

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIRECCIÓN DE NUEVAS CREACIONES

E. _____ S. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Re.: Expediente No. 10-122.816

Solicitud de Patente de Invención titulada: "ARIL/HETEROARIL[1,7]NAFTIRIDIN-5-AMINAS, ACTIVAS COMO AGONISTAS DEL RECEPTOR TIPO TOLL-7 (TLR-7)"

Solicitante: IRM LLC y NOVARTIS AG

Nuestra Ref.: P2010/000122

RESPUESTA AL OFICIO No. 04794, NOTIFICADO POR FIJACIÓN EN LISTA No. 011

DE FECHA 12 DE MARZO DE 2013

(PRIMER EXAMEN DE FONDO)

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

1. En primer lugar, con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad el Capítulo Reivindicatorio presentado el 05 de enero del 2011.

Así entonces, el Capítulo Reivindicatorio Modificado adjunto contiene los siguientes cambios:

- se eliminaron las Reivindicaciones 2-6, 10, 11, 23, 31 y 33-40;
- en la Reivindicación 1 se limitó el significado de los sustituyentes X^4 , R^4 , R^5 , R^7 , A y n.
- en la nueva Reivindicación 4 se eliminó la estructura de Fórmula XII;
- se limitó el significado de R^7 en las nuevas Reivindicaciones 7 y 8, de acuerdo con las limitaciones de la Reivindicación 1;
- en la nueva Reivindicación 10 se limitó el significado de R^{10} ;
- en la nueva Reivindicación 12 se incluyó una definición de R^{13} y L, la cual encuentra soporte en la Reivindicación 1;
- se eliminaron 53 compuestos de la nueva Reivindicación 14 (antigua Reivindicación 21), de acuerdo con las limitaciones de la Reivindicación 1;
- en la nueva Reivindicación 15 (antigua Reivindicación 22) se limitó el significado de R^4 a II;
- en la nueva Reivindicación 19 (antigua Reivindicación 27) se incluyeron los compuestos 2-(4-(isopentiloxi)-2-metilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina; y 2-(2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)etoxi)etanol, los cuales corresponden a los Ejemplos 161 y 148, respectivamente;
- la nueva Reivindicación 21 (antigua Reivindicación 29) se modificó de tal manera que se indica que el antígeno es un antígeno bacteriano, lo cual encuentra soporte en las páginas 435 a 438 del Capítulo Descriptivo y en la antigua Reivindicación 35;
- se combinaron las antiguas Reivindicaciones 30 y 31 en la nueva Reivindicación 22, particularmente se limita el agente inmunorregulador a una composición que contiene minerales y una emulsión oleosa, lo cual encuentra soporte en las páginas 435 a 438 del Capítulo Descriptivo;
- se agregaron las nuevas Reivindicaciones 24 y 25, las cuales reclaman compuestos específicos que encuentran soporte en la nueva Reivindicación 15.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican

una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial. Estas, por el contrario, corresponden a una drástica limitación de la materia reivindicada.

2. a- La Examinadora solicita que el título de la solicitud sea modificado de manera que se elimine la referencia a los usos, la cual no es permitida.

b- En atención a la anterior objeción, me permito modificar el título de la siguiente manera, la cual ha eliminado los usos de los compuestos y se refiere a la materia reclamada en el Capítulo Reivindicatorio Modificado adjunto:

“ARIL/HETTEROARIL[1,7]NAFTIRIDIN-5-AMINAS, ACTIVAS COMO AGONISTAS DEL
RECEPTOR TIPO TOLL-7 (TLR-7)”

3. a- La Examinadora señala que el Capítulo Reivindicatorio no es claro ni conciso por las siguientes razones:

- la Reivindicación 1 es demasiado amplia, abarcando un sinnúmero de compuestos. Particularmente, los significados para R^4 , R^5 , A y sustituciones para A no son concisos;
- las Reivindicaciones 29 y 30 mencionan un “antígeno” y un “agente inmunorregulador adicional”, respectivamente, los cuales no se especifican;
- la Reivindicación 33 está caracterizada por el efecto inmunoestimulante que se genera con la administración de una cantidad suficiente de un compuesto, lo cual no es claro porque una reivindicación de composición debe caracterizarse por sus componentes y no por el efecto causado por alguna de sus partes.

b- Llamo respetuosamente la atención de la Examinadora sobre las modificaciones introducidas en el Capítulo Reivindicatorio Modificado adjunto, tal y como se explican en el punto 1 del

4

presente memorial. Específicamente, en la Reivindicación 1 se limitaron las definiciones de R^4 , R^5 y Λ a $R^4 = H$, $R^5 = -(CH_2)_nR^7$ o alquilo C_2 sustituidos con $-R^7$ y $-OR^8$, y $A =$ fenilo o piridilo opcionalmente sustituidos con R^A . Adicionalmente, se limitó la definición de X^4 a CR^3 , de n a 2 y de R^7 a un grupo de sustituyentes reducido. Por su parte, en la nueva Reivindicación 23 (antigua Reivindicación 29) el término “antígeno” fue modificado por “antígeno bacteriano”, lo cual encuentra soporte en las páginas 435 a 438 del Capítulo Descriptivo. Asimismo, el término “inmunorregulador” fue modificado en la nueva Reivindicación 22 (antiguas Reivindicaciones 30 y 31) el cual se define como “un coadyuvante seleccionado de una composición que contiene minerales y una emulsión oleosa”, lo cual encuentra soporte en las páginas 435 a 438 del Capítulo Descriptivo. Finalmente, la Reivindicación 33 fue eliminada del Capítulo Reivindicatorio.

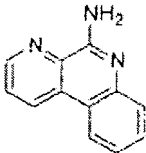
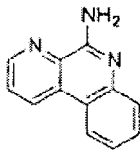
Teniendo en cuenta lo anterior, considero superadas las anteriores objeciones en tanto que el Capítulo Reivindicatorio Modificado adjunto fue limitado conforme a las observaciones y sugerencias realizadas por la Examinadora, cumpliendo con los requisitos de claridad establecidos en la Decisión 486, y a la luz del Manual Andino de Patentes (MAP) y el Instructivo de la SIC, permitiendo a la persona medianamente versada en la materia determinar inequívocamente el alcance de la materia reclamada.

4. a- i) La Examinadora señala que el documento de Ferraris Dana et al¹ (D1) afecta la novedad de la Reivindicación 1.

La Examinadora argumenta que los compuestos de la Reivindicación 1 fueron completamente divulgados por D1, especialmente cuando en la Fórmula I-a de la presente invención, X^4 es CH , X^5 es $CH=CH$, X^3 es N y Λ es arilo. La Examinadora muestra la siguiente tabla comparativa:

¹ Ferraris D. *et al*: “Design and synthesis of poly ADP-ribose polymerase-1 inhibitors. 2. Biological evaluation of aza-5(II)-phenanthridin-6-ones as potent, aqueous-soluble compounds for the treatment of ischemic injuries”. Journal of Medicinal Chemistry, Vol. 46, No. 14, 2003, pp. 3138-3151.

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Características esenciales	D1
	

ii) Adicionalmente, la Examinadora señala que las patentes US5741909 (D2) y WO2007109813 (D3) afectan el nivel inventivo de las Reivindicaciones 1 a 40.

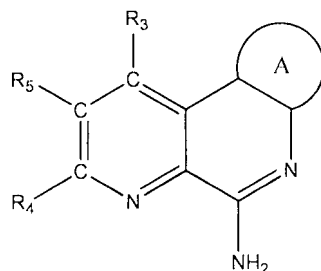
La Examinadora argumenta que la diferencia entre D2 y la presente invención es que D2 divulga compuestos de amino-imidazo-piridinas fusionadas, mientras que la materia aquí reivindicada comprende compuestos del tipo amino-pirido o pirazino-piridinas fusionadas en donde se encuentra un átomo de carbono adicional en el anillo de imidazol (cuando $X^4=N$) o un átomo de carbono adicional en el anillo de imidazol y el intercambio de un átomo de nitrógeno por uno de carbono (cuando $X^4=CH$).

Respecto a D3, la Examinadora observa que la diferencia ente dicha anterioridad y la presente invención es que los compuestos del arte previo se relacionan con benzo-amino-imidazo-pirazinas fusionadas, mientras que la materia reclamada se refiere a amino-pirido o pirazino-piridinas fusionadas.

El problema técnico que encuentra la Examinadora es cómo modificar los compuestos de D2 para lograr compuestos alternativos potenciadores inmunológicos para el tratamiento del cáncer, y concluye que una persona medianamente versada en la materia podría haber resuelto dicho problema mediante la aplicación de las enseñanzas de los compuestos de D3, haciendo obvia la presente invención.

b- i) En primer lugar, llamo respetuosamente la atención de la Examinadora sobre las limitaciones incluidas en el Capítulo Reivindicatorio Modificado adjunto. En particular, la

limitación del radical R^4 y del radical R^5 . Así, cuando se reemplaza el significado de los radicales $X3$, $X4$ y $X5$ en la Fórmula I-A, se obtiene la siguiente estructura:



A la luz del significado restringido del sustituyente R^5 , la posición 3 del núcleo benzo[f][1,7]naftiridina o la posición 8 del núcleo pirido[3,2-f][1,7]naftiridina está necesariamente sustituida por un grupo $-(CH_2)_nR^7$ o alquilo C_2 , sustituidos con $-R^7$ y $-OR^8$. Es decir, luego de las modificaciones introducidas R^5 ya no significa hidrógeno, por lo que el alcance de la materia reclamada ya no comprende el compuesto señalado por la Examinadora.

En este punto, me permito recalcar que para fundamentar una afectación válida contra la novedad de una invención, el arte previo debe divulgar explícitamente todas y cada una de las características de la invención.² Asimismo, el Tribunal Andino de Justicia (TAJ) ha determinado que para establecer una objeción de novedad válida, la “divulgación debe ser detallada y, en todo caso, suficiente para que una persona del oficio pueda utilizar esa información para ejecutar o explotar la invención”.³ Teniendo en cuenta las modificaciones realizadas, y a la luz de lo establecido el MAP y el TAJ, se puede concluir que la presente invención es nueva sobre lo divulgado en D1.

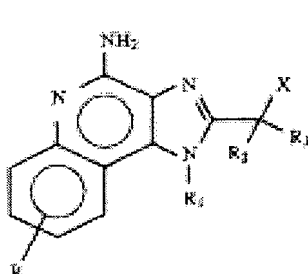
ii) Ahora bien, me permito resaltar a continuación las características y enseñanzas principales de los documentos D2 y D3 citados por la Examinadora:

- D2 divulga compuestos de núcleo 1-sustituido/2-sustituido-1H-imidazo[4,5-c]-quinolin-4-aminas, activos como **agentes antivirales**, lo cual difiere de los compuestos de la presente invención que presentan un núcleo benzo/

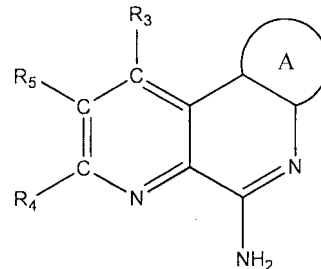
² Manual Andino de Patentes (2004). Sección 9.4. (disponible en <http://www.comunidadandina.org/public/patentes.pdf>).

³ Proceso 06-IP-89 (<http://intranet.comunidadandina.org/Documentos/Procesos/209-IP-2005.doc>) y Proceso 36-IP-2004 (<http://www.notinet.com.co/serverfiles/servicios/archivos/30jul04/ca36-04.htm>)

pirido[1,7]naftiridin-5-aminas útiles como en el tratamiento de enfermedades o desórdenes presentados cuando la modulación del receptor TLR se encuentra implicada:

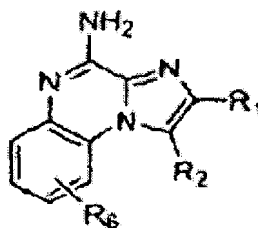


D2



presente invención

- D3 divulga compuestos del tipo imidazoquinoxalina y análogos del mismo, útiles como agentes inmunoterapéuticos para enfermedades proliferativas, infecciosas, autoinmunes, alergias y/o asma, que presentan la siguiente estructura completamente diferente a los compuestos aquí reclamados:

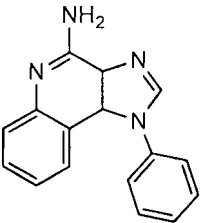
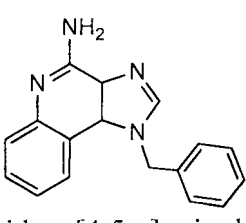
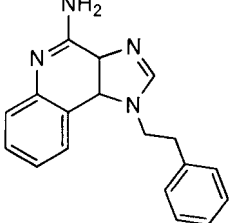


Teniendo en cuenta las diferencias estructurales que saltan a la vista, se puede concluir que nada en D2 y D3 habría motivado a la persona medianamente versada en la materia a producir específicamente benzo/ pirido[1,7]naftiridin-5-aminas, cuando dichos documentos divulgan 1-sustituido/2-sustituido-1H-imidazo[4,5-c]-quinolin-4-aminas o imidazoquinoxalinas que presentan también una actividad farmacológica no relacionada con los compuestos de la presente invención.

En adición a lo anterior, me permito mostrar en la siguiente tabla los resultados del artículo anexo de Gerster J. F. et al⁴ (ANEXO 1) desarrollado, entre otros, por dos de los autores de D2, en el cual se muestra que el uso del sustituyente fenilo en el centro de 1H-imidazo[4,5-c]quinolina reduce las capacidades moduladoras del compuesto:

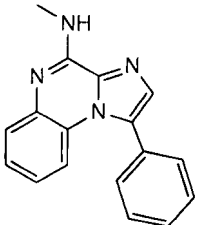
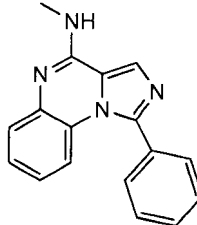
⁴ Gerster J. F. et al: "Synthesis and Structure-Activity-Relationships of 1H-imidazo[4,5-c]quinolines That Induce Interferon Production". Journal of Medicinal Chemistry, vol. 48, no. 10, 2005, pp. 3481-3491

8

Compuesto	 imidazo[4,5-c]quinolin-4-amina	 imidazo[4,5-c]quinolin-4-amina	 imidazo[4,5-c]quinolin-4-amina
Concentración efectiva mínima para observar inducción sobre IFN (agonista)	No hubo inducción	0.1 µg/mL	0.5 µg/mL

Con base en estos resultados, junto con el hecho que D2 enseñe el uso de un sustituyente bencilo y en ninguna parte de su contenido ilustre el uso de un sustituyente fenetilo, una persona medianamente versada en la materia no estaría motivada o guiada a utilizar un núcleo naftiridin dicho sustituido por un grupo fenetilo, sino a lo sumo un núcleo de 1*H*-imidazo[4,5-*c*]quinolina sustituido por un grupo bencilo con el fin de mejorar el efecto farmacológico del compuesto.

Me permito además resaltar los resultados de la siguiente tabla mostrados en los artículos anexos de Moarbess G. et al⁵ y Deleuze-Masquefa C. et al⁶ (ANEXOS 2 y 3) en los cuales se presentan las capacidades inhibitorias de la imidazo[1,2-*a*]quinoxalin-4-amina y la imidazo[1,5-*a*]quinoxalin-4-amina sobre la proliferación de células A375:

Compuesto	 imidazo[1,2-a]quinoxalin-4-amina (7a) en Deleuze-Masquefa C. et al	 imidazo[1,5-a]quinoxalin-4-amina (7a) en Moarbess G. et al
IC ₅₀ (µM)	2.19	> 400

⁵ Moarbess G. et al: "In vitro and in vivo anti-tumoral activities of imidazo[1,2-*a*]quinoxaline, imidazo[1,5-*a*]quinoxaline, and pyrazolo[1,5-*a*]quinoxaline derivatives". Bioorganci & Chemical Chemistry, vol. 16, 2008, pp. 6601-6610

⁶ Deleuze-Masquefa C. et al: "New imidazo[1,2-*a*]quinoxaline derivatives: Synthesis and in vitro activity against human melanoma". European Journal of Medicinal Chemistry, vol. 44, 2009, pp. 3406-3411

En concordancia con lo argumentado anteriormente, los compuestos ilustrados presentan actividad **inhibitoria de A375**, por lo que la persona medianamente versada en la materia no podría prever que al desarrollar un núcleo benzo/piridido[1,7]naftiridin-5-amina los compuestos presentarían actividad **agonista de TLR-7**. Además, la relación estructural de los compuestos de dichos artículos con los compuestos divulgados en D2 y D3 sugieren a la persona medianamente versada en la materia a cambiar la posición del nitrógeno del anillo imidazol con el fin de obtener diferentes respuestas biológicas a los compuestos divulgados. Por lo tanto, no resulta obvia la obtención de los compuestos tipo benzo/piridido[1,7]naftiridin-5-aminas reclamados, a partir de las imidazo[4,5-c]quinolin-4-aminas de D2 y/o las imidazo[1,2-a]quinoxalin-4-aminas de D3 más los conocimientos generales citados.

Adicionalmente, el artículo de Moarbess G. et al (ANEXO 2) establece que el grupo metilamina en la posición 4 de la imidazo[1,2-a]quinoxalina es crucial para una alta actividad biológica del compuesto, mientras que el artículo de Gerster J. F. et al (ANEXO 1) sugiere que las sustituciones en el grupo amina en posición 4 de la imidazo[4,5-c]quinolina reducen la actividad biológica de los compuestos. Por ende, la persona medianamente versada en la materia no encontraría en estos artículos sugerencia alguna de cómo mejorar la actividad biológica, pues los compuestos reclamados en la presente invención presentan un grupo amina sin sustituir en la posición 5 de un núcleo completamente diferente al de D2 y D3 (benzo/pirido[1,7]naftiridina).

Adicionalmente, llamo respetuosamente la atención de la Examinadora sobre los resultados de EC₅₀ descritos en la presente invención, que listo a continuación:

- El Ejemplo 5 muestra resultados de EC₅₀ de 15.8μM, 9.01μM y 0.396μM en los ensayos de HEK293-TLR7, HEK293-TLR8 y IL-6hPBMC, respectivamente;
- el Ejemplo 14 muestra resultados de EC₅₀ de 37μM y 6.38μM en ensayos de HEK293-TLR8 y IL-6hPBMC, respectivamente;

- el Ejemplo 15 muestra resultados de EC_{50} de $24.1\mu M$ en un ensayo de HEK293-TLR7;
- el Ejemplo 30 provee resultados de EC_{50} de $8.64\mu M$, $13\mu M$ y $2.28\mu M$ en ensayos de HEK293-TLR7, HEK293-TLR8 y IL-6hPBMC, respectivamente;
- el Ejemplo 33 provee resultados de EC_{50} de $2.44\mu M$ y $2.28\mu M$ en ensayos HEK293-TLR7 y IL-6hPMBC, respectivamente; y
- los resultados de EC_{50} de la estimulación de TLR-7 y TLR-8 para ciertos compuestos de la Fórmula (I) enlistados en las tablas 2 y 3, respectivamente.

Dados estos resultados, una persona medianamente versada en la materia no habría esperado este nivel de actividad biológica con base en las enseñanzas de D2 y D3.

En este punto vale la pena señalar que, tal y como lo establece el MAP, las invenciones en el campo de la química tienen nivel inventivo cuando los compuestos tienen una **estructura inesperada** (no derivable por la persona medianamente versada en la materia a partir del arte previo) y cuando presentan un **efecto técnico inesperado** o mejorado impredecible.⁷

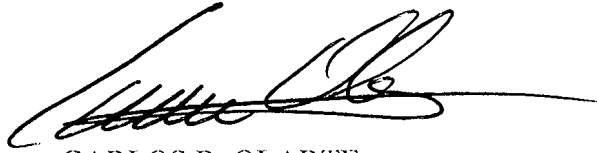
En conclusión, y de acuerdo con los lineamientos citados anteriormente, **nada** en el arte previo (D2 y D3) habría guiado inequívocamente a la persona medianamente versada en la materia a sintetizar los compuestos aquí reivindicados y mucho menos la misma persona podría esperar con alguna expectativa de éxito que dichos compuestos presentaran actividad agonista sobre TLR-7, por lo que su nivel inventivo debe ser reconocido.

6. Por todo lo anterior, considero que las objeciones presentadas por la Examinadora en su examen de fondo han sido resueltas y por consiguiente solicito respetuosamente que el Despacho tenga por cumplidos los requisitos de patentabilidad y se proceda con la concesión de la patente.

⁷ Ibídem. Sección 10.8.

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Atentamente,



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

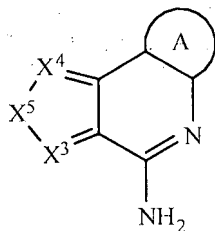
Ancxos:

- Capítulo Reivindicatorio Modificado.
- ANEXO 1: Gerster J. F. *et al*: "Synthesis and Structure-Activity-Relationships of 1*H*-imidazo[4,5-*c*]quinolines That Induce Interferon Production". *Journal of Medicinal Chemistry*, vol. 48, no. 10, 2005, pp. 3481-3491
- ANEXO 2: Moarbess G. *et al*: "In vitro and in vivo anti-tumoral activities of imidazo[1,2-*a*]quinoxaline, imidazo[1,5-*a*]quinoxaline, and pyrazolo[1,5-*a*]quinoxaline derivatives". *Bioorganic & Chemical Chemistry*, vol. 16, 2008, pp. 6601-6610
- ANEXO 3: Deleuze-Masquefa C. *et al*: "New imidazo[1,2-*a*]quinoxaline derivatives: Synthesis and in vitro activity against human melanoma". *European Journal of Medicinal Chemistry*, vol. 44, 2009, pp. 3406-3411

CAPITULO REIVINDICATORIO MODIFICADO

(Primer Examen de Fondo)

1. Un compuesto de la Fórmula (I-A), o una sal aceptable para uso farmacéutico:



Fórmula (I-A)

en donde:

X^3 es N;

X^4 es CR^3 ;

X^5 es $-CR^4=CR^5-$;

R^3 es H;

R^4 es H;

R^5 es $-(CH_2)_nR^7$ o alquilo C_2 sustituidos con $-R^7$ y $-OR^8$;

cada L se selecciona de manera independiente de un enlace, $-(O(CH_2)_m)-$, alquilo C_1-C_6 , alquenileno C_2-C_6 y alquinileno C_2-C_6 , donde los grupos alquilo C_1-C_6 , alquenileno C_2-C_6 y alquinileno C_2-C_6 de L están, cada uno, opcionalmente sustituidos con 1 a 4 sustituyentes seleccionados independientemente de halógeno, $-R^8$, $-OR^8$, $-N(R^9)_2$, $-P(O)(OR^8)_2$, $-OP(O)(OR^8)_2$, $-P(O)(OR^{10})_2$, y $-OP(O)(OR^{10})_2$;

R^7 se selecciona entre fenilo, naftilo, heteroarilo, cicloalquilo C_3-C_8 y heterocicloalquilo C_3-C_8 , donde los grupos fenilo, naftilo, heteroarilo, cicloalquilo C_3-C_8 y heterocicloalquilo C_3-C_8 de R^7 están, cada uno, opcionalmente sustituidos con 1 a 3 grupos R^{13} , y cada R^{13} se selecciona independientemente entre halógeno, $-CN$, $-LR^9$, $-LOR^9$, $-OLR^9$, $-LR^{10}$, $-LOR^{10}$, $-OLR^{10}$, $-LR^8$, $-LOR^8$, $-OLR^8$, $-LSR^8$, $-LSR^{10}$, $-LC(O)R^8$, $-OLC(O)R^8$, $-LC(O)OR^8$, $-LC(O)R^{10}$, $-LOC(O)OR^8$, $-LC(O)NR^9R^{11}$, $-LC(O)NR^9R^8$, $-LN(R^9)_2$, $-LNR^9R^8$, $-LNR^9R^{10}$, $-LC(O)N(R^9)_2$, $-LS(O)_2R^8$, $-LS(O)R^8$, $-LC(O)NR^8OH$, $-LNR^9C(O)R^8$, $-LNR^9C(O)OR^8$, $-LS(O)_2N(R^9)_2$, $-OLS(O)_2N(R^9)_2$, $-LNR^9S(O)_2R^8$, $-LC(O)NR^9LN(R^9)_2$, $-LP(O)(OR^8)_2$, $-LOP(O)(OR^8)_2$, $-LP(O)(OR^{10})_2$ y $-OLP(O)(OR^{10})_2$;

cada R^8 se selecciona independientemente entre H, $-CH(R^{10})_2$, alquilo C_1-C_8 , alqueno C_2-C_8 , alquino C_2-C_8 , haloalquilo C_1-C_6 , alcoxi C_1-C_6 , heteroalquilo C_1-C_6 , cicloalquilo C_3-C_8 , heterocicloalquilo C_2-C_8 , hidroxialquilo C_1-C_6 , y haloalcoxi C_1-C_6 , donde los grupos alquilo C_1-C_8 , alqueno C_2-C_8 , alquino C_2-C_8 , heteroalquilo C_1-C_6 , haloalquilo C_1-C_6 , alcoxi C_1-C_6 , cicloalquilo C_3-C_8 , heterocicloalquilo C_2-C_8 , hidroxialquilo C_1-C_6 y haloalcoxi C_1-C_6 de R^8 están, cada uno, opcionalmente sustituidos

con 1 a 3 sustituyentes seleccionados independientemente entre $-\text{CN}$, R^{11} , $-\text{OR}^{11}$, $-\text{SR}^{11}$, $-\text{C}(\text{O})\text{R}^{11}$, $-\text{OC}(\text{O})\text{R}^{11}$, $-\text{C}(\text{O})\text{N}(\text{R}^9)_2$, $-\text{C}(\text{O})\text{OR}^{11}$, $-\text{NR}^9\text{C}(\text{O})\text{R}^{11}$, $-\text{NR}^9\text{R}^{10}$, $-\text{NR}^{11}\text{R}^{12}$, $-\text{N}(\text{R}^9)_2$, $-\text{OR}^9$, $-\text{OR}^{10}$, $-\text{C}(\text{O})\text{NR}^{11}\text{R}^{12}$, $-\text{C}(\text{O})\text{NR}^{11}\text{OH}$, $-\text{S}(\text{O})_2\text{R}^{11}$, $-\text{S}(\text{O})\text{R}^{11}$, $-\text{S}(\text{O})_2\text{NR}^{11}\text{R}^{12}$, $-\text{NR}^{11}\text{S}(\text{O})_2\text{R}^{11}$, $-\text{P}(\text{O})(\text{OR}^{11})_2$, y $-\text{OP}(\text{O})(\text{OR}^{11})_2$;

cada R^9 se selecciona independientemente entre H , $-\text{C}(\text{O})\text{R}^8$, $-\text{C}(\text{O})\text{OR}^8$, $-\text{C}(\text{O})\text{R}^{10}$, $-\text{C}(\text{O})\text{OR}^{10}$, $-\text{S}(\text{O})_2\text{R}^{10}$, alquilo $\text{C}_1\text{-C}_6$, heteroalquilo $\text{C}_1\text{-C}_6$ y cicloalquilo $\text{C}_3\text{-C}_6$; o cada R^9 es independientemente un alquilo $\text{C}_1\text{-C}_6$ que junto con el N al que está unido forman un heterocicloalquilo $\text{C}_3\text{-C}_8$, en donde el anillo heterocicloalquilo $\text{C}_3\text{-C}_8$ contiene opcionalmente un heteroátomo adicional seleccionado entre N, O y S, y donde los grupos alquilo $\text{C}_1\text{-C}_6$, heteroalquilo $\text{C}_1\text{-C}_6$, cicloalquilo $\text{C}_3\text{-C}_6$, o heterocicloalquilo $\text{C}_3\text{-C}_8$ de R^9 están, cada uno, opcionalmente sustituidos con 1 a 3 sustituyentes seleccionados independientemente de $-\text{CN}$, R^{11} , $-\text{OR}^{11}$, $-\text{SR}^{11}$, $-\text{C}(\text{O})\text{R}^{11}$, $-\text{OC}(\text{O})\text{R}^{11}$, $-\text{C}(\text{O})\text{OR}^{11}$, $-\text{NR}^{11}\text{R}^{12}$, $-\text{C}(\text{O})\text{NR}^{11}\text{R}^{12}$, $-\text{C}(\text{O})\text{NR}^{11}\text{OH}$, $-\text{S}(\text{O})_2\text{R}^{11}$, $-\text{S}(\text{O})\text{R}^{11}$, $-\text{S}(\text{O})_2\text{NR}^{11}\text{R}^{12}$, $-\text{NR}^{11}\text{S}(\text{O})_2\text{R}^{11}$, $-\text{P}(\text{O})(\text{OR}^{11})_2$, y $-\text{OP}(\text{O})(\text{OR}^{11})_2$;

cada R^{10} se selecciona independientemente entre arilo, heterocicloalquilo $\text{C}_3\text{-C}_8$ y heteroarilo, donde los grupos arilo, heterocicloalquilo $\text{C}_3\text{-C}_8$ y heteroarilo están opcionalmente sustituidos con 1 a 3 sustituyentes seleccionados entre halógeno, $-\text{R}^8$, $-\text{OR}^8$, $-\text{LR}^9$, $-\text{LOR}^9$, $-\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{C}(\text{O})\text{R}^8$, $-\text{NR}^9\text{CO}_2\text{R}^8$, $-\text{CO}_2\text{R}^8$, $-\text{C}(\text{O})\text{R}^8$ y $-\text{C}(\text{O})\text{N}(\text{R}^9)_2$;

