalquilo C₁₋₈), -NHC(O)O(arilo), -NHC(O)O(heteroarilo), -NHC(O)O(heterociclilo), -NHC(O)NH(alquilo C₁₋₉), -NHC(O)NH(alquenilo C₂₋₆), -NHC(O)NH(alquinilo C₂₋₆), -NHC(O)NH(cicloalquilo C₃₋₁₅), -NHC(O)NH(haloalquilo C₁₋₈), -NHC(O)NH(arilo), -NHC(O)NH(heteroarilo), -NHC(O)NH(heterociclilo), -SH, -S(alquilo C₁₋₉), -S(alquenilo C₂₋₆), -S(alquinilo C₂₋₆), -S(cicloalquilo C₃₋₁₅), -S(haloalquilo C₁₋₈), -S(arilo), -S(heteroarilo), -S(heterociclilo), -NHS(O)(alquilo C₁₋₉), -N(alquilo C₁₋₉)S(O)(alquilo C₁₋₉), -S(O)N(alquilo C₁₋₉)₂, -S(O)(alquilo C₁₋₉), -S(O)(NH)(alquilo C₁₋₉), -S(O)(alquenilo C₂₋₆), -S(O)(alquinilo C₂₋₆), -S(O)(cicloalquilo C₃₋₁₅), -S(O)(haloalquilo C₁₋₈), -S(O)(arilo), -S(O)(heteroarilo), -S(O)(heterociclilo), -S(O)₂(alquilo C₁₋₉), -S(O)₂(alquenilo C₂₋₆), -S(O)₂(alquinilo C₂₋₆), -S(O)₂(cicloalquilo C₃₋₁₅), -S(O)₂(haloalquilo C₁₋₈), -S(O)₂(arilo), -S(O)₂(heteroarilo), -S(O)₂(heterociclilo), -S(O)₂NH(alquilo C₁₋₉), o -S(O)₂N(alquilo C₁₋₉)₂;

en donde cualquier alquilo, cicloalquilo, arilo, heteroarilo, o heterociclilo está opcionalmente sustituido con uno a cuatro halo, alquilo C₁₋₉, haloalquilo C₁₋₈, -OH, -NH₂, -NH(alquilo C₁₋₉), -NH(cicloalquilo C₃₋₁₅), -NH(haloalquilo C₁₋₈), -NH(arilo), -NH(heteroarilo), -NH(heterociclilo), -N(alquilo C₁₋₉)₂, -N(cicloalquilo C₃₋₁₅)₂, -NHC(O)(cicloalquilo C₃₋₁₅), -NHC(O)(haloalquilo C₁₋₈), -NHC(O)(arilo), -NHC(O)(heteroarilo), -NHC(O)(heterociclilo), -NHC(O)O(alquilo C₁₋₉), -NHC(O)O(alquinilo C₂₋₆), -NHC(O)O(cicloalquilo C₃₋₁₅), -NHC(O)O(haloalquilo C₁₋₈), -NHC(O)O(arilo), -NHC(O)O(heteroarilo), -NHC(O)O(heterociclilo), -NHC(O)NH(alquilo C₁₋₉), -S(O)(NH)(alquilo C₁₋₉), -S(O)₂(alquilo C₁₋₉), -S(O)₂(cicloalquilo C₃₋₁₅), -S(O)₂(haloalquilo C₁₋₈), -S(O)₂(arilo), -S(O)₂(heteroarilo), -S(O)₂(heterociclilo), -S(O)₂NH(alquilo C₁₋₉), -S(O)₂N(alquilo C₁₋₉)₂, -O(cicloalquilo C₃₋₁₅), -O(haloalquilo C₁₋₈), -O(arilo), -O(heteroarilo), -O(heterociclilo), o -O(alquilo C₁₋₉);

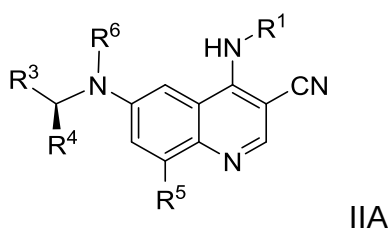
m es 0, 1, o 2;

o una sal, estereoisómero, mezcla de estereoisómeros, o análogo deuterado farmacéuticamente aceptable del mismo.

2. El compuesto de la reivindicación 1, en donde

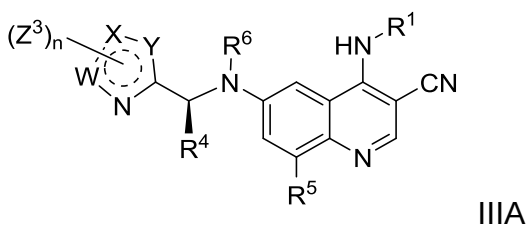
(a) R² es hidrógeno y/o m es 0, o una sal farmacéuticamente aceptable del mismo;

(b) el compuesto tiene la Formula IIA:



en donde R^1 , R^3 , R^4 , R^5 y R^6 son como se definen en la reivindicación 1, o una sal farmacéuticamente aceptable del mismo; o

(c) el compuesto tiene la Formula IIIA:



en donde R^1 , R^4 , R^5 , R^6 y Z^3 son como se definen en la reivindicación 1, W, X y Y son cada uno independientemente N o C; y n es 1, 2, o 3;

o una sal, estereoisómero, mezcla de estereoisómeros, o análogo deuterado farmacéuticamente aceptable del mismo.