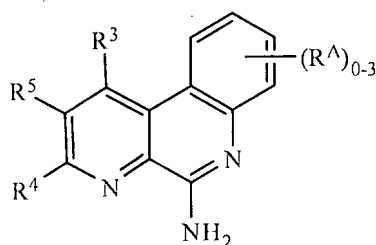
R^{11} y R^{12} se seleccionan independientemente entre H , alquilo $\text{C}_1\text{-C}_6$, heteroalquilo $\text{C}_1\text{-C}_6$, haloalquilo $\text{C}_1\text{-C}_6$, arilo, heteroarilo, cicloalquilo $\text{C}_3\text{-C}_8$ y heterocicloalquilo $\text{C}_3\text{-C}_8$, en donde los grupos alquilo $\text{C}_1\text{-C}_6$, heteroalquilo $\text{C}_1\text{-C}_6$, haloalquilo $\text{C}_1\text{-C}_6$, arilo, heteroarilo, cicloalquilo $\text{C}_3\text{-C}_8$ y heterocicloalquilo $\text{C}_3\text{-C}_8$ de R^{11} y R^{12} cada uno está opcionalmente sustituido con 1 a 3 sustituyentes seleccionados independientemente entre halógeno, $-\text{CN}$, R^8 , $-\text{OR}^8$, $-\text{C}(\text{O})\text{R}^8$, $-\text{OC}(\text{O})\text{R}^8$, $-\text{C}(\text{O})\text{OR}^8$, $-\text{N}(\text{R}^9)_2$, $-\text{NR}^8\text{C}(\text{O})\text{R}^8$, $-\text{NR}^8\text{C}(\text{O})\text{OR}^8$, $-\text{C}(\text{O})\text{N}(\text{R}^9)_2$, heterocicloalquilo $\text{C}_3\text{-C}_8$, $-\text{S}(\text{O})_2\text{R}^8$, $-\text{S}(\text{O})_2\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{S}(\text{O})_2\text{R}^8$, haloalquilo $\text{C}_1\text{-C}_6$ y haloalcoxi $\text{C}_1\text{-C}_6$; o

R^{11} y R^{12} son independientemente alquilo $\text{C}_1\text{-C}_6$, y tomados junto con el átomo de N al cual están unidos forman un anillo heterocicloalquilo $\text{C}_3\text{-C}_8$ opcionalmente sustituido, que contiene opcionalmente un heteroátomo adicional seleccionado de N, O y S;

el anillo A es fenilo o piridilo, donde los grupos fenilo y piridilo están opcionalmente sustituidos con 1 a 3 grupos R^A , donde cada R^A se selecciona independientemente entre $-\text{R}^8$, $-\text{R}^7$, $-\text{OR}^7$, $-\text{OR}^8$, $-\text{R}^{10}$, $-\text{OR}^{10}$, $-\text{SR}^8$, $-\text{NO}_2$, $-\text{CN}$, $-\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{C}(\text{O})\text{R}^8$, $-\text{NR}^9\text{C}(\text{S})\text{R}^8$, $-\text{NR}^9\text{C}(\text{O})\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{C}(\text{S})\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{CO}_2\text{R}^8$, $-\text{NR}^9\text{NR}^9\text{C}(\text{O})\text{R}^8$, $-\text{NR}^9\text{NR}^9\text{C}(\text{O})\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{NR}^9\text{CO}_2\text{R}^8$, $-\text{C}(\text{O})\text{C}(\text{O})\text{R}^8$, $-\text{C}(\text{O})\text{CH}_2\text{C}(\text{O})\text{R}^8$, $-\text{CO}_2\text{R}^8$, $-(\text{CH}_2)_n\text{CO}_2\text{R}^8$, $-\text{C}(\text{O})\text{R}^8$, $-\text{C}(\text{S})\text{R}^8$, $-\text{C}(\text{O})\text{N}(\text{R}^9)_2$, $-\text{C}(\text{S})\text{N}(\text{R}^9)_2$, $-\text{OC}(\text{O})\text{N}(\text{R}^9)_2$, $-\text{OC}(\text{O})\text{R}^8$, $-\text{C}(\text{O})\text{N}(\text{OR}^8)\text{R}^8$, $-\text{C}(\text{NOR}^8)\text{R}^8$, $-\text{S}(\text{O})_2\text{R}^8$, $-\text{S}(\text{O})_3\text{R}^8$, $-\text{SO}_2\text{N}(\text{R}^9)_2$, $-\text{S}(\text{O})\text{R}^8$, $-\text{NR}^9\text{SO}_2\text{N}(\text{R}^9)_2$, $-\text{NR}^9\text{SO}_2\text{R}^8$, $-\text{P}(\text{O})(\text{OR}^8)_2$, $-\text{OP}(\text{O})(\text{OR}^8)_2$, $-\text{P}(\text{O})(\text{OR}^{10})_2$, $-\text{OP}(\text{O})(\text{OR}^{10})_2$, $-\text{N}(\text{OR}^8)\text{R}^8$, $-\text{CH}=\text{CHCO}_2\text{R}^8$, $-\text{C}(=\text{NH})\text{-N}(\text{R}^9)_2$ y $-(\text{CH}_2)_n\text{NHC}(\text{O})\text{R}^8$;

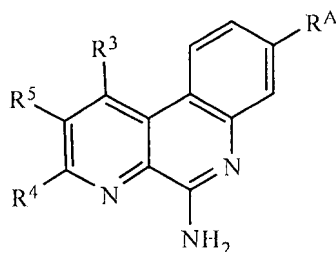
n es, de manera independiente en cada aparición, 2;
cada m se selecciona de manera independiente entre 1; 2; 3; 4; 5 y 6; y
t es 1, 2, 3, 4, 5, 6, 7 u 8.

2. El compuesto de acuerdo con la Reivindicación 1, en donde el compuesto tiene la estructura de la Fórmula (X-A):



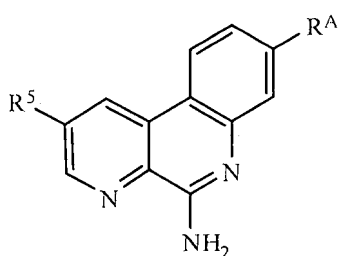
Fórmula (X-A).

3. El compuesto de acuerdo con las Reivindicaciones 1 o 2, en donde el compuesto tiene la estructura de la Fórmula (XI):



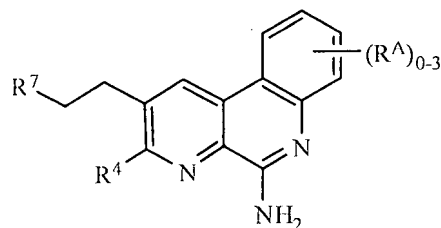
Fórmula (XI).

4. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-3, en donde el compuesto tiene la estructura de la Fórmula (XIII):



Fórmula (XIII).

5. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-2, en donde el compuesto tiene la estructura de la Fórmula (XIV):

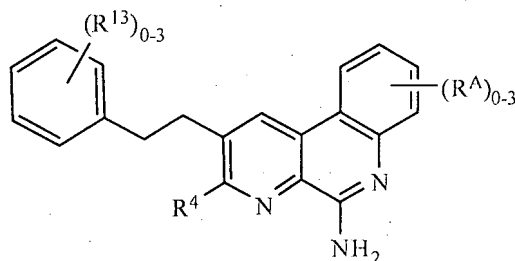


Fórmula (XIV).

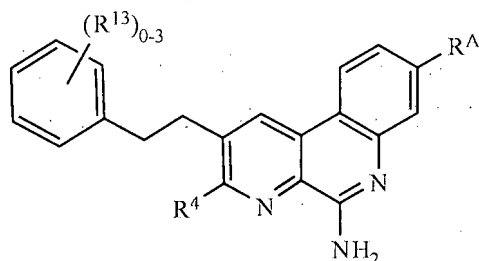
6. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-5, en donde R^7 se selecciona entre fenilo, naftilo y heteroarilo, donde los grupos fenilo, naftilo y heteroarilo de R^7 cada uno está opcionalmente sustituido con 1 a 3 grupos R^{13} , y cada R^{13} se selecciona independientemente entre halógeno, $-\text{CN}$, $-\text{LR}^9$, $-\text{LOR}^9$, $-\text{OLR}^9$, $-\text{LR}^{10}$, $-\text{LOR}^{10}$, $-\text{OLR}^{10}$, $-\text{LR}^8$, $-\text{LOR}^8$, $-\text{OLR}^8$, $-\text{LSR}^8$, $-\text{LSR}^{10}$, $-\text{LC(O)R}^8$, $-\text{OLC(O)R}^8$, $-\text{LC(O)OR}^8$, $-\text{LC(O)R}^{10}$, $-\text{LOC(O)OR}^8$, $-\text{LC(O)NR}^9\text{R}^{11}$, $-\text{C(O)NR}^9\text{R}^8$, $-\text{LN(R}^9)_2$, $-\text{LNR}^9\text{R}^8$, $-\text{LNR}^9\text{R}^{10}$, $-\text{LC(O)N(R}^9)_2$, $-\text{LS(O)}_2\text{R}^8$, $-\text{LS(O)R}^8$, $-\text{LC(O)NR}^8\text{OH}$, $-\text{LNR}^9\text{C(O)R}^8$, $-\text{LNR}^9\text{C(O)OR}^8$, $-\text{LS(O)}_2\text{N(R}^9)_2$, $-\text{OLS(O)}_2\text{N(R}^9)_2$, $-\text{LNR}^9\text{S(O)}_2\text{R}^8$, $-\text{LC(O)NR}^9\text{LN(R}^9)_2$, $-\text{LP(O)(OR}^8)_2$, $-\text{LOP(O)(OR}^8)_2$, $-\text{LP(O)(OR}^{10})_2$ y $-\text{OLP(O)(OR}^{10})_2$.

7. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-6, en donde R^7 es fenilo opcionalmente sustituido con 1-3 grupos R^{13} , y cada R^{13} se selecciona independientemente entre halógeno, haloalquilo $\text{C}_1\text{-C}_6$, $-\text{LR}^8$, LR^9 , $-\text{LOR}^8$ y $-\text{OLR}^8$.

8. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-7, en donde el compuesto tiene la estructura de la Fórmula (XV) o la Fórmula (XVI):



Fórmula (XV)



Fórmula (XVI).

9. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-8, en donde R^{13} se selecciona de $-\text{LR}^9$, $-\text{LOR}^9$, $-\text{OLR}^9$, $-\text{LR}^{10}$, $-\text{LOR}^{10}$, $-\text{OLR}^{10}$, $-\text{LR}^8$, $-\text{LOR}^8$, $-\text{OLR}^8$, $-\text{LSR}^8$, $-\text{LSR}^{10}$, $-\text{LC(O)R}^8$, $-\text{OLC(O)R}^8$, $-\text{LC(O)OR}^8$, $-\text{LC(O)R}^{10}$, $-\text{LOC(O)OR}^8$, $-\text{LC(O)NR}^9\text{R}^{11}$, $-\text{LC(O)NR}^9\text{R}^8$, $-\text{LN(R}^9)_2$, $-\text{LNR}^9\text{R}^8$, $-\text{LNR}^9\text{R}^{10}$, $-\text{LC(O)N(R}^9)_2$, $-\text{LS(O)}_2\text{R}^8$, $-\text{LS(O)R}^8$, $-\text{LC(O)NR}^8\text{OH}$, $-\text{LNR}^9\text{C(O)R}^8$, $-\text{LNR}^9\text{C(O)OR}^8$, $-\text{LS(O)}_2\text{N(R}^9)_2$, $-\text{OLS(O)}_2\text{N(R}^9)_2$, $-\text{LNR}^9\text{S(O)}_2\text{R}^8$, $-\text{LC(O)NR}^9\text{LN(R}^9)_2$, $-\text{LP(O)(OR}^8)_2$, $-\text{LOP(O)(OR}^8)_2$, $-\text{LP(O)(OR}^{10})_2$ y $-\text{OLP(O)(OR}^{10})_2$.

10. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-9, en donde R^{10} es fenilo o un heteroarilo, en donde los grupos fenilo y heteroarilo están opcionalmente sustituidos con 1 a 3 sustituyentes seleccionados entre halógeno, $-R^8$, $-OR^8$, $-LR^9$, $-LOR^9$ y $-N(R^9)_2$.

11. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-10, en donde R^{10} es fenilo opcionalmente sustituido con 1 a 3 sustituyentes seleccionados entre halógeno, $-R^8$, $-OR^8$ y $-N(R^9)_2$.

12. El compuesto de acuerdo con cualquiera de las Reivindicaciones 1-11, en donde cada R^A se selecciona independientemente entre $-R^7$, $-OR^7$, $-R^8$, $-OR^8$, $-R^{10}$, $-OR^{10}$, $-SR^8$, $-N(R^9)_2$, $-S(O)_2R^8$, $-S(O)_3R^8$, $-SO_2N(R^9)_2$, $-S(O)R^8$, $-NR^9SO_2N(R^9)_2$, $-CH=CHCO_2R^8$, $(CH_2)_nCO_2R^8$, $-NR^9SO_2R^8$, $-P(O)(OR^8)_2$, $-OP(O)(OR^8)_2$, $-P(O)(OR^{10})_2$ y $-OP(O)(OR^{10})_2$;

cada R^{13} está seleccionado independientemente entre $-LR^8$, $-LOR^8$ y $-LP(O)(OR^8)_2$;

y

L es un enlace o $-(O(CH_2)_m)_l-$

13. El compuesto de cualquiera de las Reivindicaciones 1-19, caracterizado porque R^8 se selecciona de H, alquilo C_1-C_6 y haloalquilo C_1-C_6 .

14. El compuesto de la Reivindicación 1, seleccionado de:

- 2-(3-metoxifenetil)benzo[f][1,7]naftiridin-5-amina;
- 8-metil-2-fenetilbenzo[f][1,7]naftiridin-5-amina;
- 2-(2-metilfenetil)benzo[f][1,7]naftiridin-5-amina;
- 2-(3-metilfenetil)benzo[f][1,7]naftiridin-5-amina;
- 2-(4-metilfenetil)benzo[f][1,7]naftiridin-5-amina;
- 2-fenetilbenzo[f][1,7]naftiridin-5-amina;
- 8-metil-2-(2-(naftalen-1-il)etil)benzo[f][1,7]naftiridin-5-amina;
- 8-metil-2-(2-(naftalen-2-il)etil)benzo[f][1,7]naftiridin-5-amina;
- ácido 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)benzoico;
- ácido 3-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)benzoico;
- 2-(3-clorofenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
- 2-(2-clorofenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
- (3-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)metanol;
- 2-(4-clorofenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
- 2-(4-butilfenetil)benzo[f][1,7]naftiridin-5-amina;
- 2-(4-butilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(4-propilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(trifluormetil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(2,5-dimetilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-propilfenetil)benzo[f][1,7]naftiridin-5-amina;
8-metil-2-(2,4,5-trimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(2,5-dimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-isopropilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-heptilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-isobutoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-((2-metoxietoxi)metoxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-(2-fenoxietoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-(4-fenilbutoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(aliloxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-(3-fenilpropoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(heptan-4-iloxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-(4-metilpent-3-eniloxi)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(2-ciclohexiletoxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-isopropoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-(3,3-dimetilbutoxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-(2-ciclopropiletil)-2-(4-(dimetilamino)fenetil)benzo[f][1,7]naftiridin-5-amina;
8-(2-ciclopropiletil)-2-(2,4-dimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
N-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)acetamida;
N-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)fenil)acetamida;
N-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)-4-metilbencenosulfonamida;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzonitrilo;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-aminoetil)-3-metilbenzamida;
8-metil-2-(2-metil-4-(1H-tetrazol-5-il)fenetil)benzo[f][1,7]naftiridin-5-amina;
metil 2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamido)-4-metilpentanoato;
metil-2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamido)acetato;
ácido 2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamido)-4-metilpentanoico;
ácido 2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamido)acético;
etil 2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)acetato;

ácido 2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)acético;

ácido 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)propanoico;

ácido 6-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)hexanoico;

8-metil-2-(2-metil-4-(metiltio)fenetil)benzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-(metilsulfonil)fenetil)benzo[f][1,7]naftiridin-5-amina;

2-(4-(hexiloxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-fenetoxyfenetil)benzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-(pentiloxi)fenetil)benzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-(4-metilpentiloxi)fenetil)benzo[f][1,7]naftiridin-5-amina;

2-(2-fluorfenetil)benzo[f][1,7]naftiridin-5-amina;

2-(3-fluorfenetil)benzo[f][1,7]naftiridin-5-amina;

2-(4-fluorfenetil)benzo[f][1,7]naftiridin-5-amina;

2-(2-(tiofen-3-il)etil)benzo[f][1,7]naftiridin-5-amina;

2-(3,4-dimetilfenetil)benzo[f][1,7]naftiridin-5-amina;

2-(3,4-dimetilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(3,5-dimetilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(2-(benzofuran-5-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)-1-feniletanol;

2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)-1-(4-metoxifenil)etanol;

2-(2-(2,6-dimetilpiridin-3-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(2-(5-metoxipiridin-2-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

ácido 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)propanoico;

5-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-4-metilpiridin-2(1H)-ona;

6-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)piridin-3-ol;

8-metil-2-(4-(trifluormetoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;

2-(2-(2,3-dihidro-1H-inden-5-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(2-(2,3-dihidro-1H-inden-5-il)etil)benzo[f][1,7]naftiridin-5-amina;

ácido (E)-3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)acrílico;

(E)-etil 3-(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)acrilato;

(E)-8-(2-ciclopropilvinil)-2-fenetilbenzo[f][1,7]naftiridin-5-amina;

8-(2-ciclopropiletil)-2-fenetilbenzo[f][1,7]naftiridin-5-amina;

(5-amino-2-fenetilbenzo[f][1,7]naftiridin-8-il)metanol;

(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)metanol;

3-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)fenol;

2-(2-metoxifenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-etilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-etilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-(dimetilamino)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(piperidin-1-il)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-ter-butylfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-(piperidin-1-il)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-metoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(3,5-dimetoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(2-(trifluormetil)fenetil)benzo[f][1,7]naftiridin-5-amina;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-hidroxibencimidamida;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)benzonitrilo;
8-metil-2-(4-(1-morfolinoetil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-aminofenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)guanidina;
8-metil-2-(4-(1-(fenetilamino)etil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)acetonitrilo;
2-(4-(piperidin-1-ilmetil)fenetil)benzo[f][1,7]naftiridin-5-amina;
1-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)bencil)piperidin-4-ol;
2-(4-(aminometil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-((etilamino)metil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(2-aminopropan-2-il)fenetil)benzo[f][1,7]naftiridin-5-amina;
ácido 1-(1-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)pirrolidina-3-carboxílico;
8-metil-2-(4-(1-(fenilamino)etil)fenetil)benzo[f][1,7]naftiridin-5-amina;
8-metil-2-propilbenzo[f][1,7]naftiridin-5-amina;
2-(2-(1H-indol-5-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-etoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
8-metil-2-(4-fenoxifenetil)benzo[f][1,7]naftiridin-5-amina;
2-(2,4-dimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(2,4-dimetilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-metoxi-2-metilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenol;
2-(2-(2,3-dihidrobenzofuran-5-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)-1-(2,4-dimetilfenil)etanol;
2-(2-(6-metoxi-4-metilpiridin-3-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
4-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)butan-1-ol;
metil 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)propanoato;

3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)propan-1-ol;
4-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)-2-metilbutan-2-ol;
2-(4-(aminometil)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
(E)-etil 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)acrilato;
etil 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)propanoato;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbencil)propano-1,3-diol;
ácido 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)propanoico;
5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridina-8-carbaldehído;
etil 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)benzoato;
8-metil-2-(4-metilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)propan-2-ol;
(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)metanol;
etil 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzoato;
ácido 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzoico;
(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)metanol;
8-metil-2-(2,4,6-trimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)propan-2-ol;
8-metil-2-(4-propoxifenetil)benzo[f][1,7]naftiridin-5-amina;
ácido (E)-3-(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)acrílico;
etil 3-(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)propanoato;
ácido 3-(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)propanoico;
3-(5-amino-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-8-il)propan-1-ol;
(5-amino-2-(4-metoxifenetil)benzo[f][1,7]naftiridin-8-il)metanol;
8-fenetilbenzo[f][1,7]naftiridin-5-amina;
(5-amino-2-(4-bromofenetil)benzo[f][1,7]naftiridin-8-il)metanol;
2-(4-metoxi-2-metilfenetil)-8-pentilbenzo[f][1,7]naftiridin-5-amina;
8-(2-ciclopropiletíl)-2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-5-amina;
(5-amino-2-(2,4,6-trimetilfenetil)benzo[f][1,7]naftiridin-8-il)metanol;
(5-amino-2-(4-propoxifenetil)benzo[f][1,7]naftiridin-8-il)metanol;
(2-(2-(1H-indol-5-il)etil)-5-aminobenzo[f][1,7]naftiridin-8-il)metanol;
metil 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzoato;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N,3-dimetilbenzamida;

N-(2-acetamidoetil)-4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamida;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-(dimetilamino)etil)-N,3-dimetilbenzamida;

2-(4-metoxifenetil)benzo[f][1,7]naftiridin-5-amina;

2-(4-metoxi-2-metilfenetil)benzo[f][1,7]naftiridin-5-amina;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzamida;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N,N,3-trimetilbenzamida;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-hidroxi)etil)-3-metilbenzamida;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-(dimetilamino)etil)-3-metilbenzamida;

(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)(pirrolidin-1-il)metanona;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-(dictilamino)etil)-3-metilbenzamida;

(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)(4-etilpiperazin-1-il)metanona;

(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)(piperazin-1-il)metanona;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metil-N-(2-(pirrolidin-1-il)etil)benzamida;

4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)-N-(2-(dimetilamino)etil)-N,3-dimetilbenzamida;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-N-(2-(dimetilamino)etil)-N-metilbenzamida;

2-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)propan-2-ol;

2-(4-butoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(2-(bifenil-4-il)etil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-(2-metilaliloxi)fenetil)benzo[f][1,7]naftiridin-5-amina;

2-(4-(isopentiloxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil propil carbonato;

etil 5-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)pentanoato;

2-(4-(ciclopentiloxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(4-(ciclobutilmetoxi)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

8-metil-2-(4-(2-morfolinoetoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;

2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)-1-fenilctanona;

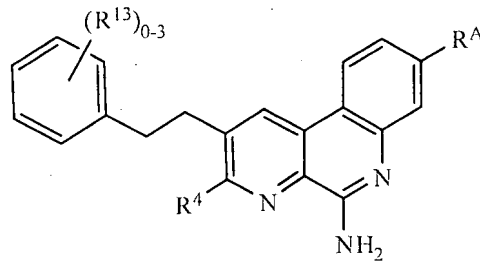
ácido 5-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)pentanoico;

18

2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)etanol;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)-N,N-
dimetilacetamida;
8-metil-2-(2-metil-4-(2-morfolinoetoxi)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)etoxi)etanol;
dietil 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)propilfosfonato;
ácido 3-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)propilfosfónico;
2-(4-butoxi-2-metilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)etanol;
2-(2-(4-(2-(5-aminobenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)etoxi)etanol;
etil 5-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)pentanoato;
ácido 5-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)pentanoico;
2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenoxi)etanol;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil etil carbonato;
metil 4-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)butanoato;
ácido 4-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenoxi)butanoico;
ácido 4-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)butanoico;
2-(4-(isopentiloxi)-2-metilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil hexil carbonato;
2-(2,4,6-trimetilfenetil)benzo[f][1,7]naftiridin-5-amina;
(5-amino-2-(2,4-dimetilfenetil)benzo[f][1,7]naftiridin-8-il)metanol;
dietil 3-(2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)etoxi)propilfosfonato;
dietil 3-(2-(2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-
metilfenoxi)etoxi)etoxi)propilfosfonato;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil
dimetilsulfamato;
(5-amino-2-(4-(dimetilamino)fenetil)benzo[f][1,7]naftiridin-8-il)metanol;
2-(4-(dimetilamino)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenol;
1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etanona;
2-(4-((dimetilamino)metil)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(4-(1-(dimetilamino)etil)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etanona oxima;
8-metil-2-(4-((metilamino)metil)fenetil)benzo[f][1,7]naftiridin-5-amina;
(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)bencilamino)ctanol;
8-metil-2-(4-(pirrolidin-1-ilmetil)fenetil)benzo[f][1,7]naftiridin-5-amina;
2-(3,4-dimetoxifenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
2-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etilamino)etanol;
1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etanol;
8-metil-2-(4-(oxazol-5-il)fenetil)benzo[f][1,7]naftiridin-5-amina;
3-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etilamino)propanonitrilo;
(2R)-2-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etilamino)propan-1-ol;
8-metil-2-(4-(1-(piperazin-1-il)etil)fenetil)benzo[f][1,7]naftiridin-5-amina;
((2S)-1-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)pirrolidin-2-il)metanol;
N¹-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)-N²,N²-dimetiletano-1,2-diamina;
ácido 3-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etilamino)propanoico;
8-metil-2-(4-(1-(4-metilpiperazin-1-il)etil)fenetil)benzo[f][1,7]naftiridin-5-amina;
N²-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)-N¹,N¹-dimetilpropano-1,2-diamina;
8-metil-2-(4-(1-(2-(piridin-4-il)etilamino)etil)fenetil)benzo[f][1,7]naftiridin-5-amina;
N¹-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)-N²,N²-dietiletano-1,2-diamina;
2-(4-(dimetilamino)-2-metilfenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina;
ácido 1-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)pirrolidina-3-carboxílico;
4-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etilamino)fenol;
1-(1-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)fenil)etil)pirrolidin-3-ol;
y
2-(4-(2-aminopropan-2-il)fenetil)-8-metilbenzo[f][1,7]naftiridin-5-amina.

15. Un compuesto de la fórmula (XVI):



(XVI)

en donde R^4 es H, y cada R^A y R^{13} se selecciona independientemente entre H, $-CH_3$, $-CH_2CH_3$, $-CF_3$, $-CH_2OH$, $-OCH_3$, $-COOCH_3$, $-COOCH_2CH_3$, F, Cl, Br, $-CH_2OCH_3$, $CH_2OCH_2CH_3$, $-N(CH_3)_2$, $-((O(CH_2)_2)_{2-4}OH)$, $-O(CH_2)_{2-4}OH$, $-O(CH_2)_{2-4}(PO_3H_2)$, $-O(CH_2)_{2-4}COOH$, $-O(CH_2)_{2-4}CH(CH_3)_2$, alquilo C_2-C_6 sustituido con 1-3 sustituyentes seleccionados entre $-OH$, $-CH_3$, ciclopropilo, $-O(CH_2)_{2-4}COOH$, $-O(CH_2)_{2-4}(PO_3H_2)$, $-COOH$, $COOCH_3$, y $-COOCH_2CH_3$;

o una sal farmacéuticamente aceptable del mismo.

16. El compuesto de acuerdo con la Reivindicación 15, en donde el compuesto está sustituido con uno a tres grupos R^{13} .

17. El compuesto de acuerdo con cualquiera de la Reivindicación 15 o Reivindicación 16, en donde R^A es H o $-CH_3$.

18. El compuesto de acuerdo con cualquiera de las Reivindicaciones 15-17, que tiene dos grupos R^{13} seleccionados del grupo que consiste de $-CH_3$, $-CH_2CH_3$, $-CF_3$, $-CH_2OH$, $-OCH_3$, $-COOCH_3$, $-COOCH_2CH_3$, F, Cl, Br, $-CH_2OCH_3$, $CH_2OCH_2CH_3$, $-N(CH_3)_2$, $-((O(CH_2)_2)_{2-4}OH)$, $-O(CH_2)_{2-4}OH$, $-O(CH_2)_{2-4}(PO_3H_2)$, $-O(CH_2)_{2-4}COOH$, $-O(CH_2)_{2-4}CH(CH_3)_2$, alquilo C_2-C_6 sustituido con 1-3 sustituyentes seleccionados entre $-OH$, $-CH_3$, ciclopropilo, $-O(CH_2)_{2-4}COOH$, $-O(CH_2)_{2-4}(PO_3H_2)$, $-COOH$, $COOCH_3$, y $-COOCH_2CH_3$.

19. El compuesto de acuerdo con la Reivindicación 15 seleccionado entre:

2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilfenil)propan-2-ol;

2-(4-metoxi-2-metilfenil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(2,4-dimetilfenil)benzo[f][1,7]naftiridin-5-amina;

etil 4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-metilbenzoato;

2-(4-(dimetilamino)fenil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(4-metoxifenil)-8-metilbenzo[f][1,7]naftiridin-5-amina;

2-(4-(isopentiloxi)-2-metilfenil)-8-metilbenzo[f][1,7]naftiridin-5-amina; y

2-(2-(4-(2-(5-amino-8-metilbenzo[f][1,7]naftiridin-2-il)etil)-3-

metilfenoxi)etoxi)etanol.

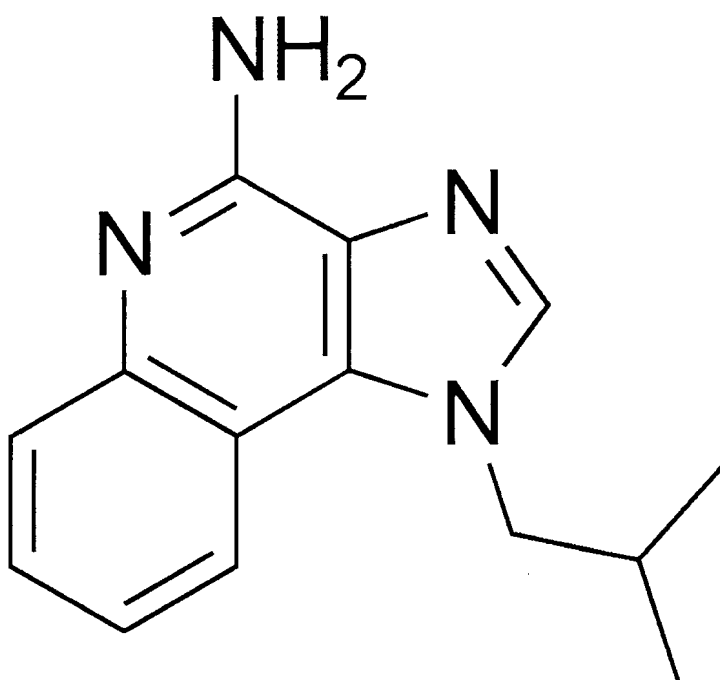
20. Una composición farmacéutica que comprende un compuesto de acuerdo con cualquiera de las Reivindicaciones 1-19 y un vehículo farmacéuticamente aceptable.
21. Una composición farmacéutica que comprende un compuesto de acuerdo con cualquiera de las Reivindicaciones 1-19, un antígeno bacteriano y un vehículo farmacéuticamente aceptable.
22. La composición farmacéutica de acuerdo con la Reivindicación 21, en donde la composición farmacéutica comprende además un agente inmunorregulador adicional, donde el agente inmunorregulador adicional es un coadyuvante seleccionado de una composición que contiene minerales y una emulsión oleosa.
23. La composición farmacéutica de acuerdo con la Reivindicación 22, caracterizada porque el coadyuvante es una emulsión oleosa.
24. El compuesto de acuerdo con la Reivindicación 15, en donde
 R^A es $-R^8$ o $(CH_2)_nCO_2R^8$;
cada R^{13} es seleccionado independientemente entre $-LR^8$, $-LOR^8$ y $-LP(O)(OR^8)_2$; y
L es un enlace o $-(O(CH_2)_m)_l-$
25. El compuesto de acuerdo con la Reivindicación 24, en donde R^8 es H o alquilo C_1 - C_6 .

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Synthesis and Structure–Activity-Relationships of 1*H*-Imidazo[4,5-*c*]quinolines That Induce Interferon Production

John F. Gerster,[‡] Kyle J. Lindstrom,[‡] Richard L. Miller,^{*,†} Mark A. Tomai,[‡] Woubalem Birmachu,[‡] Shannon N. Bomersine,[‡] Shiela J. Gibson,[‡] Linda M. Imbertson,[‡] Joel R. Jacobson,[‡] Roy T. Knafla,[‡] Peter V. Maye,[‡] Nickolas Nikolaidis,[‡] Folakemi Y. Oneyemi,[‡] Gwen J. Parkhurst,[‡] Sharon E. Pecore,[‡] Michael J. Reiter,[‡] Lisa S. Scribner,[‡] Tracy L. Testerman,[‡] Natalie J. Thompson,[‡] Tammy L. Wagner,[‡] Charles E. Weeks,[‡] Jean-Denis Andre,[‡] Daniel Lagain,[‡] Yvon Bastard,[‡] and Michel Lupu[‡]

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1*H*-Imidazo-[4,5-*c*]quinolines were prepared while investigating novel nucleoside analogues as potential antiviral agents. While these compounds showed no direct antiviral activity when tested in a number of cell culture systems, some demonstrated potent inhibition of virus lesion development in an intravaginal guinea pig herpes simplex virus-2 assay. We have determined that the in vivo antiviral activity can be attributed to the ability of these molecules to induce the production of cytokines, especially interferon (IFN), in this model. Subsequently, we found that the compounds also induce in vitro production of IFN in human peripheral blood mononuclear cells (hPBMCs). The in vitro results reported herein and the in vivo results reported previously led to the discovery of imiquimod, **26**, which was developed as a topical agent and has been approved for the treatment of genital warts, actinic keratosis, and superficial basal cell carcinoma.

Introduction

Since the discovery of interferon (IFN) in 1957¹ and its potential for the treatment of viral infections and cancer, there has been a search for small-molecule inducers of this cytokine. A number of compounds that induce IFN in mice have been identified, but few are active in human cells.² While working on potential anti-herpes agents, we discovered a series of 1*H*-imidazo[4,5-*c*]quinolines that induce IFN, as well as other cytokines, in mice, rats, guinea pigs, monkeys, and humans.^{3,4}

At the initiation of this work it was known that (*S*)-2,3-dihydroxypropyladenine ((*S*)-DHPA) (Figure 1) exhibits weak antiviral activity in an in vitro herpes simplex virus-1 (HSV-1) assay with a minimum inhibitory concentration (MIC) of 10–20 $\mu\text{g/mL}$.⁵ Our initial efforts targeted the synthesis of 3-deazaadenine analogues as potential antiviral agents. Our approach was to modify the adenine ring by preparing a 3-deazaadenine with a benzene ring fused across the 2 and 3 positions to give the 1*H*-imidazo[4,5-*c*]quinoline ring system (Figure 1). On the basis of the reported activity of (*S*)-DHPA, we had hoped that compound **1** would give similar results; however, it was found to be inactive. On the other hand, the methyl analogue **2** did exhibit weak in vitro antiviral activity and cytotoxicity at the same concentration (MIC = 5 mg/mL). Compound **2** was

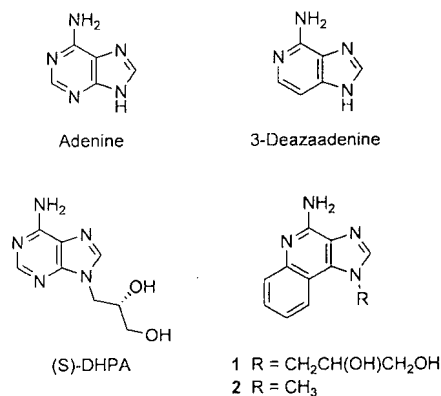


Figure 1.

further evaluated in an in vivo intravaginal guinea pig HSV-2 infection model and was found to prevent lesion development. Additional analogues were synthesized and many continued to show in vivo anti-HSV-2 activity in the guinea pig model but did not exhibit in vitro activity against HSV-1.⁶ These surprising results led to the discovery that the active compounds were potent inducers of IFN and other cytokines, thus explaining the in vivo activity. At this point, we abandoned the search for in vitro antiviral activity and instead focused on preparing compounds that induced IFN production in an in vitro assay using human peripheral blood mononuclear cells (hPBMCs).⁷ Herein, we report on the initial synthetic efforts of this novel series of immune

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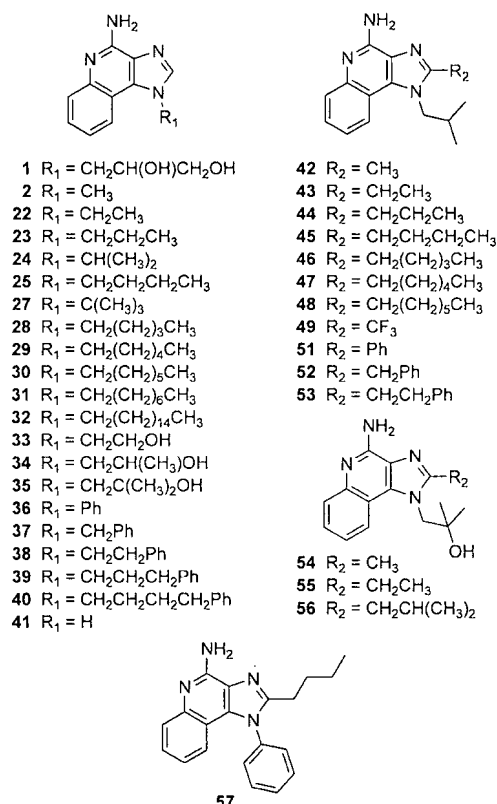


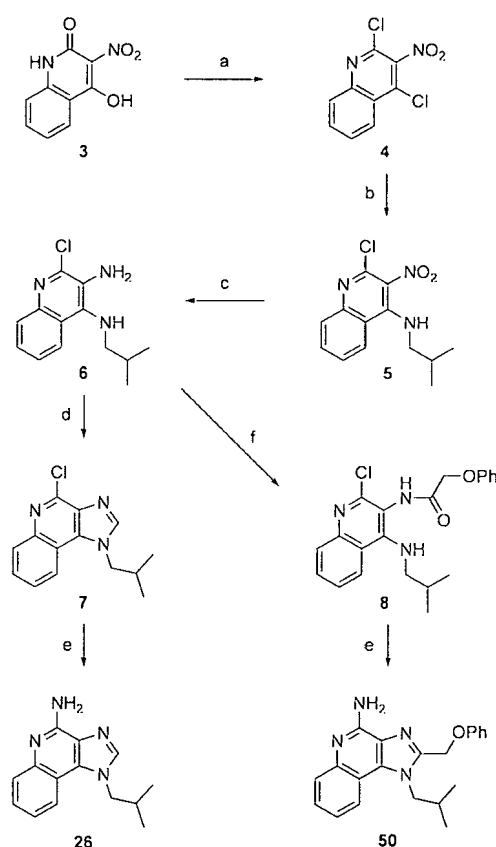
Figure 2.

response modifiers and the structure-activity-relationships (SARs) that influence the induction of IFN.

Chemistry

The 1*H*-imidazo[4,5-*c*]quinolin-4-amines were synthesized using two main synthetic routes. Common features in both routes involve the displacement of a 4-chloroquinoline with primary amines and the condensation with acid chlorides or ortho esters to form the imidazole ring. While we show several methods to prepare 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine, **26**, similar reaction conditions were utilized to prepare many of the other analogues in our SAR study (Figure 2).

The first route, Scheme 1, began with the chlorination of 4-hydroxy-3-nitro-1*H*-quinolin-2-one, **3**, employing a modification of the method of Gabriel,⁸ using phosphorus oxychloride in toluene, with triethylamine as a base, to provide 2,4-dichloro-3-nitroquinoline, **4**. Alternatively, the chlorination can be accomplished using phenylphosphonic dichloride.⁹ By careful control of the reaction temperature, the 4-chloro substituent was selectively displaced by a number of primary amines to give the corresponding nitroamines, as demonstrated by the reaction with isobutylamine to give **5**. Catalytic hydrogenation of **5** gave the corresponding diamine **6**. To form the imidazole, diamine **6** was treated with either triethyl orthoformate or diethoxymethyl acetate in refluxing toluene to generate **7**. Amination of the chloro analogue with methanolic ammonia at 150 °C gave 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine, **26**. By a change of the primary amine in step b of this route, compounds **1**, **27**, and **33–35** (see Figure 2) were also prepared. The 1-*H* analogue, **41**, was prepared by the

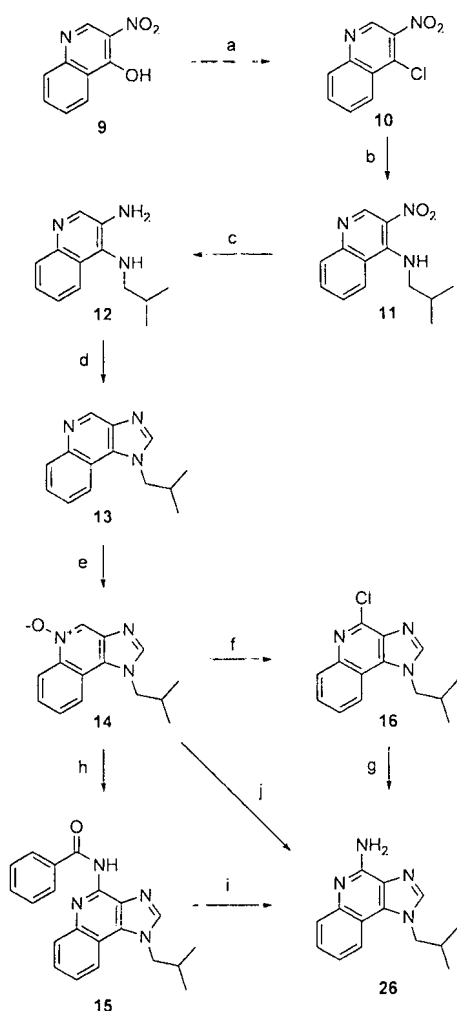
Scheme 1^a

^a Reagents and conditions: (a) POCl₃, toluene, Et₃N, heat or PhPOCl₂, heat; (b) isobutylamine, Et₃N, CH₂Cl₂, reflux; (c) 5% Pt/C, H₂, ethyl acetate; (d) HC(OEt)₃, toluene, reflux; (e) NH₃/MeOH, 150 °C; (f) PhOCH₂COCl, Et₃N, CH₃CN.

acid-induced cleavage of the corresponding 1-*tert*-butyl analogue, **27**, in refluxing 6 N HCl.

Diamine **6** could also be reacted with acid chlorides or ortho esters to give the intermediate amides as illustrated by the condensation with phenoxyacetyl chloride to give **8**. Treatment of **8** with methanolic ammonia at 150 °C resulted in the formation of the imidazole ring as well as the displacement of the 4-chloro substituent to give the corresponding C-2 substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amine **50**. By use of the appropriate acid chlorides in step f of this sequence, analogues **45**, **55**, and **56** (see Figure 2) were also prepared to provide examples with substitution at the C-2 position.

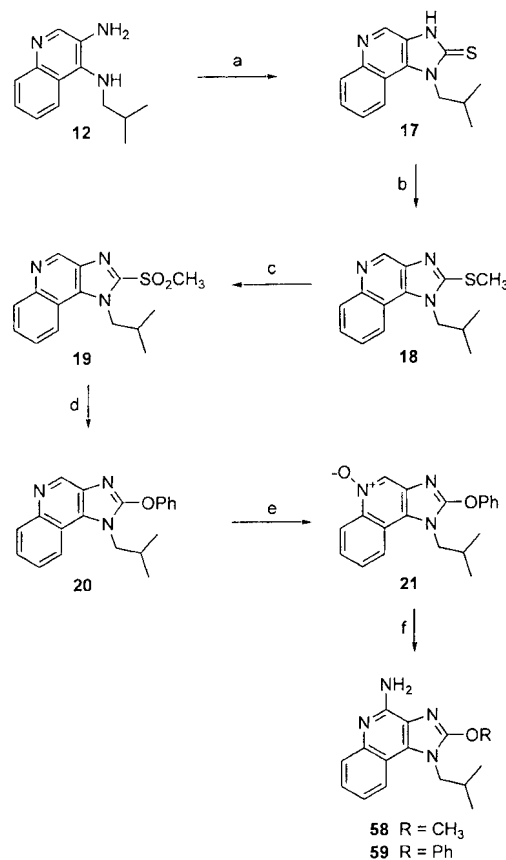
A second route to 1*H*-imidazo[4,5-*c*]quinolines (Scheme 2) started with 3-nitro-4-quinolinol, **9**, which was chlorinated using either thionyl chloride and *N,N*-dimethylformamide in dichloromethane or phosphorus oxychloride in *N,N*-dimethylformamide to give **10**. The 4-chloro substituent was displaced with isobutylamine to give *N*-(2-methylpropyl)-3-nitroquinolin-4-amine (**11**), which was catalytically reduced to provide the diamine **12**. Heating a solution of **12** in diethoxymethyl acetate gave the imidazoquinoline **13**. The *N*-oxide **14** was prepared by oxidation with either peracetic acid or *m*-chloroperbenzoic acid. Reaction of the *N*-oxide with POCl₃ in dichloromethane gave the 4-chloro-1*H*-imidazo[4,5-*c*]quinoline analogue **16**, which was converted to **26** by treatment with NH₃ in methanol at 150 °C.

Scheme 2^a

^a Reagents and conditions: (a) SOCl_2 , DMF, CH_2Cl_2 , reflux or POCl_3 , DMF, heat; (b) isobutylamine, CH_2Cl_2 , reflux; (c) 5% Pt/C, H_2 , ethyl acetate; (d) diethoxymethyl acetate, heat or $\text{HC}(\text{OEt})_3$, toluene, reflux; (e) $\text{CH}_3\text{CO}_3\text{H}$, EtOH, heat; (f) POCl_3 , CH_2Cl_2 , reflux; (g) NH_3/MeOH , 150 °C; (h) benzoyl isocyanate, CH_2Cl_2 , heat; (i) NaOCH_3 , CH_3OH ; (j) concentrated NH_4OH , CH_2Cl_2 , tosyl chloride.

Compounds **2**, **22**, **24**, **25**, **28–32**, **36–38**, **48**, **51–53** (see Figure 2) were also prepared by this route by reacting the appropriate primary amines with **10** in step b and by using the appropriate acid chlorides or ortho esters for the formation of the imidazole ring in step d. In the case of the 2-trifluoromethyl analogue **49**, the imidazole ring was formed by refluxing the diamine in trifluoroacetic acid in step d.

Alternatively, *N*-oxide **14** was treated with benzoyl isocyanate in dichloromethane to provide the corresponding benzoyl derivative **15**. Subsequent saponification with sodium methoxide gave the free 4-amino analogue **26**. In addition, the *N*-oxides could be converted directly to the corresponding 4-amines in a single step using a modification of the Hamana and Hoshino procedure.¹⁰ Compounds **23**, **39**, **40**, **42–44**, **46**, **47**, **54**, **57** (see Figure 2) were prepared from their corresponding *N*-oxides by dissolving in dichloromethane and treating sequentially with ammonium hydroxide and tosyl chloride.

Scheme 3^a

^a Reagents and conditions: (a) 1,1'-thiocarbonyldiimidazole, DMF, heat; (b) CH_3I , NaOMe, MeOH; (c) KMnO_4 , acetic acid; (d) potassium phenoxide, DMF; (e) $\text{CH}_3\text{CO}_3\text{H}$, MeOAc; (f) concentrated NH_4OH , CH_2Cl_2 , tosyl chloride.

A method to prepare 1*H*-imidazo[4,5-*c*]quinoline derivatives with heteroatoms directly attached to the C-2 position is described in Scheme 3. The reaction of *N*⁴-(2-methylpropyl)quinoline-3,4-diamine, **12**, with 1,1'-thiocarbonyldiimidazole gave the thione **17**. Treatment of **17** with iodomethane provided the *S*-methylated product **18**. Oxidation to the methyl sulfone, **19**, was achieved with potassium permanganate in acetic acid. The methyl sulfone was readily displaced with nucleophiles, as exhibited by treatment with potassium phenoxide to give intermediate **20**. *N*-Oxidation of the quinoline nitrogen, followed by reaction with tosyl chloride in ammonium hydroxide/dichloromethane, provided the corresponding 4-amino derivative **59**. Compound **58** was prepared in a similar manner by using sodium methoxide as the nucleophile in step d.

The 4-chloro group of **7** was displaced by a variety of nucleophiles, as shown in Scheme 4, to give the corresponding 4-substituted 1*H*-imidazo[4,5-*c*]quinolines **60–62**. The preparation of the 4-hydroxy analogue, **63**, was achieved by acid hydrolysis of the chloro group.