3. El compuesto de la reivindicación 1 o 2, en donde

- (i) R^5 es hidrógeno, halo, -CN, $O-R^7$, $-S(O)-R^7$, $-S(O)_2R^7$, $-S(O)_2N(R^7)_2$, $-C(O)R^7$, $-C(O)N(R^7)_2$, alquilo C_{1-9} , alquenilo C_{2-6} , alquinilo C_{2-6} , cicloalquilo C_{3-15} , arilo, heterociclilo, o heteroarilo;
en donde cada alquilo C_{1-9} , alquenilo C_{2-6} , alquinilo C_{2-6} , cicloalquilo C_{3-15} , arilo, heterociclilo, y heteroarilo puede estar opcionalmente sustituido con uno a cuatro Z^5 , o una sal farmacéuticamente aceptable del mismo, o

R^5 es hidrógeno, halo, -CN, $-C(O)R^7$, $-O-R^7$, $-S(O)_2R^7$ o heteroarilo, o una sal farmacéuticamente aceptable del mismo; y/o

- (ii) R^6 es hidrógeno, o una sal farmacéuticamente aceptable del mismo; y/o

- (iii) R^1 es $-O-R^7$, alquilo C_{1-9} , cicloalquilo C_{3-15} , heterociclilo, arilo, o heteroarilo; y dicho alquilo C_{1-9} , cicloalquilo C_{3-15} , heterociclilo, arilo, o heteroarilo puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en halo, -CN, $-O-R^{12}$, $-S(O)_2R^{12}$, alquilo C_{1-9} , haloalquilo C_{1-9} , cicloalquilo C_{3-15} ,

heterociclilo, y arilo, en donde dicho cicloalquilo C₃₋₁₅ puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en alquilo C₁₋₉, y haloalquilo C₁₋₉, o una sal farmacéuticamente aceptable del mismo, o

R¹ es alquilo C₁₋₉, opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consistente en halo, -CN, -O-R¹², -S(O)₂R¹², cicloalquilo C₃₋₁₅, heterociclilo, y arilo, en donde dicho cicloalquilo C₃₋₁₅ o heterociclilo puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consistente en alquilo C₁₋₉, y haloalquilo C₁₋₉, o una sal farmacéuticamente aceptable del mismo, o

R¹ es cicloalquilo C₃₋₁₅, heterociclilo, o heteroarilo, en donde dicho cicloalquilo C₃₋₁₅, heterociclilo, o heteroarilo esta opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en halo, -CN, -O-R¹², alquilo C₁₋₉, y arilo, o una sal farmacéuticamente aceptable del mismo, o

R¹ es heterociclilo o heteroarilo, en donde dicho heterociclilo o heteroarilo está opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consistente en halo, y alquilo C₁₋₉, o una sal farmacéuticamente aceptable del mismo, o

R¹ es arilo, opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en halo, -CN, -O-R⁷, alquilo C₁₋₉, y arilo, o una sal farmacéuticamente aceptable del mismo, o

R¹ es arilo, opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en halo, -O-R⁷, y alquilo C₁₋₉, o una sal farmacéuticamente aceptable del mismo.

4. El compuesto de la reivindicación 2 (c), en donde

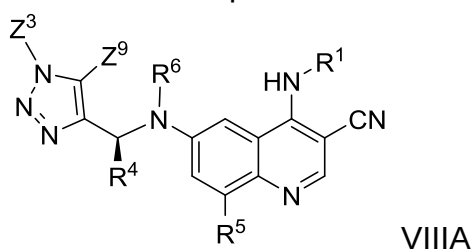
(1) W es N, X es N-Z³, y Y es C-Z³, o una sal farmacéuticamente aceptable del mismo; y/o

(2) Z³ es hidrógeno o alquilo C₁₋₉ opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste

en -CN, halo, -O-R¹², -C(O)O-R¹², -OC(O)-R¹², -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -C(O)N(R¹²)-S(O)₂R¹², alquilo C₁₋₉, heterociclilo, arilo, y heteroarilo, o una sal farmacéuticamente aceptable del mismo, o

Z³ es cicloalquilo C₃₋₁₅, heterociclilo, arilo, o heteroarilo; y dicho cicloalquilo C₃₋₁₅, heterociclilo, arilo, o heteroarilo, puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en -CN, halo, -O-R¹², -C(O)-R¹², -C(O)O-R¹², -OC(O)-R¹², -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, alquilo C₁₋₉, haloalquilo C₁₋₈, hidroxialquilo C₁₋₈, cicloalquilo C₃₋₁₅, heterociclilo, y heteroarilo, o una sal farmacéuticamente aceptable del mismo.

5. El compuesto de la reivindicación 1 que tiene la Formula VIIIA:



en donde Z³, R¹, R⁴, R⁵ y R⁶ son como se definen en la reivindicación 1, y Z⁹ es hidrógeno, halo, -CN, o -O-R¹²; o una sal, estereoisómero, mezcla de estereoisómeros, o análogo deuterado farmacéuticamente aceptable del mismo.