Compounds with substitution on the benzene ring were also prepared. The following molecules, **64**, **67**, **70**, and **73**, were prepared with a single methyl group attached to the C-6, C-7, C-8, or C-9 position of the ring system (Figure 3). These compounds were synthesized from the appropriately substituted quinolines using the routes shown in Schemes 1 and 2. The C-6, C-7, and

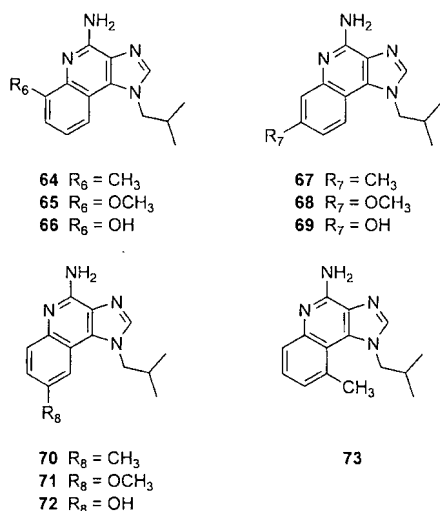
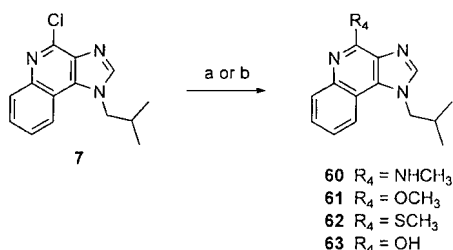
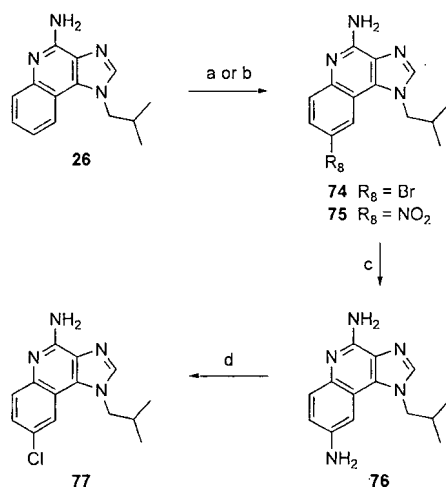


Figure 3.

Scheme 4^a

^a Reagents and conditions: for 60–62, (a) nucleophile, solvent, heat; for 63, (b) 6 N HCl, reflux.

Scheme 5^a

^a Reagents and conditions: (a) Br_2 , HOAc; or (b) HNO_3 , H_2SO_4 ; (c) SnCl_2 , HCl, EtOH, reflux; (d) NaNO_2 , HCl, CuCl.

C-8 methoxy analogues, **65**, **68**, and **71**, were prepared in a similar manner. The hydroxyl-substituted derivatives, **66**, **69**, and **72**, were prepared by acid hydrolysis of the corresponding methoxy compounds.

The 1*H*-imidazo[4,5-*c*]quinolin-4-amines can also be modified by direct electrophilic substitution of the tricyclic ring system, as shown in Scheme 5. Bromination or nitration of **26** gave the corresponding 8-bromo (**74**) and 8-nitro (**75**) analogues, respectively.¹¹ Catalytic reduction of **75** gave the corresponding 8-amino ana-

Table 1. Effect of R_1 Substitution on IFN Induction

compd	R_1	MEC ^a
1	$\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$	5.0
2	CH_3	0.5
22	CH_2CH_3	0.5
23	$\text{CH}_2\text{CH}_2\text{CH}_3$	0.5
24	$\text{CH}(\text{CH}_3)_2$	0.5
25	$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	0.5
26	$\text{CH}_2\text{CH}(\text{CH}_3)_2$	0.5
27	$\text{C}(\text{CH}_3)_3$	NI
28	$\text{CH}_2(\text{CH}_2)_3\text{CH}_3$	0.5
29	$\text{CH}_2(\text{CH}_2)_4\text{CH}_3$	0.5
30	$\text{CH}_2(\text{CH}_2)_5\text{CH}_3$	0.5
31	$\text{CH}_2(\text{CH}_2)_6\text{CH}_3$	NI
32	$\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3$	NI
33	$\text{CH}_2\text{CH}_2\text{OH}$	0.5
34	$\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$	0.5
35	$\text{CH}_2\text{C}(\text{CH}_3)_2\text{OH}$	0.5
36	Ph	NI
37	CH_2Ph	0.1
38	$\text{CH}_2\text{CH}_2\text{Ph}$	0.5
39	$\text{CH}_2\text{CH}_2\text{CH}_2\text{Ph}$	NI
40	$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Ph}$	0.1
41	H	0.5

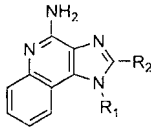
^a Minimum effective concentration at which IFN induction was observed ($\mu\text{g/mL}$). NI = no induction of IFN at the highest concentration tested (5.0 $\mu\text{g/mL}$).

logue **76**. The 8-chloro derivative, **77**, was prepared from the amine via the Sandmeyer reaction.

Results and Discussion

The assay used to measure IFN production has been previously described in detail.⁷ Human peripheral blood mononuclear cells (hPBMCs) were cultured overnight with each test compound in RPMI medium containing 10% autologous serum at 37 °C. Culture supernatants were collected and analyzed for IFN- α by a virus cytopathic effect assay, using A549 human lung carcinoma cells and encephalomyelitis virus. The compounds were evaluated according to the minimum effective concentration (MEC) at which they induced IFN production. While we observed some variability among hPBMC donors in the amounts of IFN produced, there was generally good consistency in the MEC for a given compound.

The SAR of the 1*H*-imidazo[4,5-*c*]quinoline core with differing substituents at the N-1 position is shown in Table 1. Most compounds with alkyl substituents induce IFN at a similar concentration (0.5 $\mu\text{g/mL}$), the exceptions being the longer straight-chain analogues **31** and **32** that fail to induce IFN. A single branching substituent at the α position of the carbon backbone is tolerated as seen with *N*¹-isopropyl, **24**; however, when the substituent is the bulkier *tert*-butyl, **27**, no IFN induction is observed. When the alkyl chain contains hydroxyl substituents, most of the examples (**33**–**35**) also induce IFN production at 0.5 $\mu\text{g/mL}$, the exception being the dihydroxypropyl analogue **1**, which is weakly active. Direct attachment of a phenyl group at the N-1 position, **36**, gives an analogue that does not induce IFN; however, introduction of a phenyl group at the terminus of an alkyl chain (**37**, **38**, **40**) gives active IFN inducers

Table 2. Effect of R₂ Substitution on IFN Induction


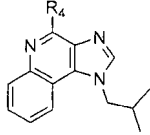
compd	R ₁	R ₂	MEC ^a
26	CH ₂ CH(CH ₃) ₂	H	0.5
42	CH ₂ CH(CH ₃) ₂	CH ₃	0.05
43	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₃	0.05
44	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ CH ₃	0.05
45	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ CH ₂ CH ₃	0.01
46	CH ₂ CH(CH ₃) ₂	CH ₂ (CH ₂) ₃ CH ₃	0.05
47	CH ₂ CH(CH ₃) ₂	CH ₂ (CH ₂) ₄ CH ₃	0.5
48	CH ₂ CH(CH ₃) ₂	CH ₂ (CH ₂) ₅ CH ₃	1.0
49	CH ₂ CH(CH ₃) ₂	CF ₃	NI
50	CH ₂ CH(CH ₃) ₂	CH ₂ OPh	0.1
51	CH ₂ CH(CH ₃) ₂	Ph	NI
52	CH ₂ CH(CH ₃) ₂	CH ₂ Ph	0.05
53	CH ₂ CH(CH ₃) ₂	CH ₂ CH ₂ Ph	1.0
35	CH ₂ C(CH ₃) ₂ OH	H	0.5
54	CH ₂ C(CH ₃) ₂ OH	CH ₃	0.05
55	CH ₂ C(CH ₃) ₂ OH	CH ₂ CH ₃	0.05
56	CH ₂ C(CH ₃) ₂ OH	CH ₂ CH(CH ₃) ₂	0.01
57	Ph	CH ₂ CH ₂ CH ₂ CH ₃	0.5
58	CH ₂ CH(CH ₃) ₂	OCH ₃	NI
59	CH ₂ CH(CH ₃) ₂	OPh	NI

^a Minimum effective concentration at which IFN induction was observed (μg/mL). NI = no induction of IFN at the highest concentration tested (5.0 μg/mL).

(MEC = 0.1–0.5 μg/mL). The exception to this trend in activity is the 3-phenylpropyl analogue **39**, which is surprisingly inactive in repeated assays. In addition, the unsubstituted derivative **41** is also an active IFN inducer (MEC = 0.5 μg/mL).

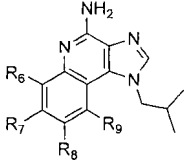
To investigate how substitution at other positions of the imidazoquinoline core influences IFN induction, we focused primarily on analogues of compound **26**. While the activity of **26** is similar to many of the compounds shown in Table 1, this molecule was effective in animal models and had progressed as a development candidate. During *in vivo* studies it was discovered that **26** is metabolized to the 1-(2-hydroxy-2-methylpropyl) derivative **35**,¹² which has similar potency in the hPBMC assay. Therefore, the SAR was further developed using 1-(2-methylpropyl) or 1-(2-hydroxy-2-methylpropyl) as the N-1 substituent.

To explore the effect that C-2 substituents have on IFN induction, we prepared a series of compounds with differing substitution at this position (Table 2). Compared to compound **26**, straight-chain alkyl substituents (C_{1–5}) increase the IFN induction activity by 10- to 50-fold (MEC = 0.01–0.05 μg/mL). Activity begins to decrease when the alkyl substituent is longer (C_{6–7}). While the methyl derivative **42** shows increased activity compared to the parent **26**, the trifluoromethyl substituent at C-2 (**49**) completely eliminates activity. Direct aryl substitution at C-2 (**51**) also abrogates IFN production. However, the phenoxymethyl and benzyl derivatives **50** and **52** show a 5- and 10-fold increase in activity, respectively, when compared to **26**. The phenethyl derivative **53** is 2-fold less active than **26**. The series with the 1-(2-hydroxy-2-methylpropyl) substitution at N-1 showed a similar trend in activity compared to the 1-(2-methylpropyl) series. Compounds **54–56** increase the IFN induction activity 10- to 50-fold when compared to **35**. In the case where the N-1 substituent

Table 3. Effect of R₄ Substitution on IFN Induction


compd	R ₄	MEC ^a
7	Cl	NI
13	H	NI
15	NHC(O)Ph	NI
60	NHCH ₃	NI
61	OCH ₃	NI
62	SCH ₃	NI
63	OH	NI
26	NH ₂	0.5

^a Minimum effective concentration at which IFN induction was observed (μg/mL). NI = no induction of IFN at the highest concentration tested (5.0 μg/mL).

Table 4. Effect of R₆, R₇, R₈, and R₉ Substitution on IFN Induction


compd	R ₆	R ₇	R ₈	R ₉	MEC ^a
26	H	H	H	H	0.5
64	CH ₃	H	H	H	NI
65	OCH ₃	H	H	H	NI
66	OH	H	H	H	1.0
67	H	CH ₃	H	H	0.5
68	H	OCH ₃	H	H	1.0
69	H	OH	H	H	0.5
70	H	H	CH ₃	H	NI
71	H	H	OCH ₃	H	NI
72	H	H	OH	H	NI
73	H	H	H	CH ₃	NI
74	H	H	Br	H	NI
75	H	H	NO ₂	H	NI
76	H	H	NH ₂	H	NI
77	H	H	Cl	H	NI

^a Minimum effective concentration at which IFN induction was observed (μg/mL). NI = no induction of IFN at the highest concentration tested (5.0 μg/mL).

is phenyl, the 2-H-analogue **36** is inactive. However, addition of a 2-butyl group (**57**) gives an active IFN inducer (MEC = 0.5 μg/mL). This clearly illustrates that simple alkyl substitution at the C-2 position is beneficial for IFN activity. On the other hand, having an oxygen atom directly attached to the C-2 position eliminates activity because both the 2-methoxy, **58**, and 2-phenoxy, **59**, derivatives fail to induce IFN.

The C-4 position of the 1*H*-imidazo[4,5-*c*]quinoline core is unique in that only a primary amino substituent leads to IFN induction (Table 3). Even monomethylation of the primary amino group, **60**, or replacement with a hydroxy group, **63**, completely eliminates the IFN inducing activity. All other C-4 substituents investigated fail to induce IFN production. From these results, it appears that having the unsubstituted 4-amino group is the most important aspect for IFN induction.

Substitution on the benzene ring gives analogues that either retain the IFN induction of the unsubstituted compound or decrease or eliminate the activity (Table 4). Substitution at the C-6 position eliminates activity (**64**, **65**), the exception being the hydroxyl-substituted

analogue **66** that is 2-fold less active than the parent. Substitution at the C-8 position (**70–72**, **74–77**) and the C-9 position (**73**) also eliminates IFN-inducing activity. However, compounds substituted at the C-7 position have IFN induction (**67–69**) comparable to that of the parent **26**.

As previously mentioned, compound **26** was chosen early in the development process as a small molecule with the potential to produce IFN in vivo. While other molecules, especially those with additional substitution at the C-2 position, are more active in the hPBMC assay, compound **26** had the right balance of activity and tolerability for the prevention of lesions caused by HSV-2 infection in the guinea pig model.⁶ The observed in vivo antiviral activity is associated with the production of IFN in a cell-mediated immune response. Subsequent studies showed that compound **26** is also a potent inducer of IFN, as well as other cytokines, in a number of species including mice, rats, monkeys, and humans via topical, oral, or parenteral administration.^{3b}

Conclusions

1*H*-Imidazo[4,5-*c*]quinolin-4-amines have been prepared and evaluated for the ability to induce IFN- α production in hPBMCs. Investigation of the N-1 substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amines lacking substitution at C-2 reveals that most simple alkyl and hydroxyalkyl substituents are tolerated to give active IFN inducers. However, activity is not enhanced compared to the nonsubstituted compound **41**. When longer alkyl chains or bulkier alkyl substituents (i.e., *tert*-butyl) are attached to N-1, activity is eliminated at the concentrations tested. This is also the case when a phenyl group is directly attached to N-1. Straight-chain alkyl substitution at the C-2 position enhances IFN induction for the smaller chains, with butyl being the most active. When the chain length reaches heptyl, activity begins to decrease. Phenoxyethyl and benzyl groups at C-2 also increase activity. The C-2 substituents that are not tolerated include trifluoromethyl, phenyl, and the methoxy and phenoxy derivatives. The amine at the C-4 position appears to be the most crucial requirement for activity. All other modifications at this position eliminate IFN production. In general, simple substitution on the benzene ring appears to be unfavorable because most of the compounds prepared fail to induce IFN. The exception is substitution at the C-7 position where the compounds retain the activity of the unsubstituted parent molecule.

In summary novel interferon inducers have been discovered. These studies have led to the development of imiquimod, **26**, for clinical use. It has been demonstrated that topical application of imiquimod in humans induces synthesis of IFN and other cytokines in skin at the application site.^{3b} Clinical utility has been demonstrated in treating genital warts,¹³ basal cell carcinoma,¹⁴ and actinic keratosis.¹⁵ Several related conditions are also reported to respond.¹⁶ We are continuing to explore this novel series of immune response modifiers in an effort to find small molecules that treat diseases involving the immune system.¹⁷

Experimental Section

Melting points were taken using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton reso-

nance (¹H NMR) spectra were recorded on a Bruker 300 NMR spectrometer at 300 MHz. Chemical shifts are reported in δ relative to tetramethylsilane. Carbon, hydrogen, and nitrogen analyses were performed by Oneida Research Services, Whitesboro, NY; Robertson Microlit Laboratories, Madison, New Jersey; or 3M Analytical Services and were within $\pm 0.4\%$ of the theoretical values unless otherwise indicated. Solvents employed in developing thin-layer chromatography plates (TLC) are noted in parentheses. The structures of all compounds were consistent with their spectral data. Unless noted otherwise, the reported yields were not optimized.

2-Chloro-N-(2-methylpropyl)-3-nitroquinoline (5). Triethylamine (63.5 mL, 0.46 mol) was added in small portions to a well-stirred suspension of 4-hydroxy-3-nitro-1*H*-quinolin-2-one (**3**) (20.8 g, 100 mmol) in toluene (100 mL). Phosphorus oxychloride (15.7 mL, 168 mmol) was added dropwise to the stirred mixture over a 3–5 min period. The temperature of the reaction mixture was maintained below 55 °C during the addition. After the addition was complete, the reaction mixture was heated at 80–90 °C for 4 h, cooled to ambient temperature, diluted with dichloromethane (200 mL), and poured into water with stirring while maintaining the temperature below 60 °C. The resulting mixture was made basic with aqueous sodium hydroxide and stirred at ambient temperature for 2 h. The organic phase was separated from the mixture, washed thoroughly with water, and dried over magnesium sulfate. A solution of triethylamine (15.7 g, 155 mmol) and isobutylamine (8.8 g, 120 mmol) was added to the dried organic phase, and the solution was refluxed for 30 min. The reaction mixture was concentrated in vacuo to dryness. The residue was slurried in water, and the resulting solid was collected by vacuum filtration. The yellow solid was washed thoroughly with water and dried to yield 17.8 g (64%) of product that was pure enough for further use. Recrystallization from propyl acetate gave an analytically pure product as a bright-yellow solid: mp 85–88 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.98–7.88 (m, 2 H), 7.75 (m, 1 H), 7.53 (m, 1 H), 6.19 (br s, 1 H), 3.38–3.30 (m, 2 H), 1.96 (m, 1 H), 1.04 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₃H₁₄ClN₂O₂: C, 55.82; H, 5.04; N, 15.02. Found: C, 55.69; H, 5.1; N, 14.9.

Alternative Chlorination Procedure Using Phenylphosphonic Dichloride: 2,4-Dichloro-8-methyl-3-nitroquinoline. A mixture of 4-hydroxy-8-methyl-3-nitroquinolin-2-(1*H*)-one¹⁸ (11.0 g, 50 mmol) and phenylphosphonic dichloride (30 mL) was slowly heated to 140 °C with stirring. The temperature of the mixture was maintained between 135 and 140 °C for 3 h, and the mixture was poured into cold water (600 mL) with vigorous stirring. The syrupy mixture gradually solidified upon continued stirring. The solid was collected by vacuum filtration, washed with water, and dried to give 12.2 g (95%) of unpurified product as a tan solid. The solid was recrystallized from heptanes/dichloromethane to give 9.0 g (70%) of pure product as brownish crystals: mp 178–180 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.10 (dd, *J* = 0.4, 8.4 Hz, 1 H), 7.78 (d, *J* = 7.8 Hz, 1 H), 7.68 (t, *J* = 7.8 Hz, 1 H), 2.79 (s, 3 H). Anal. Calcd for C₁₀H₈Cl₂N₂O₂: C, 46.72; H, 2.35; N, 10.90. Found: C, 46.81; H, 2.09; N, 10.61.

2-Chloro-N⁴-(2-methylpropyl)quinoline-3,4-diamine (6). A mixture of **5** (7.5 g, 27 mmol), ethyl acetate (300 mL), magnesium sulfate (5.0 g), and 5% platinum on carbon (200 mg) was hydrogenated in a Parr apparatus at ~50 psi initial pressure of hydrogen gas. When hydrogen uptake ceased, the solids were filtered from the mixture and the filtrate was concentrated in vacuo to yield 5.8 g of unpurified product. The unpurified solid was reprecipitated from dilute hydrochloric acid by the addition of concentrated ammonium hydroxide to give 4.8 g (71%) of colorless product that was pure enough for further use. Recrystallization of the unpurified solid from ethanol gave analytically pure product as colorless platelets: mp 145–148 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.02 (m, 1 H), 7.69 (m, 1 H), 7.46–7.35 (m, 2 H), 5.29 (t, *J* = 6.6 Hz, 1 H), 5.03 (s, 2 H), 3.11 (t, *J* = 6.7 Hz, 2 H), 1.77 (m, 1 H), 0.91 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₃H₁₆ClN₃: C, 62.52; H, 6.46; N, 16.83. Found: C, 62.54; H, 6.44; N, 16.75.

4-Chloro-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (7). A mixture of **6** (3.2 g, 12.8 mmol), triethyl orthoformate (2.0 g, 13.2 mmol), and toluene was refluxed for 1.5 h while allowing the ethanol to be distilled from the solution. The volatiles were removed under vacuum to yield an oil that solidified upon cooling to ambient temperature. The solid was crushed in *tert*-butyl methyl ether, collected by vacuum filtration, and dried to yield 3.1 g (94%) of a solid that was judged to be homogeneous by TLC (47:3, dichloromethane/*tert*-butyl methyl ether). The product was recrystallized from 2-propanol to give 2.0 g (61%) of an analytically pure product as a cream-colored solid: mp 139–140 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.20 (dd, *J* = 1.5, 7.7 Hz, 1 H), 8.09 (dd, *J* = 1.2, 7.9 Hz, 1 H), 7.93 (s, 1 H), 7.70 (ddd, *J* = 1.5, 7.0, 8.5 Hz, 1 H), 7.64 (ddd, *J* = 1.4, 7.1, 8.4 Hz, 1 H), 4.36 (d, *J* = 7.4 Hz, 2 H), 2.36 (m, 1 H), 1.05 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₄H₁₄ClN₃: C, 64.74; H, 5.43; N, 16.18. Found: C, 64.47; H, 5.34; N, 16.16.

1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (26).¹⁹ A mixture of **7** (3.0 g, 11.6 mmol) and 2 M methanolic ammonia (25 mL, 50 mmol) was placed in a glass tube and heated in a Parr pressure vessel at 150 °C for 7 h. The cooled mixture was reduced in volume in vacuo to remove ammonia and treated with potassium hydroxide (750 mg, 11.6 mmol) dissolved in methanol (10 mL). The mixture was stirred at ambient temperature for 1 h, and the resulting solid was collected by vacuum filtration. The solid was washed consecutively with methanol, water, and methanol and dried to yield 2.5 g (89%) of unpurified product as a colorless solid, which was homogeneous as judged by TLC (47:3, dichloromethane/*tert*-butyl methyl ether). The unpurified product was recrystallized from *N,N*-dimethylformamide to give 2.3 g (82%) of an analytically pure product as colorless crystals: mp 292–294 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.18 (s, 1 H), 8.00 (dd, *J* = 1.0, 8.1 Hz, 1 H), 7.62 (dd, *J* = 1.1, 8.3 Hz, 1 H), 7.44 (ddd, *J* = 1.3, 7.0, 8.3 Hz, 1 H), 7.27 (ddd, *J* = 1.3, 7.1, 8.2 Hz, 1 H), 6.58 (br s, 2 H), 4.40 (d, *J* = 7.4 Hz, 2 H), 2.18 (heptet, *J* = 6.7 Hz, 1 H), 0.92 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₆N₄: C, 69.97; H, 6.71; N, 23.31. Found: C, 69.9; H, 6.6; N, 23.4.

1*H*-Imidazo[4,5-*c*]quinolin-4-amine (41).²⁰ A mixture of **27** (1.5 g, 6.2 mmol) and 6 N HCl (25 mL) was refluxed for 30 min. The mixture was cooled to ambient temperature. The solid that had precipitated was collected by vacuum filtration and slurried in an aqueous saturated sodium carbonate solution. The solid was collected by vacuum filtration, washed with water, and dried. The unpurified solid was recrystallized from ethanol to give 0.6 g (52%) of an analytically pure product as a colorless solid: mp >250 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.35 (s, 1 H), 8.30 (dd, *J* = 1.2, 8.4 Hz, 1 H), 8.13 (dd, *J* = 0.9, 7.8 Hz, 1 H), 7.92 (s, 1 H), 7.73–7.62 (m, 2 H), 7.27 (s, 2 H). Anal. Calcd for C₁₀H₈N₄: C, 65.21; H, 4.37; N, 30.42. Found: C, 65.30; H, 4.4; N, 30.3.

1-(2-Methylpropyl)-2-phenoxyethyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (50). A solution of **6** (7.5 g, 300 mmol) in acetonitrile (100 mL) was cooled to 5–10 °C. Phenoxyacetyl chloride (5.3 g, 310 mmol) was added dropwise to the vigorously stirred solution. The solution turned yellow and exhibited a slight initial exotherm. The mixture was stirred at ambient temperature for 18 h. The solid that had formed was collected by vacuum filtration, washed with acetonitrile, and dried. The solid was suspended in 7% methanolic ammonia (100 mL), and the mixture was heated in a steel pressure vessel for 8 h at 150–160 °C. The resulting mixture was reduced in volume to remove ammonia and made strongly basic with 25% methanolic sodium methoxide. After the mixture was cooled to ambient temperature, the resulting solid was collected by vacuum filtration, washed successively with methanol and water, and dried to provide 9.3 g (89%) of the unpurified product as a fluffy, white solid that was judged to be homogeneous by TLC (ethyl acetate/methanol). Recrystallization from ethanol/water (90:10) gave an analytically pure sample: mp 195–197 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.02 (d, *J* = 8.1 Hz, 1 H), 7.61 (d, *J* = 8.4 Hz, 1 H), 7.44 (t, *J* = 8.1 Hz, 1 H), 7.36–7.24 (m, 6 H), 6.60 (br s, 2 H), 5.40 (s, 2 H), 4.47 (d,

J = 7.8 Hz, 2 H), 2.26 (m, 1 H), 0.93 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₂₁H₂₂N₄O: C, 72.81; H, 6.40; N, 16.17. Found: C, 72.57; H, 6.58; N, 16.24.

3-Nitro-4-quinolinol (9). A mixture of 4-quinolinol (26.2 g, 180 mmol) in propionic acid (250 mL) was heated to 125 °C with stirring. Nitric acid (70%, 25.4 mL, 0.4 mol) was added dropwise to the stirred solution while maintaining the reaction mixture temperature at 125 ± 5 °C. The product began to precipitate before the addition of nitric acid was completed, and the reaction mixture became quite thick. When addition was complete, the mixture was stirred at 125 °C for 15 min and cooled to ambient temperature. The mixture was diluted with ethanol, and the solid was collected by vacuum filtration. The solid was washed successively with ethanol, water, and ethanol. The resulting light-yellow solid was heated in refluxing ethanol and filtered from the hot mixture to give 27.7 g (81%) of analytically pure product as a light-yellow solid: mp >250 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.99 (br s, 1 H), 9.19 (s, 1 H), 8.26 (dd, *J* = 0.9, 8.1 Hz, 1 H), 7.83–7.71 (m, 2 H), 7.52 (ddd, *J* = 0.9, 7.0, 8.1 Hz, 1 H). Anal. Calcd for C₉H₆N₂O₃: C, 56.85; H, 3.18; N, 14.73. Found: C, 56.68; H, 3.0; N, 14.6.

***N*-(2-Methylpropyl)-3-nitroquinolin-4-amine (11).** Thionyl chloride (8.1 mL, 110 mmol) was added dropwise to a vigorously stirred suspension of pure and finely divided **9** (19.0 g, 100 mmol), as prepared by the method described above, in dichloromethane (200 mL) and *N,N*-dimethylformamide (8.5 mL, 110 mmol). The addition resulted in an exotherm. After the addition of thionyl chloride was completed, the mixture was heated at reflux for 3 h to form intermediate **10**. The mixture was cooled to –15 °C, and a solution of isobutylamine (15.1 mL, 152 mmol) and triethylamine (20.9 mL, 150 mmol) was added to the cold mixture with vigorous stirring. The temperature of the mixture rose to ~20 °C, and the color changed to yellow. The yellow mixture was refluxed for 15 min, and the volatiles were removed in vacuo. The resulting bright-yellow residue was slurried in water. The solid was collected by vacuum filtration, washed with water, partially dried, and slurried in 75 mL of ethanol. The solid was collected by vacuum filtration, washed with a minimum amount of ethanol, and dried to provide 22.6 g (92%) of product as a bright-yellow solid. Recrystallization from ethyl acetate/hexanes gave an analytically pure sample as a bright-yellow solid: mp 119–121 °C; ¹H NMR (300 MHz, CDCl₃) δ 9.79 (br s, 1 H), 9.35 (s, 1 H), 8.30 (dd, *J* = 0.7, 8.5 Hz, 1 H), 7.97 (dd, *J* = 1.1, 8.3 Hz, 1 H), 7.78 (m, 1 H), 7.49 (m, 1 H), 3.79 (dd, *J* = 6.5 Hz, 2 H), 2.05 (m, 1 H), 1.10 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₃H₁₅N₃O₂: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.7; H, 6.2; N, 17.1.

Alternative Preparation for *N*-(2-Methylpropyl)-3-nitroquinolin-4-amine (11). Phosphorus oxychloride (9.9 g, 65 mmol) was added in a slow stream to a well-stirred suspension of **9** (10.0 g, 53 mmol) in *N,N*-dimethylformamide (75 mL). The internal temperature of the reaction mixture rose to 35 °C during the addition. The reaction mixture was further heated to 50 °C with stirring for 30 min. The resulting solution was cooled to ambient temperature and poured into an ice/water mixture (300 mL). A solid (**10**) was collected by vacuum filtration, washed thoroughly with water, and pressed dry. The moist product was added to a solution of isobutylamine (7.0 mL, 70 mmol) and triethylamine (14.0 mL, 100 mmol) in ethanol (200 mL). The mixture was refluxed for 15 min and then diluted with water to precipitate the unpurified product, which was collected by vacuum filtration and washed with water. The unpurified solid was dissolved in dilute hydrochloric acid (200 mL). A small amount of insoluble material was removed by filtration, and the product was reprecipitated by the addition of concentrated ammonium hydroxide. The solid was collected by vacuum filtration, washed thoroughly with water, and dried to give 11.0 g (85%) of product as a bright-yellow solid. The product was identical to the previously prepared material as judged by melting point and NMR analysis.

N⁴-(2-Methylpropyl)quinoline-3,4-diamine (12). A suspension of **11** (24.5 g, 100 mmol), 5% platinum on carbon (~500 mg), and magnesium sulfate (15 g) in ethyl acetate (350 mL) was hydrogenated on a Parr apparatus with an initial pressure of ~53 psi of hydrogen gas. When uptake of hydrogen ceased, the solids were collected by vacuum filtration and the filtrate was concentrated in vacuo to a volume of ~75 mL. The solution was diluted with an equal volume of hexanes, and a crystalline solid formed. The solid was collected by filtration, washed with 1:1 ethyl acetate/hexanes, and dried to give 11.0 g (51%) of a pale-yellow solid. A second, darker crop of 6.1 g (28%) was obtained by repeating the procedure. Recrystallization of the first crop from *tert*-butyl methyl ether gave analytically pure product as colorless crystals (Note: For general synthetic use, the unpurified diamines were often employed in the subsequent reaction without further purification.) Mp 97–99 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.37 (s, 1 H), 8.01 (m, 1 H), 7.73 (m, 1 H), 7.39–7.30 (m, 2 H), 4.97 (s, 2 H), 4.86 (t, *J* = 6.8 Hz, 1 H), 3.03 (t, *J* = 6.8 Hz, 2 H), 1.77 (m, 1 H), 0.90 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₃H₁₇N₃: C, 72.52; H, 7.96; N, 19.52. Found: C, 72.67; H, 8.0; N, 19.5.

1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (13). A mixture of **12** (21.5 g, 100 mmol) and diethoxymethyl acetate (30 mL, 0.18 mol) was heated on a steam bath for 1 h. The solution was diluted with water (30 mL) and acidified with 6 N aqueous HCl. The aqueous mixture was heated on a steam bath for 15 min. The resulting solution was cooled to ambient temperature and made strongly basic with 50% aqueous NaOH to provide an oily liquid that was extracted into dichloromethane. The combined extracts were dried over magnesium sulfate, filtered, and concentrated in vacuo. The oily material solidified upon cooling to yield 20.3 g (90%) of unpurified product as a tan solid that was judged to be homogeneous by TLC. This material was sufficiently pure for use in the next step. Recrystallization from ether provided an analytically pure sample as a pale-yellow solid: mp 92–95 °C; ¹H NMR (300 MHz, CDCl₃) δ 9.36 (s, 1 H), 8.30 (dd, *J* = 1.4, 8.4 Hz, 1 H), 8.10 (dd, *J* = 0.9, 7.9 Hz, 1 H), 7.90 (s, 1 H), 7.76–7.60 (m, 2 H), 4.37 (d, *J* = 7.4 Hz, 2 H), 2.36 (m, 1 H), 1.05 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₅N₃: C, 74.64; H, 6.71; N, 18.65. Found: C, 74.96; H, 6.74; N, 18.71.

1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline 5*N*-oxide (14). A solution of **13** (9.0 g, 40 mmol) was dissolved in ethanol (100 mL) containing peracetic acid (32% active oxygen, 11.4 g, 48 mmol). The solution was heated at 60 °C (internal temperature) for 2.5 h, and the reaction mixture was concentrated in vacuo. The residue was azeotroped with heptanes (3 × 100 mL) to remove acetic acid. The resulting solid was dissolved in water and made slightly basic with dilute aqueous NaOH to precipitate the *N*-oxide. The solid was collected by vacuum filtration, washed with water, and air-dried to give 9.8 g of unpurified product. The hydrated solid was suspended in 150 mL of toluene, and the resulting mixture was refluxed, while stirring, to remove the water of hydration. When water evolution ceased, the solid was collected by vacuum filtration, washed with toluene, and dried to provide 7.8 g (81%) of product as yellow crystals. Recrystallization from methanol/ether provided an analytically pure sample as yellow crystals: mp 211–214 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.10 (s, 1 H), 8.85 (d, *J* = 8.4 Hz, 1 H), 8.47 (s, 1 H), 8.37 (d, *J* = 8.1 Hz, 1 H), 7.98–7.77 (m, 2 H), 4.51 (d, *J* = 7.4 Hz, 2 H), 2.18 (m, 1 H), 0.94 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₅N₃O: C, 69.69; H, 6.27; N, 17.42. Found: C, 69.80; H, 6.3; N, 17.4.

4-Chloro-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (16). A solution of **14** (3.6 g, 14.9 mmol) was dissolved in dichloromethane (10 mL), and phosphorus oxychloride (2.5 g, 16.3 mmol) was added to the stirred solution in a slow stream. A spontaneous exotherm was observed. The resulting solution was refluxed for 15 min, diluted with 100 mL of dichloromethane, and poured into ice/water (100 mL) containing excess concentrated ammonium hydroxide. The mixture was stirred at ambient temperature for 4 h. The organic phase was separated from the mixture, dried over magnesium sulfate,

and concentrated in vacuo to yield 2.9 g (74%) of a tan powder that was pure enough for further reactions. The unpurified product was recrystallized from 2-propanol to yield 1.9 g (49%) of cream-colored crystals: mp 135–137 °C. The product was judged to be homogeneous by TLC and was identical to the product prepared by the previous route (see example 7 above).

***N*-Benzoyl-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (15).** A solution of **14** (7.3 g, 30 mmol) dissolved in dichloromethane (250 mL) was treated with benzoyl isocyanate (90%, 5.0 g, 30 mmol) at ambient temperature. The reaction mixture became exothermic and was refluxed briefly. The resulting warm solution was refluxed for 15 min and diluted with hexanes until the hot solution became cloudy. Upon standing, a crystalline solid began to form, and further cooling to ambient temperature completed the precipitation of product. The solid was collected by vacuum filtration, washed with hexanes, and dried to give 8.1 g of a colorless crystalline product. A second crop was obtained from the filtrate to give a combined yield of 9.5 g (92%). Recrystallization of the unpurified product from ethanol provided colorless crystals: mp 193–196 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.84 (s, 1 H), 8.35 (s, 1 H), 8.35 (m, 1 H), 8.14–8.02 (m, 3 H), 7.76–7.51 (m, 5 H), 4.53 (d, *J* = 7.4 Hz, 2 H), 2.23 (m, 1 H), 0.95 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₂₁H₂₆N₄O: C, 73.23; H, 5.85; N, 16.27. Found: C, 73.29; H, 5.8; N, 16.3.

1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (26). A solution of **15** (5.0 g, 14.5 mmol) in methanol (50 mL) was treated with 10 drops of 25% sodium methoxide in methanol. The solution was refluxed for 2 h during which time a solid precipitated from the solution. The mixture was cooled to ambient temperature and filtered. The resulting solid was washed successively with water and methanol and dried. The unpurified material (3.3 g, 95%) was judged to be homogeneous by TLC and was identical to the same product prepared by the two previously described methods.

Direct Conversion of *N*-Oxide (14) to 1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (26). To a solution of **14** (3.1 g, 12.9 mmol) in dichloromethane (30 mL) was added concentrated ammonium hydroxide (10 mL). *p*-Toluenesulfonyl chloride (2.5 g, 13 mmol), dissolved in dichloromethane (10 mL), was added dropwise to the vigorously stirred solution at ambient temperature over a period of 10 min. A spontaneous exotherm was observed, and a solid precipitated from the solution during addition. When the addition was complete, the mixture was stirred at ambient temperature for 15 min. The solid was collected by vacuum filtration, washed with dichloromethane and water, and pressed partially dry. The moist solid was slurried with methanol, collected by vacuum filtration, and dried to yield a cream-colored solid. The solid was suspended in 50 mL of methanol and refluxed for 5 min, and the solid was collected by vacuum filtration. The resulting colorless solid was recrystallized from *N,N*-dimethylformamide (60 mL) to yield 2.2 g (71%) of analytically pure product: mp 292–294 °C. The product was identical to the sample prepared by the previously described method.

2-Methanethio-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (18). To a solution of **12** (12.5 g, 58 mmol) in *N,N*-dimethylformamide was added 1,1'-thiocarbonyldiimidazole (90%, 12.7 g, 64 mmol). The resulting mixture was stirred and heated on a steam bath for 30 min. During this time all of the solid dissolved and a new solid began to precipitate from the solution. After the mixture was cooled to ambient temperature, the resulting solid was collected by vacuum filtration, washed with acetone, and dried to provide 13.2 g (88%) of unpurified **17** as a pale-yellow, crystalline solid. Unpurified **17** (18.0 g, 70 mmol), from several sources, was dissolved in a solution of 25% sodium methoxide in methanol (21.6 g, 100 mmol) and methanol (100 mL). To the solution was added methyl iodide (14.2 g, 100 mmol). The resulting solution was maintained at ambient temperature for 18–20 h. The solution was acidified with acetic acid and concentrated in vacuo. The resulting residue was slurried in water to give an oily suspension that was extracted with ethyl acetate. The combined extracts were washed with water, dried over magnesium sulfate, filtered,

and concentrated in vacuo to yield 17.7 g of unpurified product as a tan solid. Recrystallization from *tert*-butyl methyl ether gave 10.4 g (55%) of analytically pure product as tan crystals: mp 108–111 °C; ¹H NMR (300 MHz, CDCl₃) δ 9.27 (s, 1 H), 8.25 (dd, *J* = 1.6, 8.4 Hz, 1 H), 8.09 (dd, *J* = 1.1, 7.9 Hz, 1 H), 7.63 (m, 2 H), 4.29 (d, *J* = 7.6 Hz, 2 H), 2.85 (s, 3 H), 2.40 (m, 1 H), 1.04 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₅H₁₇N₃S: C, 66.39; H, 6.31; N, 15.48. Found: C, 65.83; H, 6.26; N, 15.25.

2-Methanesulfonyl-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (19). A solution of KMnO₄ (1.0 g, 6.3 mmol) in H₂O (35 mL) was added dropwise to a stirred solution of **18** (1.0 g, 3.7 mmol) in glacial acetic acid (15 mL) at ambient temperature. A slight exotherm was observed, and the mixture turned dark-brown. After the mixture was stirred at ambient temperature for 3 h, TLC analysis (10% methanol/ethyl acetate) showed no remaining starting material. To the dark mixture was added solid NaHSO₃ until the mixture was decolorized. The mixture was diluted with water, and the product was extracted into dichloromethane. The combined extracts were washed successively with water and saturated aqueous NaHCO₃, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 0.9 g (80%) of unpurified product as a colorless solid. Recrystallization from ether gave an analytically pure sample of **19** as a colorless solid: mp 134–136 °C; ¹H NMR (300 MHz, CDCl₃) δ 9.34 (s, 1 H), 8.33 (dd, *J* = 1.2, 8.4 Hz, 1 H), 8.21 (dd, *J* = 0.8, 8.1 Hz, 1 H), 7.79 (ddd, *J* = 1.4, 7.1, 8.3 Hz, 1 H), 7.72 (ddd, *J* = 1.5, 7.0, 8.3 Hz, 1 H), 4.85 (d, *J* = 7.7 Hz, 2 H), 3.63 (s, 3 H), 2.51 (m, 1 H), 1.05 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₅H₁₇N₃O₂S: C, 59.39; H, 5.65; N, 13.85. Found: C, 59.26; H, 5.57; N, 13.73.

1-(2-Methylpropyl)-2-phenoxy-1*H*-imidazo[4,5-*c*]quinoline (20). A solution of **19** (3.0 g, 9.9 mmol) and potassium phenoxide (1.6 g, 12 mmol) in *N,N*-dimethylformamide (50 mL) was refluxed for 1 h. The solution was cooled to ambient temperature and diluted with a large volume of water to precipitate the product. The solid was collected by filtration, washed with water, and dried to yield 2.3 g (73%) of unpurified product that was recrystallized from ether to yield an analytically pure sample as a colorless solid: mp 111–112 °C; ¹H NMR (300 MHz, CDCl₃) δ 9.17 (s, 1 H), 8.24 (dd, *J* = 2.2, 7.4 Hz, 1 H), 8.10 (dd, *J* = 2.2, 7.4 Hz, 1 H), 7.69–7.59 (m, 4 H), 7.51–7.39 (m, 2 H), 7.31 (m, 1 H), 4.41 (d, *J* = 7.6 Hz, 2 H), 2.46 (m, 1 H), 1.12 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₂₀H₁₉N₃O: C, 75.69; H, 6.03; N, 13.24. Found: C, 75.40; H, 6.04; N, 13.16.

1-(2-Methylpropyl)-2-phenoxy-1*H*-imidazo[4,5-*c*]quinolin-4-amine (59). A solution of **20** (1.2 g, 3.8 mmol) in methyl acetate (15 mL) was refluxed with peracetic acid (32% active oxygen, 1.1 g, 4.5 mmol) for 1 h. Additional peracetic acid (330 mg, 1.4 mmol) was added, and heating was continued for an additional 4 h. The mixture stood at ambient temperature overnight. The resulting solid was collected by vacuum filtration to give 1.2 g (95%) of unpurified **21** as a white powder. The unpurified *N*-oxide (1.2 g, 3.6 mmol) and concentrated NH₄OH (10 mL) were added to dichloromethane (20 mL), and the mixture was stirred vigorously at ambient temperature. *p*-Toluenesulfonyl chloride (0.76 g, 4.0 mmol) dissolved in dichloromethane (10 mL) was added dropwise to the vigorously stirred mixture at ambient temperature. An exotherm was observed, and the mixture turned yellow. The mixture was stirred at ambient temperature for 4 h and diluted with dichloromethane (50 mL). The organic phase was separated, washed with water, dried over magnesium sulfate, filtered, and concentrated in vacuo to give 1.1 g (92%) of the unpurified product that was recrystallized from toluene to give 0.9 g (75%) of product. A second recrystallization from methanol provided analytically pure material as pale-yellow crystals: mp 184–186 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.89 (dd, *J* = 1.0, 8.2 Hz, 1 H), 7.81 (dd, *J* = 0.9, 8.4 Hz, 1 H), 7.53–7.21 (m, 7 H), 5.27 (br s, 2 H), 4.31 (d, *J* = 7.6 Hz, 2 H), 2.40 (m, 1 H), 1.07 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₂₀H₂₀N₄O: C, 72.27; H, 6.06; N, 16.85. Found: C, 72.45; H, 6.10; N, 16.90.

4-Methoxy-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline (61). To a solution of **7** (2.1 g, 8.1 mmol) in methanol

(50 mL) was added 25% methanolic sodium methoxide (11.5 g, 53.2 mmol), and the solution was refluxed for 4 h. The solution was then concentrated in vacuo, and the residue was mixed with water. The oily residue solidified rapidly and was collected by vacuum filtration, washed with water, and partially dried. The solid was dissolved in ether, dried over magnesium sulfate, and filtered. The resulting ether solution was diluted with hexanes, and the total volume was reduced under vacuum until crystallization was initiated. After standing at ambient temperature for 15–30 min, the solid was collected by vacuum filtration, washed with hexanes, and dried to give 1.4 g (68%) of product as a light-yellow solid: mp 111–114 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.03–7.97 (m, 2 H), 7.81 (s, 1 H), 7.59 (t, *J* = 8.4 Hz, 1 H), 7.46 (t, *J* = 7.2 Hz, 1 H), 4.31 (d, *J* = 7.4 Hz, 2 H), 4.26 (s, 3 H), 2.35 (m, 1 H), 1.03 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₅H₁₇N₃O: C, 70.56; H, 6.71; N, 16.46. Found: C, 70.37; H, 6.89; N, 16.51.

1,5-Dihydro-1-(2-methylpropyl)-4*H*-imidazo[4,5-*c*]quinolin-4-one (63). A solution of **7** (20.0 g, 77.0 mmol) in 6 N HCl (200 mL) was refluxed for 1 h and cooled to ambient temperature. A small amount of dark, gummy material separated and was removed by vacuum filtration. The filtrate was diluted with water (500 mL) and made basic with concentrated aqueous NH₄OH to precipitate the product. The solid was collected by vacuum filtration, washed successively with water, ethanol, and ethyl acetate, and dried to give 16.0 g of unpurified product as an off-white solid. Recrystallization from methanol gave 13.3 g (72%) of analytically pure product: mp >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.59 (s, 1 H), 8.13 (s, 1 H), 7.96 (d, *J* = 8.1 Hz, 1 H), 7.51–7.43 (m, 2 H), 7.29 (m, 1 H), 4.38 (d, *J* = 7.5 Hz, 2 H), 2.13 (m, 1 H), 0.91 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₅N₃O: C, 69.69; H, 6.27; N, 17.41. Found: C, 69.70; H, 6.2; N, 17.4.

4-Amino-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-8-ol (72). A mixture of **71** (3.8 g, 14.1 mmol) and 48% hydrobromic acid (50 mL) was refluxed for approximately 18 h. The cooled solution was diluted with water and neutralized with concentrated ammonium hydroxide. The precipitated product was collected by vacuum filtration, washed with water, and dried. The solid was dissolved in dilute aqueous hydrochloric acid, and the resulting solution was treated with charcoal and filtered through a filter aid pad. The filtrate was treated with saturated aqueous sodium bicarbonate to precipitate a solid that was collected by vacuum filtration. The solid was washed thoroughly with water and dried to yield 3.1 g (85%) of product as a colorless solid: mp 202–205 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.48 (br s, 1 H), 8.03 (s, 1 H), 7.81 (d, *J* = 8.8 Hz, 1 H), 6.96 (s, 1 H), 6.80 (d, *J* = 8.8 Hz, 1 H), 6.43 (s, 2 H), 4.31 (d, *J* = 7.4 Hz, 2 H), 2.15 (m, 1 H), 0.91 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₆N₄O·1.3H₂O: C, 60.11; H, 6.70; N, 20.03. Found: C, 59.82; H, 6.72; N, 20.03.

8-Bromo-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (74). Bromine (10.0 g, 62.4 mmol) was added in a slow stream to a suspension of **26** (10.0 g, 41.6 mmol) in acetic acid at ambient temperature, and the mixture was stirred for 18 h. The resulting solid was collected by vacuum filtration, pressed partially dry, and then suspended in a saturated aqueous sodium bisulfite solution. The mixture was made strongly basic with aqueous sodium hydroxide. The resulting colorless solid was collected by vacuum filtration, washed thoroughly with water, and dried. The solid was added to methanol, and the mixture was stirred at ambient temperature for 18–24 h and collected by vacuum filtration. The solid was recrystallized from *N,N*-dimethylformamide to give 3.5 g (26%) of analytically pure product: mp 221–223 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.24 (s, 1 H), 8.06 (s, 1 H), 7.56 (s, 1 H), 7.55 (s, 1 H), 6.81 (br s, 2 H), 4.41 (d, *J* = 7.3 Hz, 2 H), 2.07–2.21 (m, 1 H), 0.94 (d, *J* = 6.7 Hz, 6 H). Anal. Calcd for C₁₄H₁₅BrN₄: C, 52.68; H, 4.74; N, 17.55. Found: C, 52.78; H, 4.73; N, 18.08.

1-(2-Methylpropyl)-8-nitro-1*H*-imidazo[4,5-*c*]quinolin-4-amine (75). A solution of **26** (24.0 g, 0.1 mol) in sulfuric acid (150 mL) was prepared by gradual addition of **26** to the sulfuric acid at 35 °C or less. To the solution was added

dropwise 70% nitric acid (10.0 g, 0.11 mol), dissolved in 20 mL of sulfuric acid, with vigorous stirring at 25–30 °C over a period of 15 min. The solution was stirred at ambient temperature for 30 min and poured over ice with stirring. The ice mixture was made basic with a 50% aqueous sodium hydroxide solution while maintaining the temperature at less than 45 °C by the addition of ice. The yellow mixture was stirred at ambient temperature for 1 h, and the solid was collected by vacuum filtration. The yellow solid was washed successively with hot water, methanol, and acetone. The semidry solid was suspended in *N,N*-dimethylformamide (400 mL), and the mixture was heated to ~100 °C and cooled slowly to ambient temperature. The resulting solid was collected by vacuum filtration, washed successively with *N,N*-dimethylformamide, methanol, and acetone and dried to yield 22.2 g (78%) of unpurified product as a bright-yellow solid. A 2.5 g sample of the unpurified product was recrystallized from *N,N*-dimethylformamide to yield 2.4 g (96% recovery) of analytically pure product as bright-yellow platelets: mp >250 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.81 (d, *J* = 2.5 Hz, 1 H), 8.34 (s, 1 H), 8.22 (dd, *J* = 2.5, 9.2 Hz, 1 H), 7.68 (d, *J* = 9.2 Hz, 1 H), 7.45 (br s, 2 H), 4.47 (d, *J* = 7.2 Hz, 2 H), 2.24 (m, 1 H), 0.99 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₅N₅O₂: C, 58.94; H, 5.30; N, 24.55. Found: C, 58.88; H, 5.14; N, 24.73.

1-(2-Methylpropyl)-1*H*-imidazo[4,5-*c*]quinoline-4,8-diamine (76). To a well-stirred mixture of **75** (17.1 g, 0.06 mol) in ethanol (500 mL) was added concentrated hydrochloric acid (100 mL). Stannous chloride (45.1 g, 0.23 mol), dissolved in water (70 mL), was added to the stirred mixture. The mixture was heated at reflux for 1 h, and an additional 7.7 g (0.04 mol) of stannous chloride was added to the hot mixture. Heating was continued for an additional 30 min, and the mixture was concentrated in vacuo to a thick paste. The residue was dissolved in water (500 mL), and a colorless solid precipitated as the solution was made strongly basic with 50% aqueous sodium hydroxide. After the mixture was stirred for 30 min, the solid was collected by vacuum filtration, washed with hot water and methanol, and pressed partially dry. The moist solid was dissolved in dilute hydrochloric acid (1 L), and the product was reprecipitated by the addition of excess aqueous sodium hydroxide. The solid was collected by vacuum filtration, washed successively with hot water, ethanol, and acetone, and dried to yield 13.6 g (89%) of unpurified product as a white powder. This material was pure enough for further use. Recrystallization of 4.0 g of the solid from methanol/dichloromethane gave 2.2 g of analytically pure product as a light-tan solid: mp 277–282 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.07 (s, 1 H), 7.36 (d, *J* = 8.8 Hz, 1 H), 7.12 (d, *J* = 2.3 Hz, 1 H), 6.84 (dd, *J* = 2.3, 8.8 Hz, 1 H), 5.99 (s, 2 H), 5.05 (s, 2 H), 4.29 (d, *J* = 7.4, 2 H), 2.16 (m, 1 H), 0.93 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₇N₅: C, 65.86; H, 6.71; N, 27.43. Found: C, 65.59; H, 6.78; N, 27.54.

8-Chloro-1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (77). Copper(I) chloride was freshly prepared by the following procedure: sodium chloride (366 mg, 6.25 mmol) was added to a solution of copper(II) sulfate pentahydrate (1.49 g, 6.00 mmol) in water (5 mL). The blue solution was cooled to 0 °C and treated with a solution of sodium bisulfite (624 mg, 6.00 mmol) dissolved in 4 mL of water. After the mixture was stirred for 10 min, the blue color had disappeared and copper(I) chloride had precipitated as a white solid. The aqueous portion was decanted, and the remaining white solid was washed with three small portions of water. The solid was then dissolved in 7.5 mL of concentrated hydrochloric acid, and the solution was set aside at 0 °C. In a separate flask, compound **76** (1.13 g, 4.43 mmol) was dissolved in 42 mL of 2.0 M HCl and cooled to –5 °C. The stirred solution was treated by the dropwise addition of sodium nitrite (336 mg, 4.87 mmol), which was dissolved in 4 mL of water, over a 15 min period. The resulting yellow suspension was then slowly added to the stirred copper(I) chloride solution at 0 °C. Rapid foaming was observed. After addition was complete, the reaction mixture was allowed to warm to ambient temperature and was then heated to reflux for 90 min. The reaction mixture

was then cooled to 0 °C and made basic by the addition of concentrated ammonium hydroxide to give a rust-colored solid. The solid was removed by filtration and treated with hot chloroform (100 mL). The hot solution was filtered to remove solids, and the filtrate was concentrated to give 1.05 g of an orange solid. The solid was dissolved in a minimum amount of chloroform and applied to column of silica gel (3 cm × 10 cm). Elution with chloroform followed by 2% ethanol in chloroform gave 0.55 g of the desired product (43%) as a yellow powder. Recrystallization from propyl acetate provided 0.35 g (27%) of analytically pure product as yellow crystals: mp 222–225 °C; ¹H NMR (300 MHz, CDCl₃) 8.23 (s, 1 H), 7.93 (d, *J* = 2.3 Hz, 1 H), 7.61 (d, *J* = 8.9 Hz, 1 H), 7.45 (dd, *J* = 2.3, 8.9 Hz, 1 H), 6.75 (s, 2 H), 4.41 (d, *J* = 7.3 Hz, 2 H), 2.14 (m, 1 H), 0.93 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for C₁₄H₁₅ClN₄: C, 61.20; H, 5.50; N, 20.39. Found: C, 60.99; H, 5.68; N, 20.48.