6. El compuesto de la reivindicación 5, en donde:

Z³ es hidrógeno, alquilo C₁₋₉, cicloalquilo C₃₋₁₅, heterociclilo, arilo, o heteroarilo; en donde dicho alquilo C₁₋₉, cicloalquilo C₃₋₁₅, o heterociclilo puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en oxo, -CN, halo, -O-R¹², -C(O)-R¹², -C(O)O-R¹², -OC(O)-R¹², -C(O)-N(R¹³)(R¹⁴), -N(R¹²)S(O)₂(R¹²), -N(R¹³)(R¹⁴), -N(R¹³)₂(R¹⁴)⁺, -C(O)N(R¹²)-S(O)₂R¹², alquilo C₁₋₉, haloalquilo C₁₋₈, hidroxialquilo C₁₋₈, cicloalquilo C₃₋₁₅, arilo, heterociclilo, y heteroarilo;

Z⁹ es hidrógeno;

R¹ es alquilo C₁₋₉, cicloalquilo C₃₋₁₅, heterociclilo, arilo, o heteroarilo; en donde dicho alquilo C₁₋₉, heterociclilo, arilo, o heteroarilo puede estar opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en halo, -CN, -O-R¹², -S(O)₂R¹², alquilo C₁₋₉, haloalquilo C₁₋₉, heterociclilo, y arilo, en donde dicho C₃₋₁₅

cicloalquilo puede estar opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en alquilo C₁₋₉, y haloalquilo C₁₋₉;

R⁴ es heterociclilo o heteroarilo;

en donde dicho heterociclilo o heteroarilo está opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en -CN, halo, -O-R¹², -C(O)-R¹², -N(R¹³)(R¹⁴), alquilo C₁₋₉, haloalquilo C₁₋₉, y heterociclilo;

R⁵ es -CN, halo, -O-R⁷ o -S(O)₂R⁷;

R⁶ es hidrógeno;

cada R⁷ es independientemente hidrógeno o alquilo C₁₋₉;
en donde dicho alquilo C₁₋₉ puede estar opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en hidroxilo, halo, -O(alquilo C₁₋₉) y arilo;

cada R¹² es independientemente hidrógeno, alquilo C₁₋₉ o heterociclilo;
en donde dicho alquilo C₁₋₉ puede estar opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en hidroxilo, halo, -O(alquilo C₁₋₉) y arilo; y

cada R¹³ y R¹⁴ es independientemente hidrógeno o alquilo C₁₋₉;
en donde dicho alquilo C₁₋₉ puede estar opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en hidroxilo, halo, -O(alquilo C₁₋₉) y arilo;

o una sal, estereoisómero, mezcla de estereoisómeros farmacéuticamente aceptable del mismo.

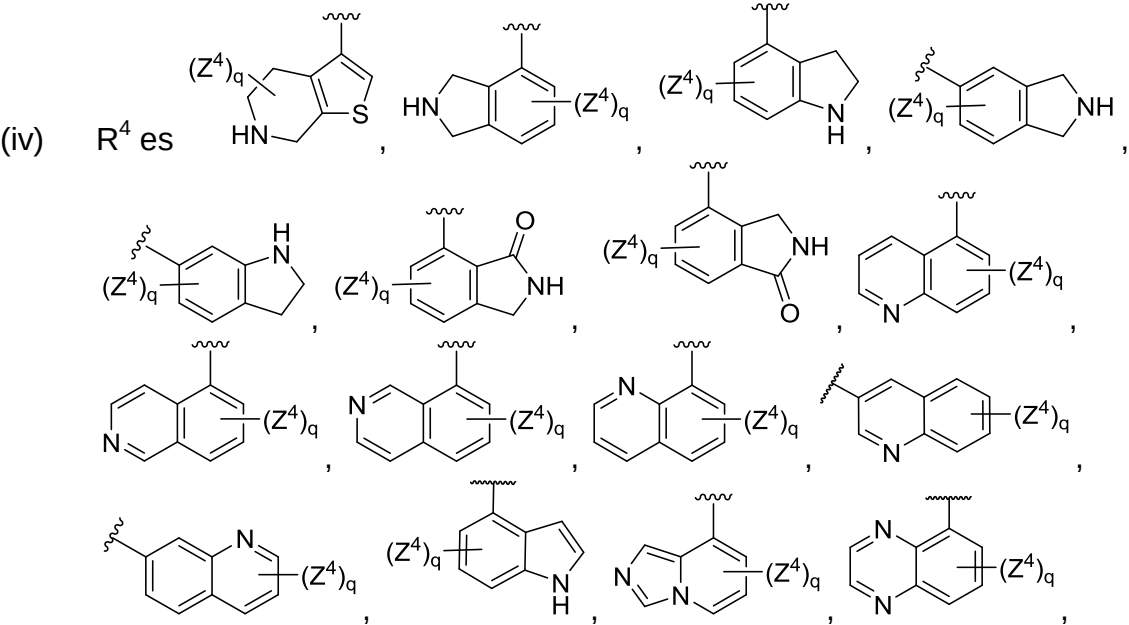
7. El compuesto de la reivindicación 6, en donde Z³ es cicloalquilo C₃₋₁₅ opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en -CN, halo, -C(O)-R¹², -OC(O)-R¹², -C(O)N(R¹³)(R¹⁴), alquilo C₁₋₉, haloalquilo C₁₋₈, hidroxialquilo C₁₋₈, cicloalquilo C₃₋₁₅, y heteroarilo, o una sal farmacéuticamente aceptable del mismo, o

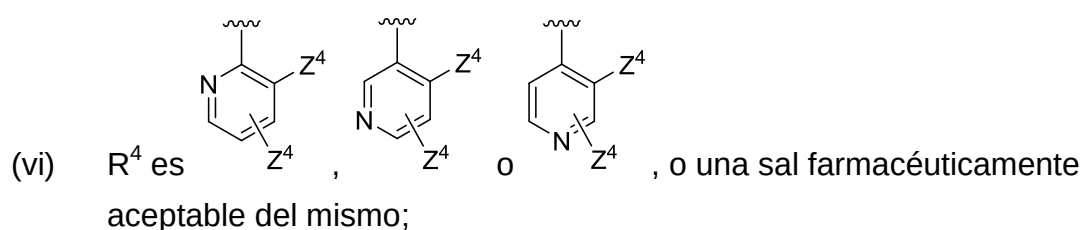
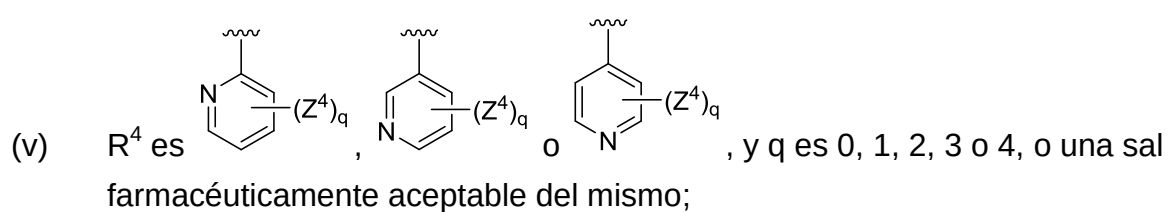
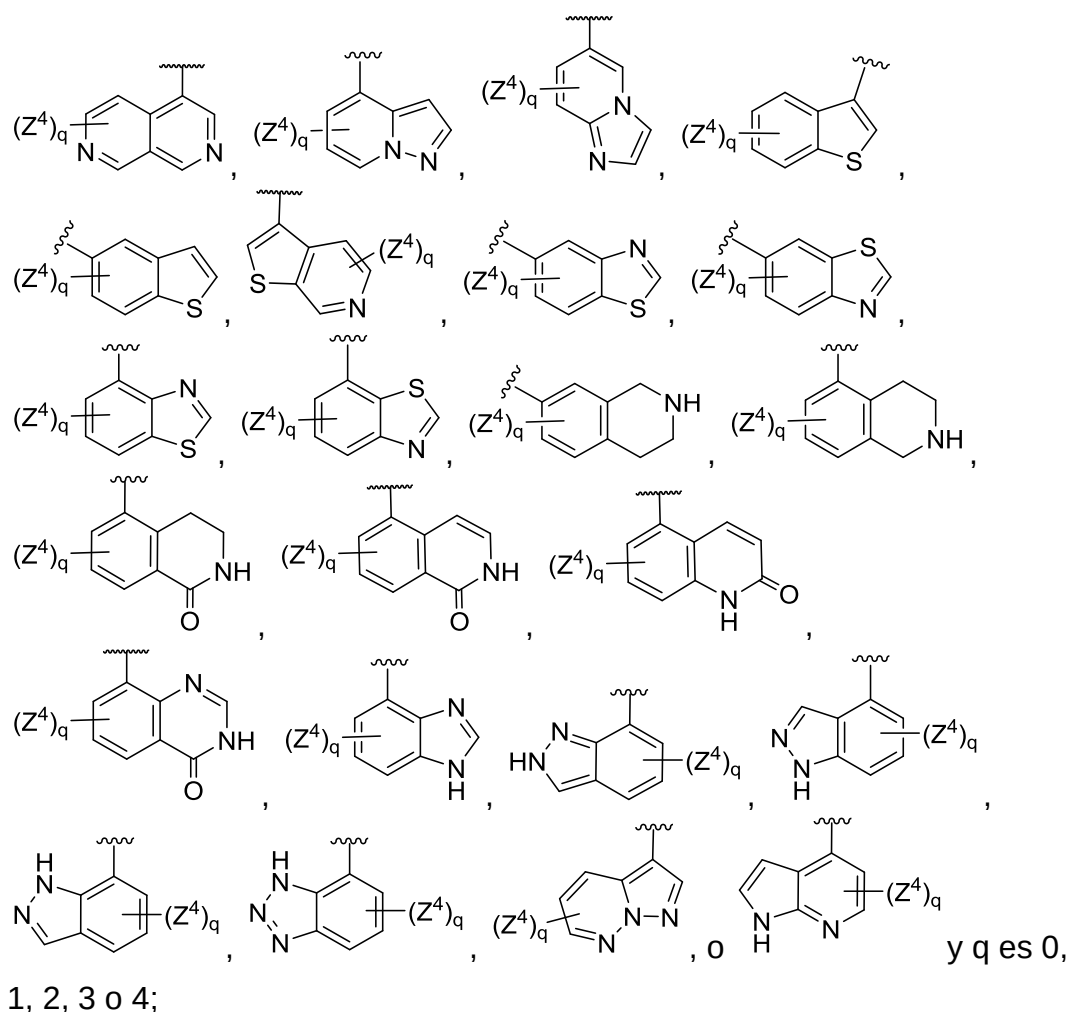
Z^3 es heterociclilo opcionalmente sustituido con uno a cuatro sustituyentes seleccionados independientemente del grupo que consiste en $-O-R^{12}$, $-C(O)O-R^{12}$, alquilo C_{1-9} , haloalquilo C_{1-8} , hidroxialquilo C_{1-8} , y heterociclilo, o una sal farmacéuticamente aceptable del mismo.

8. El compuesto de la reivindicación 5 o 6, en donde R^5 es ciano o halo, o una sal farmacéuticamente aceptable del mismo; o R^6 es hidrógeno, o una sal farmacéuticamente aceptable del mismo.