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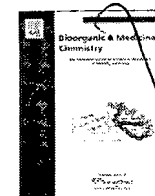
Supporting Information Available: Synthetic routes to and characterization of compounds **1**, **2**, **22–25**, **27–40**, **42–49**, **51–58**, **60**, **62**, **64–71**, and **73**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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In vitro and in vivo anti-tumoral activities of imidazo[1,2-*a*]quinoxaline, imidazo[1,5-*a*]quinoxaline, and pyrazolo[1,5-*a*]quinoxaline derivatives

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ABSTRACT

Imidazoquinoxaline and pyrazoloquinoxaline derivatives, analogues of imiquimod, were synthesized, and their in vitro cytotoxic and pharmacodynamic activities were evaluated. In vitro cytotoxicity studies were assessed against melanoma (A375, M4Be, RPMI-7591), colon (LS174T), breast (MCF7), and lymphoma (Raji) human cancer cell lines. In vivo studies were carried out in M4Be xenografted athymic mice. **EAPB0103**, **EAPB0201**, **EAPB0202**, and **EAPB0203** showed significant in vitro activities against A375 compared to fotemustine and imiquimod used as references. These compounds were 6–110 and 2–45 times more active than fotemustine and imiquimod, respectively. **EAPB0203** bearing phenethyl as substituent at position 1 and methylamine at position 4 showed the highest activity. **EAPB0203** has also a more potent cytotoxic activity than imiquimod and fotemustine in M4Be and RPMI-7591 and interesting cytotoxic activity in other tumor cell lines tested. In vivo, **EAPB0203** treatment schedules caused a significant decrease in tumor size compared to vehicle control and fotemustine treatments.

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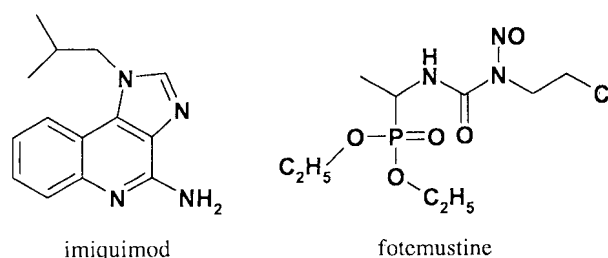
1. Introduction

Imiquimod, an imidazoquinoline amine, is an immunomodulator agent approved in 1997 by the U.S Food and Drugs Administration for treating external genital and peri-anal warts.^{1–3} In murine animal models, imiquimod has proven its efficacy in a variety of transplantable tumors including colon carcinomas, melanomas, lung sarcomas, mammary, and bladder carcinomas.² It has shown activities toward basal cell carcinomas (BCC),^{3,4} actinic keratoses,^{4,5} and some melanoma metastases,^{6–9} and its therapeutic spectrum was extended to cutaneous B-cell lymphomas.¹⁰

Extensive studies over the past years indicated that imiquimod acts both: (i) indirectly, by activating the innate as well as the adaptive immune system via binding to cell surface receptors such as Toll-like receptors (TLR) 7 and 8, thereby inducing the activation of transcription factors like nuclear factor NF- κ B and resulting in the secretion of pro-inflammatory cytokines predominantly interferon (IFN)- α , tumor necrosis factor (TNF)- α and interleukin (IL)-12,^{11,12} and (ii) directly, by inducing direct in vitro and in vivo pro-apoptotic activities in a rather tumor selective manner,^{11,13,14}

requiring the activation of the 'work horses' of apoptosis, the caspases' family of proteases.

Responsible of several thousand deaths each year, melanoma is the most frequent malignant tumor in the white human population worldwide. Its frequency increases dramatically with age and chronic sun exposure. INF- α , IL-2, some chemotherapeutic drugs, and tumor-directed vaccination have been evaluated in clinical trials. All of these approaches, unfortunately, met with rather limited success. Given the limited effectiveness of a large variety of conventional anti-cancer drugs in the treatment of melanoma such as various chemo-and/or immunotherapies, novel approaches or drugs selectively targeting melanoma cells are urgently needed.



imiquimod

fotemustine

Figure 1. Chemical structures of imiquimod and fotemustine.

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This study was related to the pharmacology of nitrogen heterocycles. The aim of this paper was to synthesize and to develop three chemical series, analogues of imiquimod (Fig. 1): the imidazo[1,2-*a*]quinoxalines, the imidazo[1,5-*a*]quinoxalines, and the pyrazolo[1,5-*a*]quinoxalines, in order to evaluate their potential anti-cancer properties, in vitro against a variety of human cancer cell lines, and in vivo on melanoma by comparison with fotemustine (Fig. 1), one of the main drug used in clinic to treat human melanoma.

2. Results

2.1. Chemistry

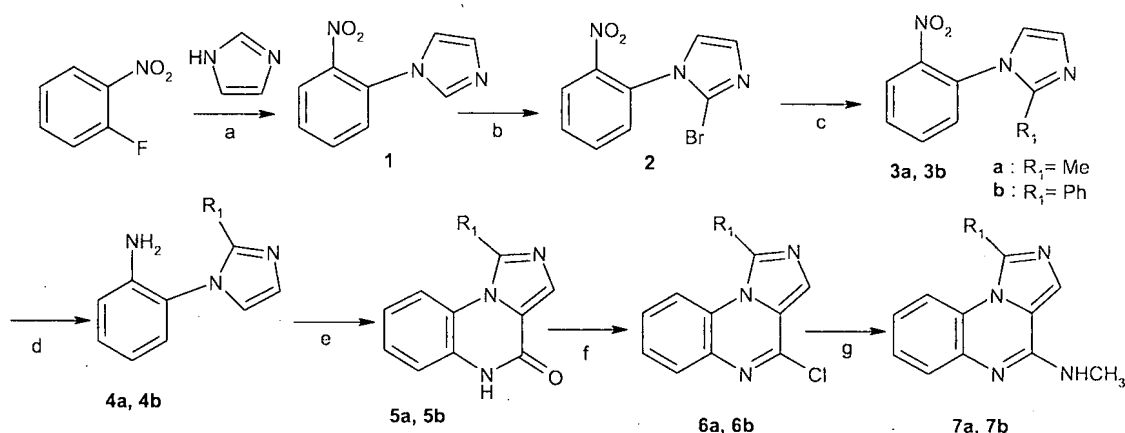
2.1.1. Library synthesis

For the preparation of the 1*H*-imidazo[1,5-*a*]quinoxaline derivatives, the procedure presented in Scheme 1 was used. In general, treatment of ortho-fluoronitrobenzene with imidazole and an excess of potassium carbonate formed compound **1** in 95% overall yield. After halogenation with *N*-bromosuccinimide, compounds **3a** and **3b** were obtained through Suzuki cross-coupling reactions

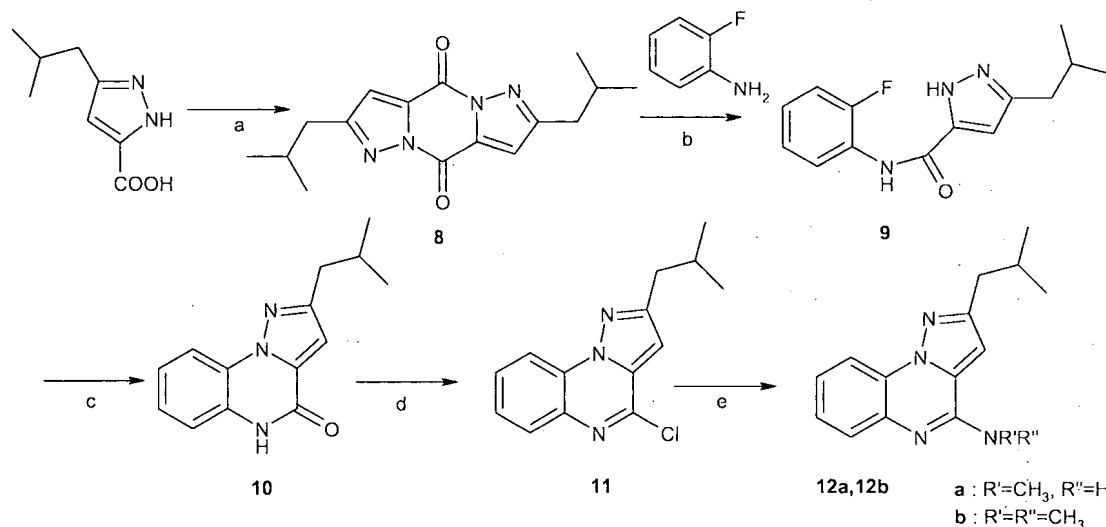
with yields of 94% and 54%, respectively. Attempted reduction of the nitro group by hydrogenation resulted in the formation of **4a** and **4b** with yields of 45% and 94%, respectively. The formation of the tricyclic compounds **5a** and **5b** was obtained after addition of carbonyldiimidazole by intramolecular cyclization of **4a** and **4b**, respectively. The chlorination of **5a** and **5b** by phosphorus oxychloride and substitution by an adapted amine formed the **7a** and **7b** compounds with yields of 53% and 86%, respectively.

The synthesis of the 1*H*-pyrazolo[1,5-*a*]quinoxaline derivatives is illustrated in Scheme 2.

Dimer **8** resulted from the bimolecular condensation of the 3-isobutyl-1*H*-pyrazole-5-carboxylic acid, in presence of thionyl chloride in 80% overall yield. Product **8** was coupled with ortho-fluoroaniline to give the intermediate **9**, with a yield of 46%. The tricyclic compound **10** was obtained via an intramolecular cyclization of **9** in the presence of sodium hydride and dimethylacetamide as solvent with 80% yield. Treatment of compound **10** with phosphorus oxychloride yielded to 50% of compound **11** which was coupled with methylamine (series **a**) and dimethylamine (series **b**) to form **12a** and **12b** with average yields of 75% and 85%, respectively. The same strategy was applied to obtain the monosubstituted 1*H*-imi-



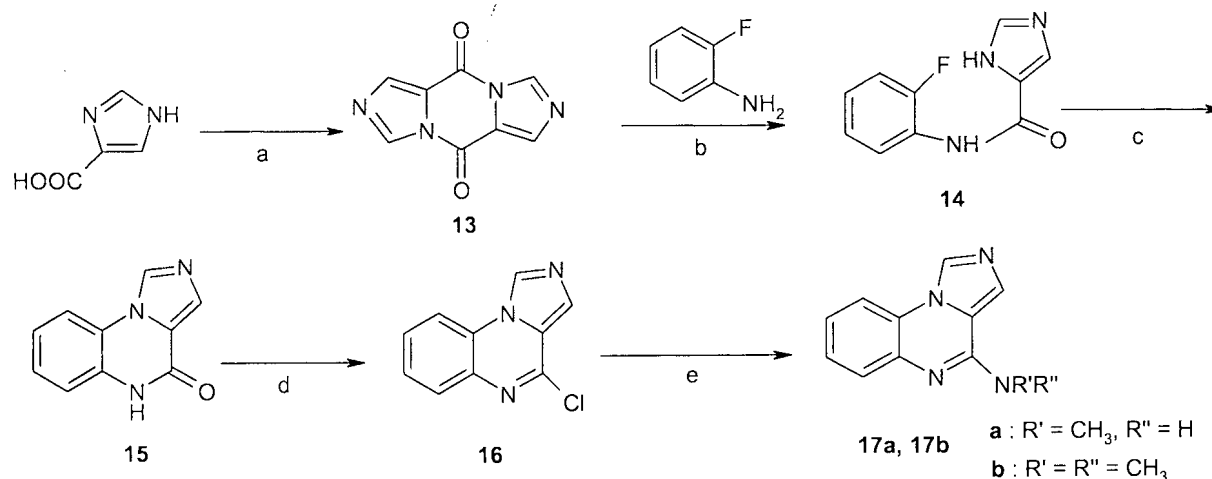
Scheme 1. Synthesis of 1*H*-imidazo[1,5-*a*]quinoxaline derivatives. Reagents and conditions: (a) MeCN, reflux; (b) NBS, MeCN, reflux; (c) $R_1\text{-B(OH)}_2$, Na_2CO_3 , DME, $\text{Pd(PPh}_3)_4$; (d) $\text{H}_2/\text{Pd-C}$, EtOH; (e) Im_2CO , PhCl_2 , reflux; (f) POCl_3 , reflux; (g) $\text{R}'\text{NH}_2$, EtOH.



Scheme 2. Synthesis of 1*H*-pyrazolo[1,5-*a*]quinoxaline derivatives. Reagents and conditions: (a) SOCl_2 , reflux, 18 h; (b) NaHMDS, THF, 5 h; (c) NaH, DMA, reflux, 10 h; (d) POCl_3 , reflux, 6 h; (e) EtOH, $\text{R}'\text{R}''\text{NH}$, 20 h, rt.

dazo[1,5-*a*]quinoxalines **17a** and **17b** starting from imidazole-4-carboxylic acid (Scheme 3).

All synthesized compounds with their corresponding pK_a and $\log P$ calculated values are presented in Table 1. The 1*H*-imi-



Scheme 3. New synthesis of the monosubstituted 1*H*-imidazo[1,5-*a*]quinoxaline derivatives. Reagents and conditions: (a) SOCl_2 reflux, 18 h; (b) NaHMDs, THF, 5 h; (c) NaH, DMA, reflux, 10 h; (d) POCl_3 , reflux, 6 h; (e) EtOH, $\text{R}'\text{R}''\text{NH}$, 20 h, rt.

Table 1
1*H*-Imidazo[1,5-*a*]quinoxaline, 1*H*-imidazo[1,2-*a*]quinoxaline and 1*H*-pyrazolo[1,5-*a*]quinoxaline: formulas and in vitro cytotoxic activities against A375 human melanoma cell line

		Imidazo[1,2- <i>a</i>]quinoxaline	Imidazo[1,5- <i>a</i>]quinoxaline	Pyrazolo[1,5- <i>a</i>]quinoxaline		
Compounds	R_1	R_4	Formula	IC_{50} (μM) ^a against A375 cell line	pK_a ^c	$\log P$ ^c
Imidazo[1,2-<i>a</i>]quinoxalines						
EAPB0103	$(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$	$\text{CH}_3\text{-NH-}$	$\text{C}_{15}\text{H}_{18}\text{N}_4$	31.6 ± 2.0	5.61 ± 0.4	3.91 ± 1.42
EAPB0203	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$	$\text{CH}_3\text{-NH-}$	$\text{C}_{19}\text{H}_{18}\text{N}_4$	1.57 ± 0.56	5.61 ± 0.4	4.91 ± 1.42
EAPB0202	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$	$\text{NH}_2\text{-}$	$\text{C}_{18}\text{H}_{16}\text{N}_4$	2.35 ± 0.15	5.13 ± 0.4	4.97 ± 1.42
EAPB0201	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$	Cl-	$\text{C}_{18}\text{H}_{14}\text{ClN}_3$	24.0 ± 0.5	-1.57 ± 0.4	4.53 ± 1.27
EAPB0104	$(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$	$(\text{CH}_3)_2\text{-N-}$	$\text{C}_{16}\text{H}_{20}\text{N}_4$	66.3 ± 7.5	5.73 ± 0.4	3.33 ± 1.42
EAPB0204	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$	$(\text{CH}_3)_2\text{-N-}$	$\text{C}_{20}\text{H}_{20}\text{N}_4$	80.1 ± 7.0	5.73 ± 0.4	4.32 ± 1.42
EAPB0206	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$	OCH_3	$\text{C}_{19}\text{H}_{17}\text{N}_3\text{O}$	47.8 ± 5.0	0.85 ± 0.4	4.56 ± 1.42
EAPB0105	$(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$		$\text{C}_{21}\text{H}_{21}\text{ClN}_4$	>400	2.25 ± 0.4	4.56 ± 1.42
EAPB0205	$\text{C}_6\text{H}_5\text{-(CH}_2)_2\text{-}$		$\text{C}_{25}\text{H}_{21}\text{ClN}_4$	329 ± 63	2.25 ± 0.4	7.25 ± 1.43
Imidazo[1,5-<i>a</i>]quinoxalines						
17a	H	$\text{CH}_3\text{-NH-}$	$\text{C}_{11}\text{H}_{10}\text{N}_4$	85.3 ± 7.0	6.50 ± 0.4	0.93 ± 1.07
17b	H	$(\text{CH}_3)_2\text{-N-}$	$\text{C}_{12}\text{H}_{12}\text{N}_4$	121 ± 17	6.62 ± 0.4	0.95 ± 1.08
7a	$\text{C}_6\text{H}_5\text{-}$	$\text{CH}_3\text{-NH-}$	$\text{C}_{17}\text{H}_{14}\text{N}_4$	>400	6.45 ± 0.4	3.38 ± 1.09
7b	$\text{CH}_3\text{-}$	$\text{CH}_3\text{-NH-}$	$\text{C}_{12}\text{H}_{12}\text{N}_4$	100 ± 9	7.33 ± 0.4	1.19 ± 1.08
Pyrazolo[1,5-<i>a</i>]quinoxalines						
12a	$(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$	$\text{CH}_3\text{-NH-}$	$\text{C}_{15}\text{H}_{18}\text{N}_4$	78.6 ± 7.8	6.20 ± 0.4	3.38 ± 1.06
12b	$(\text{CH}_3)_2\text{-CH-CH}_2\text{-}$	$(\text{CH}_3)_2\text{-N-}$	$\text{C}_{16}\text{H}_{20}\text{N}_4$	101 ± 5	6.32 ± 0.4	3.40 ± 1.06
Imiquimod				70.3 ± 4.3		
Fotemustine				173 ± 24		

^a IC_{50} , concentration of the compound (μM) producing 50% cell growth inhibition after 96 h of drug exposure, as determined by the MTT assay. Each experiment was run at least three times, and the results are presented as average values $\pm$ standard deviation.

^c pK_a and $\log P$ values are calculated using the ACD/labs software.

dazo[1,2-*a*]quinoxaline substituted derivatives were obtained as previously described.¹⁵

2.1.2. In vitro biological activity

All compounds were firstly evaluated for their cytotoxic activity in vitro against the A375 human melanoma cell line by comparison with imiquimod and fotemustine used as references. Concentrations of the compounds which produced 50% cell growth inhibition (IC_{50} values) are summarized in Table 1 for the imidazo- and pyrazoloquinoxaline compounds, and for fotemustine and imiquimod. Among the compounds tested, **EAPB0203** (IC_{50} = 1.57 μ M) and **EAPB0202** (IC_{50} = 2.35 μ M) exhibited the highest cytotoxic activities. **EAPB0203** containing phenethyle as substituent at position 1 and methylamine at position 4 was the 'lead' compound with an activity against A375 cell line 110 times higher than fotemustine (IC_{50} = 173 μ M) and 45 times higher than imiquimod (IC_{50} = 70 μ M). The **EAPB0202** (R_1 = phenethyl and R_4 = amine) had an activity very close to that of **EAPB0203** (almost 2 times more active than **EAPB0202**) with an IC_{50} value 70 times lower than fotemustine and 30 times lower than imiquimod. The **EAPB0103** compound having isobutyle at position 1 and methylamine at position 4 exhibited an interesting activity; this compound is 7 times more active than fotemustine and 3 times more active than imiquimod. The **EAPB0201** (R_1 = phenethyl and R_4 = chloro) also presents a good cytotoxic activity, this compound is 2 and 6 times more active than imiquimod and fotemustine, respectively. The other compounds, substituted or unsubstituted at position 1 and bearing dimethylamine or chloromethylaniline as substituent at position 4, are less potent and have IC_{50} values between 20 and 300 μ M. The cytotoxic activities of **7a** and **EAPB0105** have not been evaluated due to the very low solubility of these compounds in the culture medium. The pyrazolo[1,5-*a*]quinoxaline analogue **12a** showed similar activity than imiquimod.

Since **EAPB0203** exhibited the highest activity against the A375 melanoma cell line, we further evaluated its cytotoxic activity against the following human cell lines: M4Be and RPMI-7591 (melanoma), LS174T (colon cancer), MCF7 (breast cancer), and Raji (B lymphoma). The IC_{50} values are illustrated in Table 2. The most interesting results were obtained on M4Be and RPMI-7591. **EAPB0203** exhibited cytotoxic activity 30–130 times higher than fotemustine, with IC_{50} values of 2.58 and 4.23 μ M, respectively. In addition **EAPB0203** showed a cytotoxic activity (3 times less potent) similar to irinotecan on LS174T cell line.

2.1.3. In vivo activity

The 50% lethal dose (LD_{50}) of **EAPB0203** was determined, in Swiss mice, after intraperitoneal administrations at doses ranging from 30 to 300 mg/kg. At the highest doses, **EAPB0203** appeared to be non toxic.

Previous studies have shown that **EAPB0203** is a potent in vitro growth inhibitor for human melanoma cell lines with a cytotoxic

activity higher than fotemustine used as reference. To further evaluate the in vivo induction of melanoma death by **EAPB0203** we proceeded as described in materials and methods. Tumor size is the most common parameter used to assess effective anti-cancer treatment in preclinical models. The **EAPB0203** treatments were well tolerated without apparent side-effects or weight losses. As expected, M4Be xenografts grew progressively using the vehicle control. In contrast, in mice treated with **EAPB0203** or fotemustine, a highly significant delay in tumor growth was observed (Fig. 2). At highest doses (20 mg/kg), from day 18 to day 55, the simple contrast test showed a high significant difference in tumor growth between the **EAPB0203** and the control groups (P values ranging from 0.0026 to 0.00004). Lower significant differences were observed between the fotemustine and the control groups (not significant at days 18 through 24, 34, 37, and 55; and P : 0.014 to 0.0024 at days 27, 31, 41 through 51). Most interesting, **EAPB0203** treatment produced a statistically significant decrease in tumor growth at days 21, 24, 41, and 44 compared to the fotemustine treatment. Results are presented in Figure 2. At low doses (5 mg/kg), the variations in tumor growth according to the treatment group have the same profile than at high doses (data not shown) but lower significant differences were observed. Between fotemustine and **EAPB0203**, significant difference occurred on day 34.

Efficacy of treatment is dose-related. In mice receiving **EAPB0203**, the decrease in tumor growth was statistically higher on days 18 (P = 0.026), 21 (P = 0.0058), 24 (P = 0.0073), 31 (P = 0.0033), and 34 (P = 0.016) at the 20 mg/kg dose than at the 5 mg/kg dose. In animals treated by fotemustine, significant differences occurred at days 24 (P = 0.017), 27 (P = 0.019), 31 (P = 0.0046), and 34 (P = 0.0052).

In the control group, all mice were sacrificed on day 55. In the fotemustine group (20 mg/kg), all mice were killed on day 76, since tumor volumes were superior to 2 cm³, while in the **EAPB0203** group (20 mg/kg), one mouse (1/6) was still alive with a tumor volume less than 2 cm³ (the limit fixed by the protocol).

3. Discussion

Imiquimod is a low-molecular-weight immune response modifier that can induce the synthesis of interferon- α and other cytokines in a variety of cell types. Imiquimod has potent anti-viral and anti-tumor properties. Several mechanisms by which this compound exerts its activity have been mentioned. Many studies^{11,16,17} show that imiquimod activates immune cells via the Toll-like receptor 7 (TLR7)-MyD88-dependent signalling pathways. Based on some reports^{14,18} of apoptosis in skin cancer, imiquimod exerts a direct pro-apoptotic effect against human melanoma. It is able to induce apoptosis in malignant melanoma cells both in vitro and in vivo in a rather tumor-selective manner. Imiquimod-induced apoptosis in tumor cells could not be antagonized by functional blockage of various membrane-bound death receptors,

Table 2

IC_{50} values for imiquimod, fotemustine, methotrexate, irinotecan, doxorubicin, and **EAPB0203** against A375, M4Be, and RPMI-7591 (melanoma cell lines), LS174T (colon cancer), MCF7 (breast cancer), and Raji (B lymphoma) human cell lines

Compounds	IC_{50} (μ M) ^a					
	A375	M4Be	RPMI-7591	MCF7	LS174T	Raji
Fotemustine	173 $\pm$ 24	326 $\pm$ 34	125 $\pm$ 49			
Doxorubicin				0.13 $\pm$ 0.01		
Irinotecan					1.16 $\pm$ 0.07	
Methotrexate						0.04 $\pm$ 0.005
Imiquimod	70.3 $\pm$ 4.3	33.5 $\pm$ 7.7	53.7 $\pm$ 9.8	145 $\pm$ 7	34.4 $\pm$ 9.7	139 $\pm$ 12
EAPB0203	1.57 $\pm$ 0.56	2.58 $\pm$ 0.40	4.23 $\pm$ 0.48	1.08 $\pm$ 0.46	4.12 $\pm$ 0.67	6.23 $\pm$ 0.06

^a IC_{50} , concentration of the compounds (μ M) producing 50% cell growth inhibition after 96 h of drug exposure, as determined by the MTT assay. Each experiment was run at least three times, and the results are presented as average values $\pm$ standard deviation.