9. El compuesto de cualquier reivindicación anterior, en donde

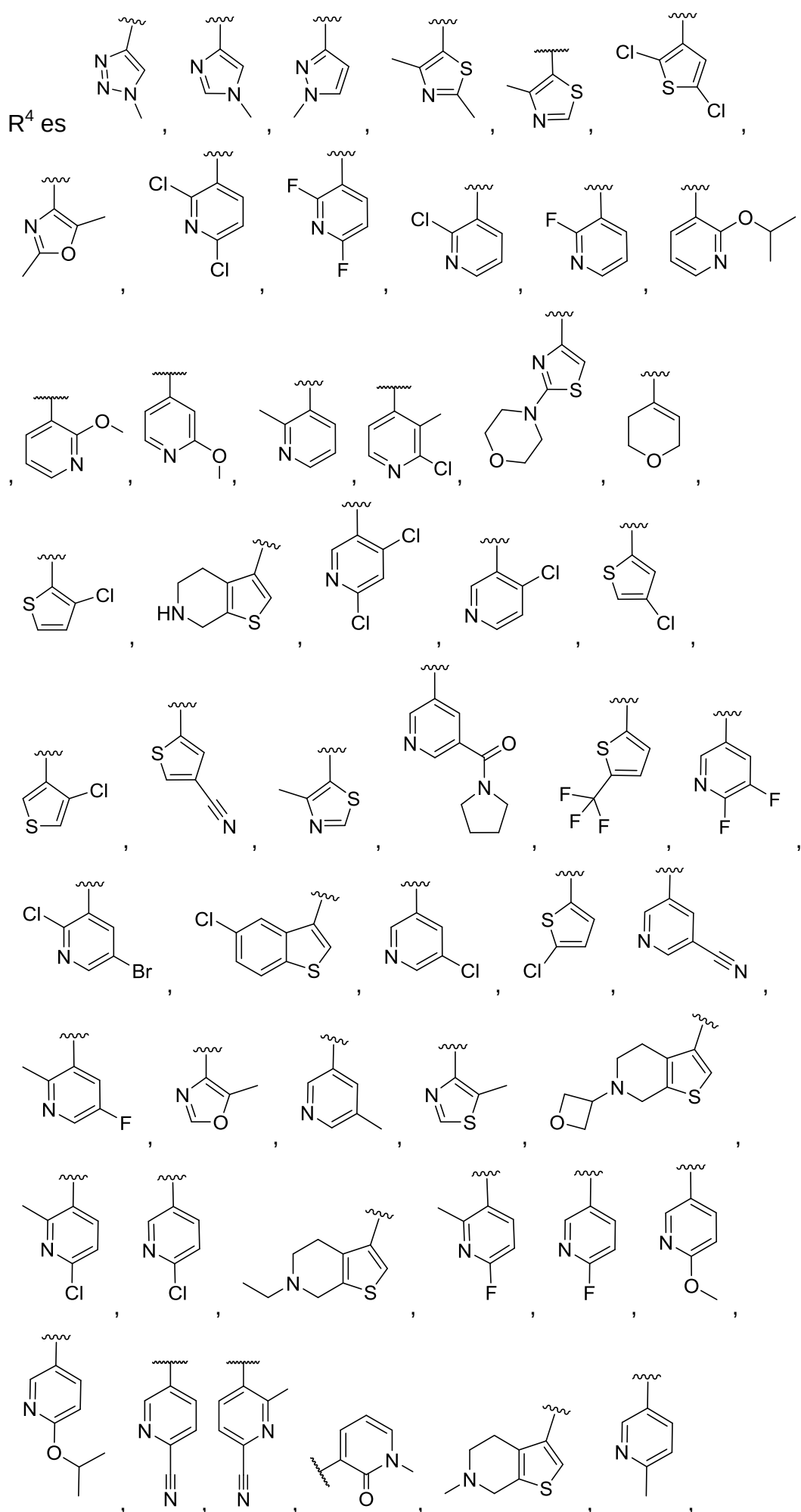
- (i) R^4 es heterociclilo o heteroarilo; y dicho heterociclilo o heteroarilo está opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en $-CN$, halo, $-O-R^{12}$, $-C(O)-R^{12}$, $-N(R^{13})(R^{14})$, alquilo C_{1-9} , haloalquilo C_{1-9} , y heterociclilo, o una sal farmacéuticamente aceptable del mismo;
- (ii) R^4 es heteroarilo opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en $-CN$, halo, $-O-R^{12}$, $-C(O)-R^{12}$, $-N(R^{13})(R^{14})$, alquilo C_{1-9} , haloalquilo C_{1-9} , y heterociclilo, o una sal farmacéuticamente aceptable del mismo;
- (iii) R^4 es heterociclilo opcionalmente sustituido con uno a tres sustituyentes seleccionados independientemente del grupo que consiste en $-CN$, halo, $-O-R^{12}$, $-C(O)-R^{12}$, $-N(R^{13})(R^{14})$, alquilo C_{1-9} , haloalquilo C_{1-9} , y heterociclilo, o una sal farmacéuticamente aceptable del mismo;

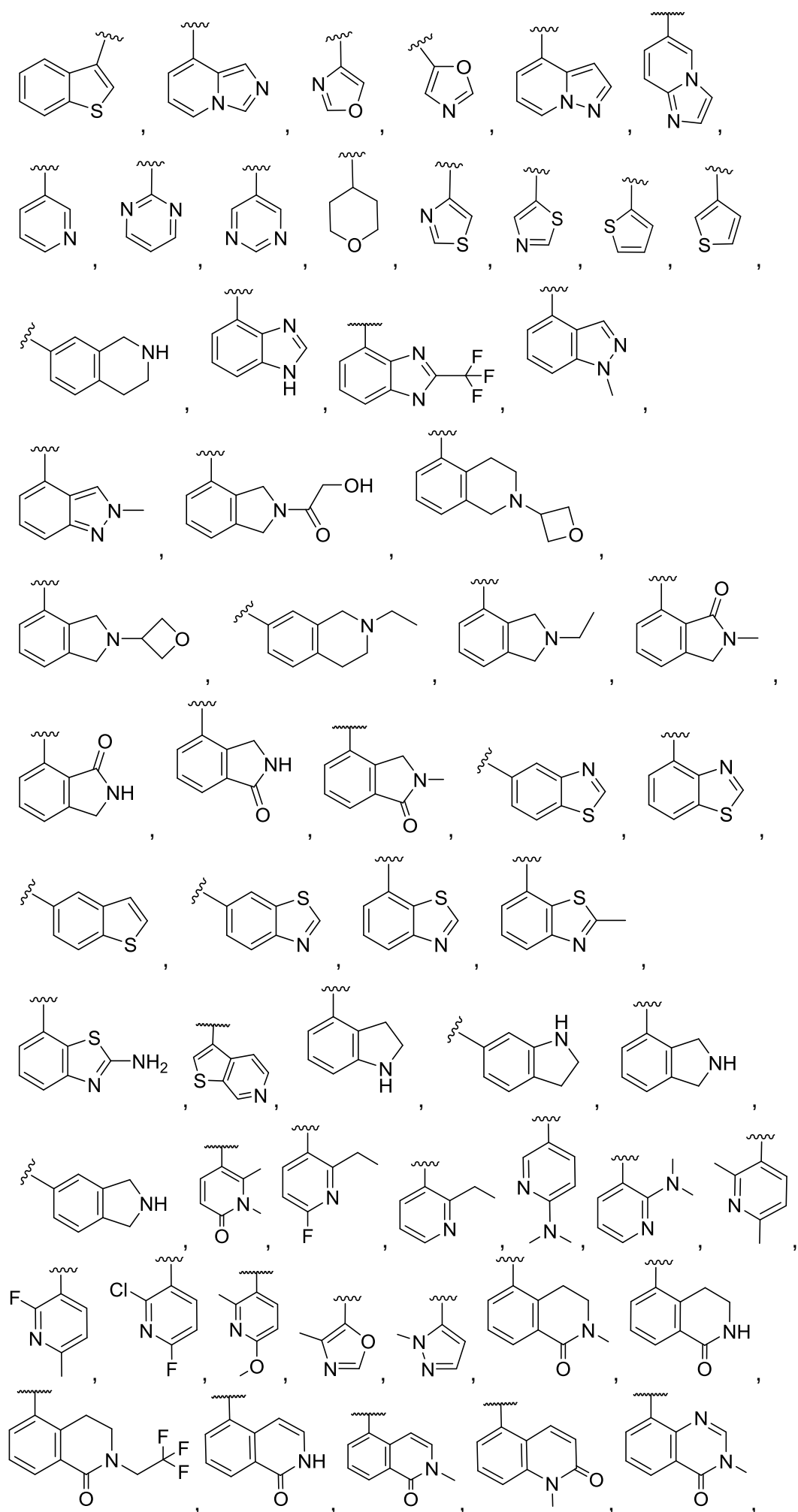


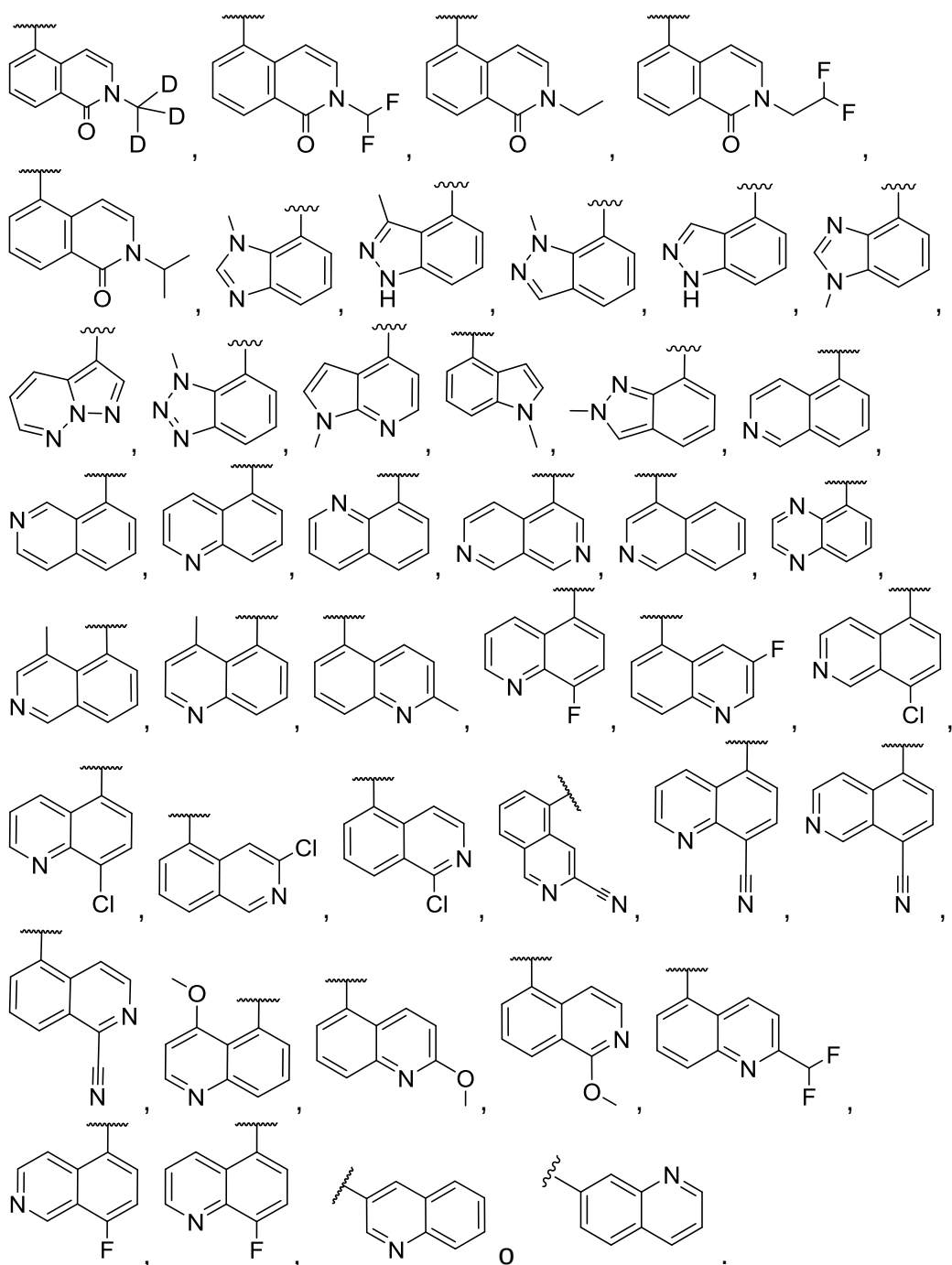


en donde cada Z^4 de (iv) a (vi) puede ser seleccionado independientemente del grupo que consiste en $-CN$, halo, $-O-R^{12}$, $-C(O)-R^{12}$, $-N(R^{13})(R^{14})$, alquilo C_{1-9} , haloalquilo C_{1-9} , y heterociclilo, o una sal farmacéuticamente aceptable del mismo;

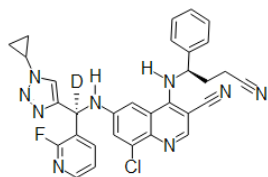
(vii) R⁴ es







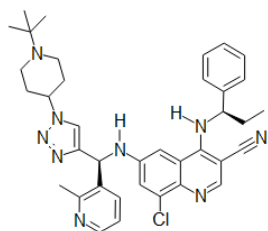
10. El compuesto de la reivindicación 1 seleccionado de la **Tabla 1**, o una sal farmacéuticamente aceptable del mismo.
11. Una composición que comprende un compuesto de cualquiera de las reivindicaciones 1-10 y un vehículo farmacéuticamente aceptable.
12. El compuesto de la reivindicación 1 o la composición de la reivindicación 11, en donde el compuesto tiene la fórmula



,

o una sal farmacéuticamente aceptable del mismo.

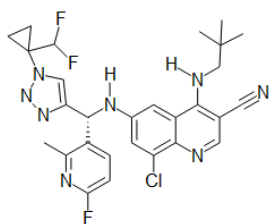
- 13.** El compuesto de la reivindicación 1 o la composición de la reivindicación 11, en donde el compuesto tiene la fórmula



,

o una sal farmacéuticamente aceptable del mismo.