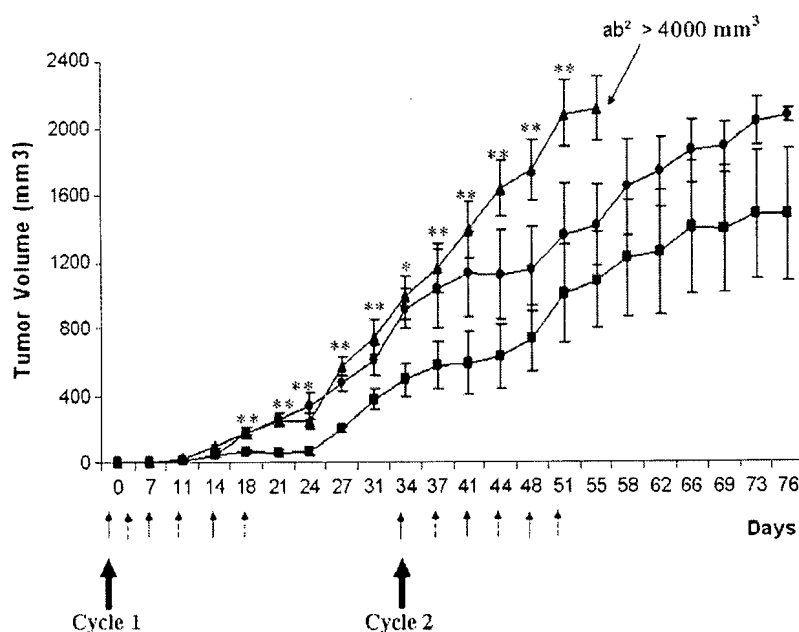


Figure 2. Effects of fotemustine (20 mg/kg once a week for 3 weeks) and **EAPB0203** (20 mg/kg twice a week for 3 weeks) on tumor growth (ab^2) in xenografted nude mice by M4Be melanoma cell lines. ↑, fotemustine and **EAPB0203** administrations; ▬, **EAPB0203** administrations ▲, control mice; ■, **EAPB0203**; ●, fotemustine. Data are the mean of one experiment carried out on six mice. Error bars represent SEM. Significant difference between the 3 treatment groups: *, $P < 0.05$; **, $0.002 < P < 0.009$; ***, $P \leq 0.001$.

including Fas/APO-1 (CD95), TRAIL, and TNF receptors, which suggests that imiquimod-inducing apoptosis is independent from apoptosis mediated by membrane-bound death receptors. Its tumor-selective pro-apoptotic activity is correlated with translocation of cytochrome c from the mitochondria to the cytosol by shifting the mitochondria-associated Bcl-2/Bax ratio, and activating caspases 9 and 3. Imidazo[1,2-*a*]quinoxalines are imiquimod analogues that inhibit cyclic nucleotide phosphodiesterase enzymes 4.¹⁵ Furthermore, in studies on murine fibroblast L929 cells, we recently showed that these imidazo[1,2-*a*]quinoxalines activate the p38 MAPK pathway, and inhibit the PI3K pathway.¹⁹ Furthermore, we recently demonstrated that one of these compounds (**EAPB0203**) induced inhibition of cell proliferation, G2/M cell cycle arrest, and apoptosis in HTLV-I transformed and HTLV-I-negative malignant T cells as well in fresh adult T-cell leukaemia (ATL) cells, while normal resting or activated T lymphocytes were resistant. **EAPB0203** initiated the apoptotic process via the intrinsic cell death pathway converging at the mitochondria.²¹

In the present study, we report the synthesis and preliminary pharmacological evaluation of a new series of imiquimod's analogues, the imidazo/pyrazoloquinoxaline derivatives. Imidazoquinoxalines were prepared via two different methods, the first one previously described¹⁵ and the second one is presented in Scheme 1. The first method¹⁵ was used to synthesize the imidazo[1,2-*a*]quinoxaline compounds which were obtained in seven steps in good yields. The second method outlined in Scheme 1 was employed for the synthesis of the imidazo[1,5-*a*]quinoxaline compounds. Using these two methods, a limited number of analogues were obtained. Actually, the alkyl or aryl substitution at the R_1 position must be done at the beginning steps of the synthesis leading to a low variability in the moieties on the imidazole cycle. Likewise, the purifications after each step caused further limitations of the synthetic process. Our framework was to optimize the methods of synthesis (i) to obtain a common strategy for the three chemical series, (ii) to reduce the number of purification steps, and (iii) to increase the structural diversity of these series essential for the SAR studies. The procedure presented in Scheme 2 was used to prepare the pyrazolo[1,5-*a*]quinoxaline derivatives.²⁰ This

method not only reduced the number of purification steps but also led us to propose a new common and efficient route for the preparation of the unsubstituted imidazo[1,5-*a*]quinoxalines on the imidazole core (Table 1) starting from the unsubstituted carboxylic acid (Scheme 3). This new strategy also permitted the introduction of the R_1 substituent at the last steps of the procedure with different commercial Suzuki cross coupling reagents (syntheses under study, data not shown).

All compounds were screened in vitro against the human melanoma cell line A375. First, we assessed the influence of the substituent at the R_4 position by the introduction of four types of amines with increasing steric hindrance **EAPB0202**, **EAPB0203**, **EAPB0204**, and **EAPB0205** (Table 1). At R_4 position, the methylamino group (**EAPB0203**) appears to be essential for the potency. Indeed, accessibility at the electronic doublet of the nitrogen on this position of the heterocycle might be critical for activity, thanks to the low steric bulk of this secondary amine. No significant differences were observed between the pK_a and $\log P$ calculated values (Table 1) of this series of compounds which are all substituted by an amino group at the R_4 position. We next focused our attention on the substitution on the imidazole core with the objective to improve in vitro potency against A375 cells. We also explored the effect of the incorporation of a phenethyl substituent at the R_1 position. **EAPB0203**, bearing phenethyl on R_1 , significantly enhanced the activity ($IC_{50} = 1.57 \mu M$) by creation of an π interaction necessary for its binding. The improved bioactivity of the **EAPB0203** compound may be attributed to the additional effect and the synergy between the methylamino and phenethyl groups in addition to the basic pharmacophore that might be defined by the sequence of the three intracyclic nitrogens of the imidazo[1,2-*a*]quinoxaline series. Overall, the other analogues appeared to be less potent. In a general way, it is noticed that the imidazo[1,2-*a*]quinoxaline derivatives have a better activity than the two other series of compounds, but this still remains to be proven by comparison with a larger variety of imidazo-/pyrazolo[1,5-*a*]quinoxaline derivatives.

Two drugs are approved in the European Union for treatment of advanced or metastatic melanoma: dacarbazine and fotemustine.²²

Furthermore, imiquimod was recently approved for treatment of different cutaneous cancers.

EAPB0203 displayed potent cellular cytotoxic activity with micromolar IC_{50} values against different human cancer cell lines and was compared to fotemustine and imiquimod (Table 2). Upon such encouraging results, **EAPB0203** was selected for detailed in vivo studies. In human melanoma (M4Be) xenografted athymic mice, **EAPB0203**, administered twice a week at 20 mg/kg, has approached a significant delay in tumor growth and also a decrease in tumor size compared to that of control and fotemustine groups (Fig. 2). Interestingly, a stabilization and even an arrest in tumor growth were observed after each treatment cycle. The efficacy of treatment is dose-related. The **EAPB0203** treatment was well tolerated without side-effects.

4. Conclusion

In this paper, we have reported the synthesis of imidazoquinoxaline and pyrazoloquinoxaline derivatives, and their in vitro cytotoxic activity against human melanoma cell lines and the in vivo anti-proliferative activity of the **EAPB0203** derivative on melanoma. The synthesis was based on a bimolecular condensation of a carboxylic acid, followed by a coupling with orthofluoroaniline, an intramolecular cyclisation and a substitution with appropriate amines. This strategy will be the starting point for the development of other compounds. The incorporation of phenethyle as moiety at the R_1 position and methylamine at the R_4 position led to the **EAPB0203** compound with enhanced cytotoxic activity against A375 human melanoma cell line. Its IC_{50} (1.57 μ M) was 110 times lower than fotemustine (IC_{50} = 173 μ M), and **EAPB0203** was 45 times more potent than imiquimod (IC_{50} = 70 μ M). Interestingly, compared to imiquimod and fotemustine, this compound also had lower IC_{50} values (30–130-fold) against the two other melanoma cell lines tested. In vivo, **EAPB0203** had potent anti-tumor activity on human melanoma (M4Be) xenografted nude mice. Our results support a potential therapeutic role of **EAPB0203** in the treatment of malignant melanoma and prompt us to modulate the chemical structure of this lead molecule to optimize its effect. Further studies are now in progress to study the mode of action and should lead to an interesting class of new anti-cancer drugs with innovating mechanism of action.

5. Experimental

5.1. Chemistry

All solvents and reagents were obtained from commercial sources and used without further purification unless indicated otherwise. ^1H and ^{13}C NMR spectra were recorded using a Bruker AC 300 spectrometer. Chemical shifts are reported in parts per million (ppm) from the tetramethylsilane resonance in the indicated solvent. Coupling constants are reported in Hertz (Hz), spectral splitting partners are designed as follow: singlet (s); doublet (d); triplet (t); multiplet (m). Column chromatography was performed on Merck silica gel 60 (200–400 mesh). Elemental analysis was carried out at the Microanalytical Central Department (Montpellier, France). All melting points are determined using köfler hot plate melting point apparatus and are not corrected. pK_a and $\log P$ values are calculated using the ACDLabs software (Toronto, Canada).

5.1.1. 2-Chloro-1-isobutyl-6-methylphenyl-*N*-methylimidazo[1,2-*a*]quinoxalin-4-amine (EAPB0105)

To a solution of 6-chloro-3-methylaniline (89 mg, 0.63 mmol) in THF (12 ml), was added a solution of sodium bis(trimethylsilyl)amide (4 mL, 4 mmol, 1.0 M in THF), and the reaction mixture

was heated to reflux for 0.5 h. The reaction mixture was cooled to room temperature and the 4-chloro-1-isobutylimidazo[1,2-*a*]quinoxaline¹⁵ (150 mg, 0.5 mmol) in THF (10 mL) was added. The mixture was again heated to reflux for 0.5 h and then cooled to room temperature and stopped with acetic acid until pH 7. The reaction mixture was concentrated in vacuum followed by addition of water and saturated sodium bicarbonate. The solid was collected by filtration, washed with water and hexane, and dried under high vacuum to give **EAPB0105** (110 mg, 60%); ^1H NMR (300 MHz, $CDCl_3$ - d_6): δ 8.01 (t, 1H), 7.6 (m, 3H), 7.5 (d, 1H), 7.38 (d, 1H), 7.06 (s, 1H), 6.67 (t, 1H), 3.00 (t, 2H), 2.2 (m, 5H), 0.81 (t, 6H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 142.61, 140.68, 139.55, 131.44, 130.32, 129.71, 129.19, 128.07, 126.78, 126.27, 125.55, 123.44, 122.79, 114.36, 33.68, 32.75, 21.99, 18.10. Anal. calcd for $C_{21}H_{21}ClN_4$: C, 69.13; H, 5.80; N, 15.35. Found: C, 69.33; H, 5.42; N, 15.65.

5.1.2. 2-Chloro-6-methylphenyl-*N*-methyl-1-phenethylimidazo[1,2-*a*]quinoxalin-4-amine (EAPB0205)

EAPB0205 was prepared with the same procedure as the one for the preparation of **EAPB0105**; using 4-chloro-1-phenethylimidazo[1,2-*a*]quinoxaline¹⁵ (200 mg, 0.65 mmol) in 10 ml of THF. **EAPB0205** was obtained as a white solid (120 mg, 45%); ^1H NMR (300 MHz, $CDCl_3$ - d_6): δ 7.93 (d, 1H), 7.49 (d, 1H), 7.56 (m, 3H), 7.39 (d, 1H), 7.24 (m, 5H), 6.73 (t, 1H), 3.3 (t, 4H), 2.23 (s, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 143.12, 142.57, 140.69, 139.77, 137.67, 131.44, 130.42, 130.12, 129.71, 129.19, 128.81, 126.78, 126.44, 126.27, 125.55, 124.38, 124.3, 114.22, 35.17, 23.83, 18.10. Anal. calcd for $C_{25}H_{21}ClN_4$: C, 72.72; H, 5.13; N, 13.57. Found: C, 72.41; H, 5.38; N, 13.39.

5.1.3. 1-(2-Nitrophenyl)-1*H*-imidazole (1)

A mixture of 2-nitrofluorobenzene (14.85 mL, 0.142 mol), imidazole (14.9 g, 0.128 mol), and 40 g of K_2CO_3 in 200 ml of CH_3CN was heated to reflux for 24 h. The solvent was removed under vacuum, and the residue was slurred in 200 mL of CH_2Cl_2 , washed twice with 200 mL of water, dried over Mg_2SO_4 , and the solvent was removed under vacuum. We obtained 95% of **1** as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 9.12 (s, 1H), 7.58 (d, 1H), 7.42 (d, 1H), 7.37 (d, 1H), 7.26 (d, 1H), 7.10 (m, 3H, ArH). Anal. calcd for $C_9H_7N_3O$: C, 57.14; H, 3.70; N, 22.22. Found: C, 56.99; H, 3.9; N, 22.06.

5.1.4. 2-Bromo-1-(2-nitrophenyl)-1*H*-imidazole (2)

To a solution of **1** (5 g, 0.27 mmol) in dry acetonitrile (250 mL), *N*-bromosuccinimide (5.65 g, 0.032 mmol) was added. The reaction mixture was stirred to reflux for 3 h. The solvent was removed under vacuum and the residue was slurred in 250 mL of CH_2Cl_2 , washed twice with a solution of 5% $NaHCO_3$, dried over $MgSO_4$, and purified by column chromatography (CH_2Cl_2 -MeOH, 98:2, v/v) to give 50% of **2**. ^1H NMR (300 MHz, $CDCl_3$): δ 8.03 (d, 1H), 7.70 (d, 1H), 7.47 (t, 1H), 7.33 (d, 1H), 7.09 (m, 2H). Anal. calcd for $C_9H_6N_3O_2Br$: C, 40.30; H, 2.24; N, 15.42. Found: C, 40.65; H, 2.01; N, 15.52.

5.1.5. 2-Methyl-1-(2-nitrophenyl)-1*H*-imidazole (3a)

To a mixture of **2** (2 g, 7.46 mmol) and $Pd(PPh_3)_4$ (0.8 g, 0.7 mmol) in 1,4-dioxane (34 mL), 1.4 mL of the trimethylboroxine was added followed by Na_2CO_3 (2.37 g, 22.38 mmol) in 5 mL of water. The reaction mixture was heated to 75 °C with vigorous stirring under nitrogen atmosphere. After 24 h, a second portion of trimethylboroxine was added. After that, the mixture was poured into water and extracted with dichloromethane. The combined organic extracts were washed with water, dried over calcium chloride, and concentrated to dryness under vacuum. The crude products were purified by column chromatography (silica gel, elu-

tion with CH_2Cl_2 –MeOH, 97:7, v/v) to give **3a** (yield, 94%). ^1H NMR (300 MHz, CDCl_3): δ 7.40 (m, 2H), 7.22 (d, 1H), 7.11 (d, 1H), 7.01 (m, 2H), 2.3 (s, 3H). Anal. calcd for $\text{C}_{10}\text{H}_9\text{N}_3\text{O}_2$: C, 59.11; H, 4.43; N, 20.69. Found: C, 59.32; H, 4.65; N, 20.51.

5.1.6. 1-(2-Nitrophenyl)-2-phenyl-1H-imidazole (3b)

3b was prepared from **2** following the protocol described for **3a** (i.e., from **2** (2 g, 7.46 mmol), $\text{Pd}(\text{PPh}_3)_4$ (0.5 g, 0.4 mmol) in DME (85 mL), 2×1 g (16.4 mmol) of phenylboronic acid and Na_2CO_3 (2.37 g, 22.38 mmol) in 5 mL of water). The crude products were purified by column chromatography (silica gel, elution with CH_2Cl_2 –MeOH, 97:7, v/v) to give **3b** (yield, 88%). ^1H NMR (300 MHz, CDCl_3): δ 8.45 (dd, 2H), 7.60 (t, 1H), 7.57 (m, 2H), 7.40 (m, 5H), 7.02 (d, 1H). Anal. calcd for $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_2$: C, 67.92; H, 4.15; N, 15.85. Found: C, 67.75; H, 4.47; N, 15.70.

5.1.7. 2-(2-Methylimidazol-1-yl)phenylamine (4a)

Compound **3a** (0.3 g, 1.48 mmol) was dissolved in 20 mL of ethanol with 0.3 g of 10% palladium on carbon and hydrogenated for 2 h. The catalyst was removed by filtration, and the residue concentrated under vacuum and purified by column chromatography on silica gel using CH_2Cl_2 –MeOH (97:3) as eluent to yield 43% of **4a**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.36 (m, 2H), 7.03 (d, 1H), 6.90 (m, 2H), 6.79 (d, 1H), 2.1 (s, 3H). Anal. calcd for $\text{C}_{10}\text{H}_{11}\text{N}_3$: C, 69.36; H, 6.36; N, 24.28. Found: C, 69.5; H, 6.11; N, 24.12.

5.1.8. 2-(2-Phenylimidazol-1-yl)phenylamine (4b)

This compound was prepared as described for **4a** to obtain **4b** (yield, 93%) (i.e., from **3b** (1.31 g, 5 mmol), EtOH (60 mL), and 0.3 g of 10% palladium on carbon). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.36 (m, 2H), 7.25 (m, 5H), 7.12 (t, 1H), 6.8 (m, 2H), 3.67 (s, 2H, $-\text{NH}_2$). Anal. calcd for $\text{C}_{15}\text{H}_{13}\text{N}_3$: C, 76.59; H, 5.53; N, 17.97. Found: C, 76.72; H, 5.34; N, 18.02.

5.1.9. 1-Methyl-5H-imidazo[1,5-a]quinoxalin-4-one (5a)

Compound **4a** (1.38 g, 8 mmol) and carbonyldiimidazole (1.55 g, 9.57 mmol) were dissolved in 83 mL of 1,2-dichlorobenzene and heated to reflux under N_2 for 2 h. After cooling to room temperature, the residue was slurred with CH_2Cl_2 , washed with water, and dried over MgSO_4 . The product was purified by chromatography on silica gel using CH_2Cl_2 –MeOH (97:3, v/v) as eluent to give 62% of **5a**. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.1 (d, 1H), 7.7 (s, 1H), 7.37 (m, 2H), 7.25 (t, 1H), 2.5 (s, 3H). Anal. calcd for $\text{C}_{11}\text{H}_9\text{N}_3\text{O}$: C, 68.38; H, 4.66; N, 16.09. Found: C, 68.49; H, 4.41; N, 16.20.

5.1.10. 1-Phenyl-5H-imidazo[1,5-a]quinoxalin-4-one (5b)

This compound was prepared as described for **5a**, that is, from **4b** (1.08 g, 4.6 mmol) and carbonyldiimidazole (0.9 g, 5.51 mmol) in 56 mL of 1,2-dichlorobenzene. After cooling to room temperature, the solid was filtered and washed with diethylether to give **5b** (yield, 42%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.9 (s, 1H), 7.72 (m, 5H), 7.35 (m, 2H), 7.01 (d, 1H), 6.9 (t, 1H). Anal. calcd for $\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}$: C, 73.31; H, 4.21; N, 16.09. Found: C, 73.42; H, 4.32; N, 16.21.

5.1.11. 4-Chloro-1-methylimidazo[1,5-a]quinoxaline (6a)

A mixture of **5a** (0.9 g, 4.5 mmol) and 10 mL of phosphorus oxychloride was heated at reflux under N_2 for 5 h. The excess of POCl_3 was removed under vacuum, and the residue dissolved in 20 mL CH_2Cl_2 , washed with 5% Na_2CO_3 , dried over MgSO_4 , and purified by chromatography on silica gel using CH_2Cl_2 –MeOH (95:5, v/v). Concentration of the pure fractions provided 30% of **6a**. ^1H NMR (300 MHz, CDCl_3): δ 8.29 (dd, 1H), 7.82 (dd, 1H), 7.7 (s, 1H), 7.54 (m, 2H), 3.04 (s, 3H). Anal. calcd for $\text{C}_{11}\text{H}_8\text{N}_3\text{Cl}$: C, 60.69; H, 3.68; N, 19.31. Found: C, 60.78; H, 3.92; N, 19.06.

5.1.12. 4-Chloro-1-phenylimidazo[1,5-a]quinoxaline (6b)

6b was prepared from **5b** following the protocol described for **6a** (i.e., from **5b** (0.57 g, 2.18 mmol) and POCl_3 (5 mL)). A yellow solid was obtained after column chromatography purification as specified for **6a** (yield, 61%). ^1H NMR (300 MHz, CDCl_3): δ 8.1 (s, 1H), 7.9 (dd, 1H), 7.5 (m, 5H), 7.4 (m, 2H), 7.32 (t, 1H). Anal. calcd for $\text{C}_{16}\text{H}_{10}\text{N}_3\text{Cl}$: C, 68.69; H, 3.58; N, 15.03. Found: C, 68.51; H, 3.39; N, 15.38.

5.1.13. N-Methyl-1-methylimidazo[1,5-a]quinoxalin-4-amine (7a)

Methylamine (0.5 mL of a 40% (w/v) aqueous solution, 4 mmol) was added dropwise to a stirred solution of **6a** (0.3 g, 1.33 mmol) in absolute EtOH (7 mL) at room temperature. After 40 h, another portion of methylamine (0.5 mL of a 40% (w/v) aqueous solution, 4 mmol) was added and stirring was maintained for additional 3 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (10 mL). The organic fraction was successively washed with 5% NaHCO_3 (15 mL) and water (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography on silica gel eluting with CH_2Cl_2 –MeOH (98:2, v/v) to yield a solid cream of **7a** (yield, 53%). ^1H NMR (300 MHz, CDCl_3): δ 8.01 (d, 1H), 7.7 (d, 1H), 7.5 (s, 1H), 7.3 (t, 1H), 7.21 (t, 1H), 3.22 (d, 3H), 2.95 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3): δ 149.30, 142.34, 126.96, 126.33, 124.9, 122.85, 120.85, 119.78, 115.20, 30.84, 27.69. Anal. calcd for $\text{C}_{12}\text{H}_{12}\text{N}_4$: C, 67.92; H, 5.66; N, 26.41. Found: C, 67.52; H, 5.95; N, 26.36.

5.1.14. N-Methyl-1-phenylimidazo[1,5-a]quinoxalin-4-amine (7b)

7b was prepared from **6b** following the protocol described for **7a**, that is, from **6b** (0.4 g, 1.4 mmol) and methylamine (0.12 g of 40% (w/v), 4 mmol). A yellow solid was obtained after column chromatography purification as specified for **7a** (yield, 86%). ^1H NMR (300 MHz, CDCl_3): δ 7.6 (m, 4H), 7.56 (m, 3H), 7.2 (m, 2H), 6.93 (t, 1H), 3.22 (d, 3H). ^{13}C NMR (300 MHz, CDCl_3): δ 149.31, 144.42, 138.31, 132.53, 129.76, 129.71, 129.00, 128.77, 126.96, 126.55, 124.11, 122.37, 122.31, 120.07, 116.25, 27.73. Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4$: C, 74.45; H, 5.11; N, 20.40. Found: C, 74.10; H, 5.22; N, 20.57.

5.1.15. 5,5-Diisobutylidipyrazolo[1,5-a]piperazine-5,10-dione (8)

The 3-isobutyl-1H-pyrazole-5-carboxylic acid (0.9 g, 5.35 mmol) in suspension in thionyl chloride (7 mL) was heated to reflux, under agitation for 18 h. The reaction mixture was cooled, then filtered and washed with toluene, and dried under high vacuum to obtain a white solid (yield, 80 %). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.9 (d, 12H), 1.9 (m, 1H), 2.5 (d, 2H), 7.4 (s, 2H). Anal. calcd for $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O}_2$: C, 64.00; H, 6.67; N, 18.67. Found: C, 64.31; H, 6.33; N, 18.56.

5.1.16. 2-Fluoroaniline-3-isobutyl-1H-pyrazole-5-carboxamide (9)

To 2-fluoroaniline (0.734 mL, 7.6 mmol) in THF (8 mL), cooled in a -10°C bath, 18.3 mL (18.30 mmol, 1.0 M) sodium bis-(trimethylsilyl)amide in THF was added. The mixture was stirred for 1 h, and a suspension of **8** (1.14 g, 3.8 mmol) in THF (15 mL) was added and let warm to room temperature. The mixture was stirred for 2 h, and acetic acid was added to pH 7. The reaction mixture was concentrated in vacuum followed by the addition of water and saturated NaHCO_3 . The solid was collected by filtration, washed with water and cyclohexane, and dried under high vacuum to give **9** as a beige solid (yield, 46%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 7.85 (m, 1H), 7.25 (m, 1H), 7.15 (m, 2H), 6.5 (s, 1H), 2.46 (d, 2H), 1.88

(m, 1H), 0.85 (d, 6H). ^{19}F NMR: 126. Anal. calcd for $\text{C}_{14}\text{H}_{16}\text{N}_3\text{OF}$: C, 64.37; H, 6.13; N, 16.09. Found: C, 64.09; H, 6.01; N, 16.25.

5.1.17. 3-Isobutylpyrazolo[1,5-*a*]quinoxaline-pyrazine-6-(5*H*)-one (10)

A mixture of **9** (0.88 g, 3.37 mmol) and sodium hydride (0.97 g, 4.04 mmol) in dimethylacetamide (25 mL) was heated to reflux for 6 h. The reaction mixture was then concentrated in vacuum followed by the addition of water and saturated ammonium chloride. The solid was collected by filtration, washed with water, and dried to give **10** (yield, 80%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.9 (d, 6H), 2 (m, 1H), 3.3 (s, 2H), 6.9 (s, 1H), 7.3 (m, 4H), 8.05 (s, 1H). Anal. calcd for $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}$: C, 69.71; H, 6.22; N, 17.43. Found: C, 69.98; H, 6.51; N, 17.38.

5.1.18. 6-Chloro-3-isobutylpyrazolo[1,5-*a*]quinoxaline (11)

To **10** (1.04 g, 4.3 mmol), 12 mL of phosphorus oxychloride was added and the mixture was heated to reflux for 6 h. The reaction mixture was concentrated under vacuum, and the residue was cooled in an ice bath. Water was added to the residue and neutralized with saturated NaHCO_3 . The solid was collected by filtration and purified using column chromatography on silica gel to yield **11** as a yellow solid (yield, 50%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.9 (d, 6H), 2 (m, 1H), 3.3 (s, 2H), 6.9 (s, 1H), 7.3 (m, 4H), 8.05 (s, 1H). Anal. calcd for $\text{C}_{14}\text{H}_{14}\text{N}_3\text{OCl}$: C, 64.74; H, 5.39; N, 16.18. Found: C, 64.52; H, 5.24; N, 16.33.