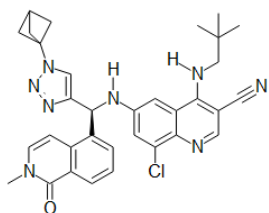
- 14.** El compuesto de la reivindicación 1 o la composición de la reivindicación 11, en donde el compuesto tiene la fórmula



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o una sal farmacéuticamente aceptable del mismo.

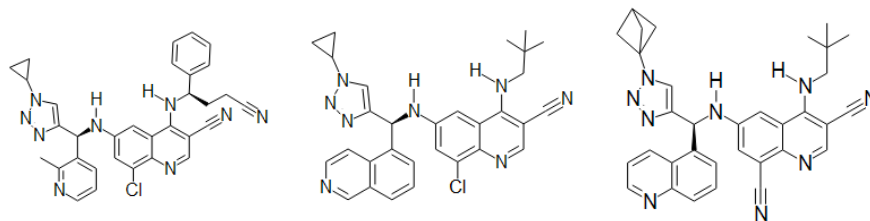
- 15.** El compuesto de la reivindicación 1 o la composición de la reivindicación 11, en donde el compuesto tiene la fórmula



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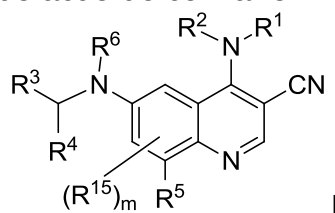
o una sal farmacéuticamente aceptable del mismo.

16. El compuesto de la reivindicación 1 o la composición de la reivindicación 11, en el que el compuesto tiene una de las siguientes fórmulas:

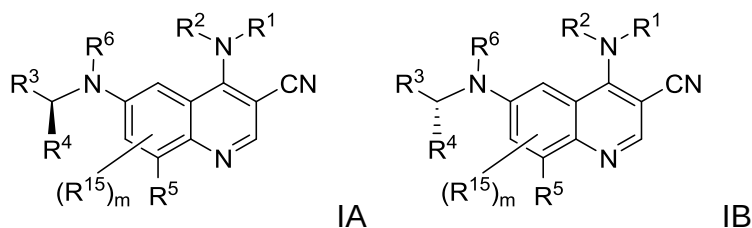


o una sal farmacéuticamente aceptable del mismo.

17. Una composición que comprende una mezcla de estereoisómeros de un compuesto de Formula I de acuerdo con la reivindicación 1:



en donde la mezcla comprende compuestos de Formula IA y IB en una proporción de al menos aproximadamente 3:1:



en donde m, R¹, R², R³, R⁴, R⁵, R⁶ y R¹⁵ son como se definen en la reivindicación 1.

18. Una composición que comprende un compuesto o composición de acuerdo a cualquiera de las reivindicaciones 1-17 y un agente anti-inflamatorio adicional, en donde dicho agente anti-inflamatorio adicional es preferiblemente un inhibidor de $\alpha 4\beta 7$, un esteroide, un anticuerpo MMP-9, un agonista de S1P1, un biológico FNT, o cualquier combinación de los mismos.
19. The compound according to any one of claims claims 1-10 and 12-16 for use in therapy.
20. The compound or composition according to any one of claims claims 1-17 for use in a method for treating a disease or condition mediated by cancer Osaka thyroid (Cot) in a human patient in need thereof, wherein the disease or condition is preferably selected from the group consisting of:

- (A) cancer;
- (B) diabetes;
- (C) an inflammatory disease such as inflammatory bowel disease (IBD);
- (D) a solid tumor selected from pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, prostate cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, gastric cancer, esophageal cancer, head and neck cancer, melanoma, neuroendocrine cancers, CNS cancers, brain tumors (e.g., glioma, anaplastic oligodendroglioma, adult glioblastoma multiforme, and adult anaplastic astrocytoma), bone cancer, and soft tissue sarcoma;
- (E) type 1 and type 2 diabetes, gestational diabetes, prediabetes, insulin resistance, metabolic syndrome, impaired fasting glycaemia and impaired glucose tolerance;
- (F) systemic lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis (RA), acute disseminated encephalomyelitis, idiopathic thrombocytopenic purpura, multiple sclerosis (MS), inflammatory bowel disease (IBD), sepsis, psoriasis, Sjögren's syndrome, autoimmune hemolytic anemia, asthma, or chronic obstructive pulmonary disease (COPD), ankylosing spondylitis, reactive arthritis, monoarticular arthritis, osteoarthritis, gouty arthritis, juvenile arthritis, juvenile onset rheumatoid arthritis, juvenile rheumatoid arthritis, and psoriatic arthritis;
- (G) diversion colitis, ischemic colitis, infectious colitis, chemical colitis, microscopic colitis (including collagenous colitis and lymphocytic colitis), atypical colitis, pseudomembranous colitis, fulminant colitis, autistic enterocolitis, indeterminate colitis, Behçet's disease, gastroduodenal CD, jejunoileitis, ileitis, ileocolitis, Crohn's (granulomatous) colitis, irritable bowel syndrome, mucositis, radiation induced enteritis, short bowel syndrome, celiac disease, stomach ulcers, diverticulitis, pouchitis, proctitis, and chronic diarrhea;
- (H) alcoholic hepatitis;

(l) systemic lupus erythematosus (SLE), lupus nephritis, lupus-related, and autoimmune disorders or a symptom of SLE, wherein said symptom of SLE is preferably selected from joint pain, joint swelling, arthritis, fatigue, hair loss, mouth sores, swollen lymph nodes, sensitivity to sunlight, skin rash, headaches, numbness, tingling, seizures, vision problems, personality changes, abdominal pain, nausea, vomiting, abnormal heart rhythms, coughing up blood and difficulty breathing, patchy skin color and Raynaud's phenomenon.

- 21.** The use of a compound or composition according to any one of claims 1-17 in the manufacture of a medicament.

LISTA DE ANTECEDENTES CITADOS POR EL EXAMINADOR DE LA OFICINA EUROPEA DE PATENTES (EPO)

- D1 WU J ET AL: "Selective inhibitors of tumor progression loci-2 (Tpl2) kinase with potent inhibition of TNF-alpha production in human whole blood", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 19, no. 13, 7 Mayo 2009 (2009-05-07), páginas 3485-3488, XP026155092, ISSN: 0960-894X, DOI: 10.1016 / J.BMCL.2009.05.009 [consultado el 2009-05-07].
- D2 KAILA ET AL: "Identification of a novel class of selective Tpl2 kinase inhibitors: 4-Alkylamino-[1,7]naphthyridine-3-carbonitriles", BIOORGANIC & MEDICINAL CHEMISTRY, PERGAMON, GB, vol. 15, no. 19, 4 julio 2007 (2007-07-04), páginas 6425-6442, XP022192446, ISSN: 0968-0896, DOI: 10.1016/J.BMC.2007.06.054
- D3 RAKESH K GOYAL ET AL: "Models for anti.inflammatory activity of 8-substitued-4-anilino-6-aminoquinoline-3-carbonitriles", MEDICINAL CHEMISTRY RESEARCH, BIRKHÄUSER-VERLAG, BOSTON, vol. 21, no. 7, 19 marzo 2011 (2011-03-19), páginas 1044-1055, XP035060999, ISSN: 1554-8120, DOI: 10.1007 / S00044-011-9613-5.
- D4 NEAL GREEN ET AL: "Inhibitors of Tumor Progression Loci.2 (Tpl2) Kinase and Tumor Necrosis Factor [alpha] (TNF-[alpha]) Production: Selectivity and in Vivo Antiinflammatory Activity of Novl 8-Substitued-4-anilino-6-aminoquinoline-3-carbonitriles", JOURNAL OF MEDICINAL CHEMISTRY, vol. 50, no. 19, 2007, páginas 4728-4745, XP055294201, US ISSN: 0022-2623, DOI: 10.1021 / jm070436q.
- D5 WO 98/43960 A1 (AMERICAN CYANAMID CO [US]) 8 de octubre de 1998 (1998-10-08)