5.1.19. 3-Isobutyl-*N*-methylpyrazolo[1,5-*a*]quinoxalin-4-amine (12a)

Methylamine (0.15 mL of a 40% (w/v) aqueous solution, 1.74 mmol) was added dropwise to a stirred solution of **11** (0.15 g, 0.57 mmol) in absolute EtOH (10 mL) at room temperature. After 40 h, another portion of methylamine (0.25 mL of a 40% (w/v) aqueous solution, 2.7 mmol) was added and stirring was maintained for additional 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (10 mL). The organic fraction was successively washed with 5% NaHCO_3 (15 mL) and water (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with $\text{C}_6\text{H}_{12}\text{--EtOAc}$ (70:30, v/v) as eluent to yield a cream solid (yield, 75%). Mp 145–150°C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.25 (dd, $J_1 = 1.37\text{ Hz}$, $J_2 = 7.8$, 1H), 7.7 (dd, $J_1 = 1.46$, $J_2 = 8.11$, 1H), 7.3 (m, 2H), 6.37 (s, 1H), 4.95 (s, 1H), 3.25 (d, 3H), 3.75 (d, 2H), 2.08 (m, 1H), 0.95 (d, 6H). ^{13}C NMR (300 MHz, $\text{DMSO}-d_6$): δ 152.43, 150.30, 139.7, 125.4, 125.16, 124.6, 123.7, 122.21, 112.21, 108.25, 35.10, 29.61, 28.57, 23.14. Anal. calcd for $\text{C}_{15}\text{H}_{18}\text{N}_4$: C, 70.84; H, 7.13; N, 22.03. Found: C, 70.37; H, 7.44; N, 21.99.

5.1.20. 3-Isobutyl-*N,N*-dimethylpyrazolo[1,5-*a*]quinoxalin-4-amine (12b)

12b was prepared from **11** following the protocol described for **12a**, that is, from dimethylamine (0.22 mL of a 40% (w/v) aqueous solution, 1.73 mmol) and **11** (0.15 g, 0.57 mmol) in absolute EtOH (10 mL). The product was purified by column chromatography on silica gel with $\text{C}_6\text{H}_{12}\text{--EtOAc}$ (70:30, v/v) as eluent to give a white solid (yield, 83%). Mp 160–165°C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.25 (dd, $J_1 = 1.32$, $J_2 = 7.9$, 1H), 7.65 (dd, $J_1 = 1.24$, $J_2 = 9.12$, 1H), 7.3 (m, 2H), 6.6 (s, 1H), 3.3 (s, 6H), 2.75 (d, 2H), 2.09 (m, 1H), 1 (d, 6H). ^{13}C NMR (300 MHz, $\text{DMSO}-d_6$): δ 149.79, 145.9, 140.85, 125.56, 125.42, 124.98, 124.00, 122.63, 114.26, 112.60, 39.48, 35.10, 29.61, 23.01. Anal. calcd: for $\text{C}_{16}\text{H}_{20}\text{N}_4$: C, 71.61; H, 7.51; N, 20.88. Found: C, 71.37; H, 7.62; N, 21.09.

5.1.21. Diimidazo[1,5-*a*]piperazine-5,10-dione (13)

13 was prepared from the imidazole-4-carboxylic acid (5 g, 44.6 mmol) and thionyl chloride (75 mL) following the protocol de-

scribed for **8**. We obtained 4.7 g of a white solid (yield, 90–95%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.85 (s, 2H); 8.2 (s, 2H). Anal. calcd for $\text{C}_8\text{H}_4\text{N}_4\text{O}_2$: C, 51.33; H, 2.13; N, 29.79. Found: C, 51.23; H, 2.01; N, 29.56.

5.1.22. (2-Fluoroaniline)-1*H*-imidazole-4-carboxamide (14)

14 was prepared from **13** following the protocol described for **9**, that is, from 2-fluoroaniline (5.9 g, 53 mmol) in THF (26 mL), sodium bis-(trimethylsilyl)amide (133.0 mmol, 1.0 M in THF) and **13** (4.7 g, 24.0 mmol) in THF (30 mL). We obtained 7.50 g of **14** as a light beige solid (yield, 42%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 9.45 (s, 2H), 8.13 (s, 1H), 8.1 (s, 1H), 8.03 (s, 1H), 7.8 (s, 1H), 7–7.25 (m, 2H). ^{19}F NMR: 128. Anal. calcd for $\text{C}_{10}\text{H}_8\text{N}_3\text{F}$: C, 63.49; H, 4.23; N, 22.22. Found: C, 63.28; H, 4.51; N, 22.09.

5.1.23. Imidazo[1,5-*a*]quinoxaline-pyrazine-6-(5*H*)-one (15)

A mixture of **14** (7.50 g, 36 mmol) and sodium hydride (1.1 g, 43 mmol) in dimethylacetamide (280 mL) was heated to reflux for 6 h. The reaction mixture was then concentrated under vacuum followed by the addition of water and saturated ammonium chloride. The solid was collected by filtration, washed with water, and dried to give 4 g of **15** (yield, 80%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 9.4 (s, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.31 (d, 1H), 7.1–7.3 (m, 3H). Anal. calcd for $\text{C}_{10}\text{H}_7\text{N}_3\text{O}$: C, 64.86; H, 3.78; N, 22.70. Found: C, 65.11; H, 3.87; N, 22.93.

5.1.24. 6-Chloroimidazo[1,5-*a*]quinoxaline (16)

16 was prepared from **15** following the protocol described for **11**, that is, from **15** (4 g, 21.6 mmol) and phosphorus oxychloride (44 mL). The solid was collected by filtration and purified using column chromatography on silica gel to yield **16** as a yellow solid (yield, 20%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 9.4 (s, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.31 (d, 1H), 7.1–7.3 (m, 3H). Anal. calcd for $\text{C}_{10}\text{H}_6\text{N}_3\text{Cl}$: C, 58.97; H, 2.95; N, 20.64. Found: C, 58.77; H, 2.72; N, 20.88.

5.1.25. *N*-Methylimidazo[1,5-*a*]quinoxalin-4-amine (17a)

17a was prepared from **16** following the protocol described for **12a**, that is, from methylamine 2 $\times$ (0.25 mL of a 40% (w/v) aqueous solution, 2.7 mmol), **16** (0.11 g, 0.54 mmol), and EtOH (8 mL). The product was purified by column chromatography on silica gel with $\text{C}_6\text{H}_{12}\text{--EtOAc}$ (70:30, v/v) as eluent to yield a cream solid (yield, 80–85%). Mp 255–258°C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.54 (s, 1H), 7.75 (dd, $J_1 = 1.33$, $J_2 = 8.07$, 1H), 7.65 (dd, $J_1 = 1.3$, $J_2 = 8.11$, 1H), 7.59 (s, 1H), 7.35 (s, 1H), 7.25 (s, 1H), 3.24 (d, 3H). ^{13}C NMR (300 MHz, $\text{DMSO}-d_6$): δ 147.50, 142.34, 135.54, 130.67, 129.16, 125.14, 124.34, 114.14, 111.24, 26.98. Anal. calcd for $\text{C}_{11}\text{H}_{10}\text{N}_4$: C, 66.65; H, 5.08; N, 28.26. Found: C, 66.54; H, 4.98; N, 28.15.

5.1.26. *N,N*-Dimethylimidazo[1,5-*a*]quinoxalin-4-amine (17b)

17b was prepared from **16** following the protocol described for **17a**, that is, from dimethylamine (0.15 mL of a 40% (w/v) aqueous solution, 1.19 mmol), **16** (0.11 g, 0.54 mmol), and EtOH (10 mL). The product was purified by column chromatography on silica gel with $\text{C}_6\text{H}_{12}\text{--EtOAc}$ (70:30, v/v) as eluent to give a white solid (yield, 96%). Mp 160–165°C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.55 (s, 1H), 7.78 (s, 1H), 7.71 (dd, $J_1 = 1.25$, $J_2 = 8.07$, 1H), 7.6 (dd, $J_1 = 1.21$, $J_2 = 8.12$, 1H), 7.35 (t, 1H), 7.15 (t, 1H), 3.43 (s, 6H). ^{13}C NMR (300 MHz, $\text{DMSO}-d_6$): δ 146.93, 143.22, 135.50, 129.56, 129.08, 128.29, 125.48, 124.75, 114.49, 111.16, 37.89. Anal. Calcd for $\text{C}_{12}\text{H}_{12}\text{N}_4$: C, 67.91; H, 5.70; N, 26.40. Found: C, 67.64; H, 5.48; N, 26.53.

5.2. In vitro cytotoxic activity

5.2.1. Materials and reagents

Imiquimod (Molekula, Wessex House, Shaftesbury, Dorset, UK), doxorubicin hydrochloride (Teva laboratory, Courbevoie, France),

fotemustine (Servier, Orléans, France), irinotecan (Aventis Pharma laboratories, Vitry-sur-Seine, France), methotrexate (Teva laboratory) were used in this study. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropyl alcohol, and hydrochloric acid were obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade and were obtained from commercial sources.

5.2.2. Cell lines and culture techniques

Melanoma (A375, M4Be and RPMI-7951), breast (MCF7), colon (LS174T), and B lymphoma (Raji) human cancer cell lines were obtained from American Type Culture Collection (Rockville, Md., USA). Cells were cultured in RPMI medium containing RPMI-1640 (Gibco Laboratories, France), with the exception of the M4Be line that required DMEM medium supplemented with 10% heat-inactivated (56 °C) foetal bovine serum (FBS) (Polylabo, Paris, France), 2 mM L-glutamine, 100 IU/ml penicillin G sodium, 100 µg/ml streptomycin sulphate, and 0.25 µg/ml amphotericin B. Cells were maintained in a humidified atmosphere of 5% CO₂ in air at 37 °C.

5.2.3. Cytotoxicity assay

Previously to the experiments, the number of cells by well, the doubling time and the MTT concentration have been optimized. In all the experiments, A375, M4Be, RPMI 7950, LS147T, MCF7, and Raji cells were seeded at a final concentration of 5000 cells/well in 96-well microtiter plates and allowed to attach overnight. After 20–24 h incubation, the medium was aspirated carefully from the plates using a sterile Pasteur pipette, and cells were exposed (i) to vehicle controls (1% DMSO/culture medium and culture medium alone), (ii) to doxorubicin, irinotecan, fotemustine, methotrexate, and imiquimod at concentrations of 10^{−4} to 10^{−10} µM, diluted in the culture medium, and (iii) to the synthesized compounds (10^{−4}–10^{−10} µM) dissolved in a mixture 1% DMSO/culture medium (v/v). After 96 h of incubation, 10 µl of MTT solution in PBS (5 mg/ml, phosphate-buffer saline pH 7.3) was added to each well, and the wells were incubated at 37 °C for 4 h. This colorimetric assay is based on the ability of live and metabolically unimpaired tumor-cell targets to reduce MTT to a blue formazan product. At the end of the incubation period, the supernatant was carefully aspirated, then, 100 µl of a mixture of isopropyl alcohol and 1 M hydrochloric acid (96/4, v/v) was added to each well. After 10 min of incubation and vigorous shaking to solubilize formazan crystals, the optical density was measured at 570 nm in a microculture plate reader (Dynatech MR 5000, France). For each assay, at least three experiments were performed in triplicate.

The individual cell line growth curves confirmed that all tumor lines in control medium remained in the log phase of cell growth 96 h after plating. Cell survival was expressed as percent of vehicle control. The IC₅₀ values defined as the concentrations of drugs which produced 50% cell growth inhibition; 50% reduction of absorbance, were estimated from the sigmoidal dose–response curves.

5.2.4. Xenografts and in vivo study protocol

This research adhered to the 'Principles of Laboratory Animal Care' (NIH publication #85-23, revised 1985). The animal study was approved by the local Animal Use Committee. Before to start pharmacodynamic studies in mouse, the 50% lethal dose (LD₅₀) of the EAPB0203 compound was evaluated in healthy animal after intraperitoneal (ip) administration. The LD₅₀ was higher than 300 mg/kg.

M4Be tumor cells at logarithmic growth in vitro were harvested and washed twice with PBS. Tumor cells (3 × 10⁶) suspended in 150 µl of DMEM medium without FBS were inoculated subcutaneously into the flank area of female Swiss Nude mice (7 weeks old, weighing 20–22 g) through a 23-gauge needle. Mice were pur-

chased from Charles River (L'Arbresle Cedex, France) and were kept under sterile conditions. They received sterile food and water. Tumor growth was monitored twice a week. Two perpendicular diameters (a and b) were measured with a Vernier caliper, and tumor volumes (V) were calculated using the following formula: $V = 0.5ab^2$. At a tumor size of ~0.7 cm³, athymic mice were randomized into three groups (6 mice per group): (i) the vehicle control group (weekly ip administration of DMSO/intralipid (50:50, v/v)), (ii) the EAPB0203 group (two protocols: protocol 1, weekly ip administration of 5 mg/kg for 3 weeks and protocol 2, administration of 20 mg/kg twice a week (monday and thursday) for 3 weeks, this cycle was repeated after 15 days), and (iii) the fotemustine group (two protocols: protocol 1, weekly ip administration of 5 mg/kg for 3 weeks and protocol 2, weekly ip administration of 20 mg/kg for 3 weeks, this cycle was repeated after 15 days). EAPB0203 being poorly soluble in water was dissolved in a mixture intralipid/DMSO (50:50, v/v). Fotemustine was dissolved in isotonic glucose. The dosing regimen of fotemustine is near to the fotemustine dose of 100 mg/m² used clinically. Animals were euthanized with CO₂ when the tumor size reached 2 cm³.

Tumor response was assessed by comparing the tumor volume in each mouse group (control, fotemustine, and EAPB0203).

5.2.5. Statistical analysis

In vivo results are presented as mean ± standard error of the mean (SEM). A one way ANOVA for repeated measurements was performed to compare (i) the efficacy of treatment according to the administered dose (5 and 20 mg/kg), both for fotemustine and EAPB0203; (ii) the variations of tumor volume between the three treatment groups (control, fotemustine, and EAPB0203) both at low and high administered doses. A simple contrast test was used to compare 2-by-2 each group; in this case, the level of significance was corrected for multiple comparisons and fixed at 0.02. This analysis was performed using the PK-fit software. Probability values of less than 0.05 were considered statistically significant.

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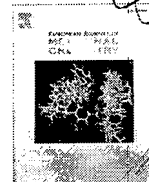
Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.05.022.

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Original article

New imidazo[1,2-*a*]quinoxaline derivatives: Synthesis and in vitro activity against human melanomaCarine Deleuze-Masquefa^{a,*}, Georges Moarbess^{a,1}, Sonia Khier^b, Nadège David^a, Stéphanie Gayraud-Paniagua^a, Françoise Bressolle^{b,c}, Frédéric Pinguet^c, Pierre-Antoine Bonnet^a^a EA 4215, Laboratoire de Chimie des Biomolécules et de l'Environnement, Pharmacochimie et Biomolécules, Université Montpellier I, Faculté de Pharmacie, 15 av. Charles Flahault, BP 14491, 34 093 Montpellier Cedex 5, France^b Pharmacocinétique Clinique, Faculté de Pharmacie, Université Montpellier I, 15 av. Charles Flahault, BP 14491, 34 093 Montpellier Cedex 5, France^c Centre Régional de Lutte contre le Cancer, Val d'Aurelle, Rue de la Croix Verte, 34000 Montpellier, France

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ABSTRACT

New imidazo[1,2-*a*]quinoxaline analogues have been synthesized in good yields via a bimolecular condensation of 2-imidazole carboxylic acid, followed by a coupling with ortho-fluoroaniline and subsequent substitution on the imidazole ring by Suzuki Cross-coupling reaction using microwave assistance. Antitumor activities of these derivatives were evaluated by growth inhibition of A375 cells in vitro. All compounds exhibited high activities compared to imiquimod and fotemustine used as references.

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1. Introduction

In our previous studies [1–3], a general synthetic strategy has been elaborated which can easily get access to three basic heterocycles imidazo/pyrazoloquinoxalines. This method led us to the intermediates which remain non-substituted on the 5-membered cycle with good yields. New imiquimod analogues in the imidazo[1,2-*a*]quinoxaline, imidazo[1,5-*a*]quinoxaline and pyrazolo[1,5-*a*]quinoxaline series have also been synthesised and tested in vitro and in vivo for their anti-cancer activities.

Imiquimod, an imidazoquinoline amine, has been initially approved for the topical treatment of external genital warts caused by human papilloma virus [4–6]. It has shown activities toward basal cell carcinomas (BCC), actinic keratoses, and some melanoma metastases [7–10]. Fotemustine is a third generation nitrosourea which is used both in melanoma and brain tumors [11]. However, its clinical application is somewhat limited both by its toxicity and by the resistance of melanoma cells [12]. BMS-345541, an imidazo[1,2-*a*]quinoxaline derivative has shown important cytotoxic activities

on melanoma in vitro and in vivo [13]. It was identified as a selective inhibitor of IκB kinase that blocked NF-κB pathway [14].

EAPB0203, a new imidazoquinoxaline amine derivative demonstrated significant in vitro activities against the human melanoma cell line A375, 110 and 45 times more active than fotemustine and imiquimod, respectively [1,2]. Tested in vivo in female Swiss Nude mice, **EAPB0203** caused a significant decrease in tumor (M4Be xenografts) growth compared to vehicle control and fotemustine treatments [1,2].

Recently, **EAPB0203** was evaluated in T-cell lymphomas and HTLV-I associated adult T-cell leukaemia/lymphoma. **EAPB0203** exhibits an important cytotoxicity in vitro on HTLV-I-infected CD4⁺ T-cell lines HuT-102, MT-2, and C91-PL as well as HTLV-I-negative malignant CD4⁺ T-cell lines CEM, Jurkat, HuT-78, and MOLT-4. The imidazoquinoxaline derivative exhibits inhibition of cell proliferation, G2/M cell cycle arrest, and induction of apoptosis in HTLV-I transformed and HTLV-I negative malignant T cells [15].

In continuation of our studies on the reactivity of nitrogen heterocycles, we focused on the preparation of imidazo[1,2-*a*]quinoxaline derivatives using the new synthetic strategy described previously [1,2]. To develop a convenient pathway in good agreement with rapid pharmacomodulation, we were interested in using the Suzuki Cross-coupling reaction. The application of this coupling to these series was reported on the bromo derivatives [16]. In this

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work, we turned our interest to study the incorporation of different arylboronic acids on the cytotoxic activity, in order to afford a variety of new derivatives of our lead compound **EAPB0203**.

2. Results

2.1. Chemistry

2.1.1. Library synthesis

For the preparation of the 1*H*-imidazo[1,2-*a*]quinoxaline derivatives, the procedure presented in Scheme 1 was used. Carbonyl-imidazole dimer **1** resulted from the bimolecular condensation of the 2-imidazole carboxylic acid, in presence of thionyl chloride in 95% overall yield. Product **1** was coupled with *ortho*-fluoroaniline to give the intermediate **2**, with a yield of 40%. The tricyclic compound **3** was obtained via an intramolecular cyclization of **2** accomplished under strong basic conditions with 80% yield. Treatment of compound **3** with phosphorus oxychloride and *N,N*-diethylaniline yielded to 80% of compound **4** which was coupled with methylamine to give compound **5** with average yields of 93% [1,3]. The bromination of compound **5** by *N*-bromosuccinimide led to compound **6**. The position of the bromine on the imidazole ring was confirmed by n.O.e study. Utilizing the appropriate arylboronic acid allowed for introduction of various groups on the imidazole ring of compound **6** via Suzuki reaction in the presence of palladium catalyst, basic conditions and under microwave assistance to give compounds **7a–7n** in good yields.

2.1.2. In vitro cytotoxic activity

All compounds were evaluated for their cytotoxic activity in vitro against A375 human melanoma cell line by comparison with imiquimod, fotemustine used as references and **EAPB0203**. Table 1 gives the IC₅₀ values for all of the imidazo[1,2-*a*]quinoxaline derivatives as determined by the microculture tetrazolium dye assay. All the tested imidazo[1,2-*a*]quinoxalines caused a significant inhibition of cell growth, their cytotoxicities were superior to that of imiquimod and fotemustine. Compounds **7d**, **7k–7n** were considerably 10–100 times less active than the present **EAPB0203** known to be 110 and 45 times more active than fotemustine and imiquimod on human melanoma cell line (A375), respectively [1,2]. Compounds **7h–7j** showed a similar activity to **EAPB0203**. Five

compounds **7b**, **7c**, **7e–7g** are 7–9 times more potent against A375 than **EAPB0203** (Table 1).

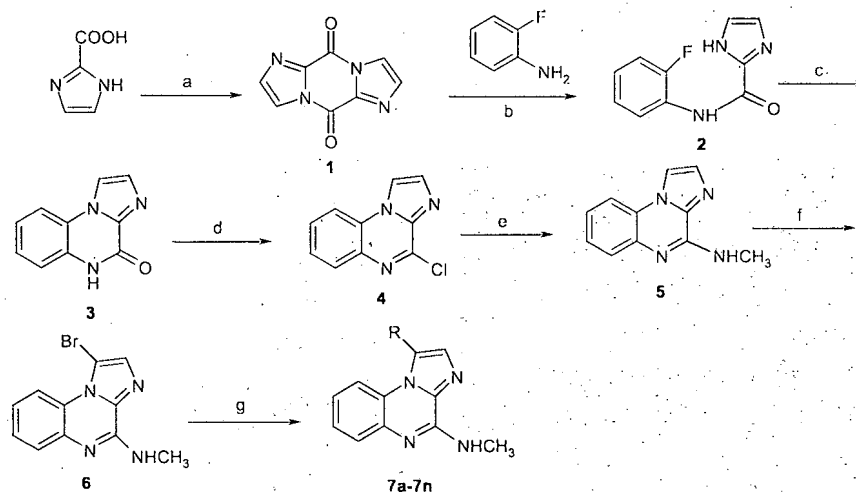
3. Discussion

The procedure presented in Scheme 1 was used to prepare the imidazo[1,2-*a*]quinoxaline derivatives. This method not only reduced the number of purification steps but also led us to propose a new common and efficient route for the preparation of the unsubstituted imidazo[1,2-*a*]quinoxalines on the imidazole core starting from the unsubstituted carboxylic acid. This new strategy also permitted the introduction of R substituent at the last steps of the procedure with different commercial Suzuki cross-coupling reagents using microwave assistance.

The exact chemical structure of these imidazo[1,2-*a*]quinoxaline derivatives and the assignments of the position of substitution on the heterocycle using ¹H and ¹³C NMR data were described previously [17].

In order to confirm the bromine position on the imidazole ring, we carried out a n.O.e study. The irradiation of H-9 at 9.10 ppm showed a correlation with only one proton H-8 at 7.5 ppm and the signal intensity of n.O.e effect was increased up to 15%. No correlation was observed between H-9 and protons of the imidazole ring. The same results were obtained by irradiation of H-2 at 7.65 ppm. These results led us to confirm that the bromine was in the position 1 of the imidazole ring.

We reported previously [1,2] that **EAPB0203** bearing phenethyl as substituent at position 1 was a lead compound with an activity against A375 cell line 110 times higher than fotemustine (IC₅₀ = 173 μM) and 45 times higher than imiquimod (IC₅₀ = 70 μM). In this paper, replacement of phenethyl (**EAPB0203**) by phenyle (compound **7a**) on the imidazole ring resulted in no change in cytotoxicity. The presence of the carbon chain (**EAPB0203**) between the phenyl and imidazole core appeared to be less interesting, the activity was not influenced by the flexibility of the substituent on R position. Introduction of an electron-donating group (compounds **7b**, **7c**, **7e**, **7f**) enhanced activity. This increase could be explained by additional interaction type hydrogen bond with the biological target. The substitution of an electron-withdrawing group on the phenyl core (compounds **7k–7m**) displayed decreased potency. The methoxy group at position 2 on the phenyl ring (compound **7d**) has proved to be less favorable than the 3 and 4 positions



Scheme 1. Synthesis of 1*H*-imidazo[1,2-*a*]quinoxaline derivatives. Reagents and conditions: (a) SOCl₂, reflux, 18 h; (b) NaHMDs, THF, 5 h; (c) NaH, DMA, reflux, 10 h; (d) POCl₃, reflux, 6 h; (e) EtOH, NHCH₃, 20 h, rt; (f) NBS, CHCl₃, reflux, 2 h; (g) arylboronic acid (R-B(OH)₂), Pd(PPh₃)₄, Na₂CO₃, DME, MW (140 °C, 20 min).

Table 1
Imidazo[1,2-*a*]quinoxaline derivatives: formula, IC₅₀ against A375 (human melanoma cell lines), pK_a and log *P* calculated values.

Rc1nc2c(c1)nc3ccccc3n2NC
imidazo[1,2-*a*]quinoxaline

Compounds	R ₁	Formula	IC ₅₀ ^a (μM)	pK _a [*]	log <i>P</i> [*]
7a	C ₆ H ₅ –	C ₁₇ H ₁₄ N ₄	2.19 ± 0.08	5.18 ± 0.4	3.68 ± 1.43
7b (EAPB0503)	3-OCH ₃ –C ₆ H ₄ –	C ₁₈ H ₁₆ N ₄ O	0.20 ± 0.09	5.10 ± 0.4	3.45 ± 1.43
7c	4-OCH ₃ –C ₆ H ₄ –	C ₁₈ H ₁₆ N ₄ O	0.37 ± 0.4	5.45 ± 0.4	3.50 ± 1.43
7d	2-OCH ₃ –C ₆ H ₄ –	C ₁₈ H ₁₆ N ₄ O	122 ± 25	4.89 ± 0.4	3.35 ± 1.43
7e	3-OC ₂ H ₅ –C ₆ H ₄ –	C ₁₉ H ₁₈ N ₄ O	0.30 ± 0.01	5.11 ± 0.4	3.98 ± 1.43
7f	3-OH–C ₆ H ₄ –	C ₁₇ H ₁₄ N ₄ O	0.56 ± 0.12	5.14 ± 0.4	2.94 ± 1.43
7g	3-Br–C ₆ H ₄ –	C ₁₇ H ₁₃ BrN ₄	0.65 ± 0.02	4.96 ± 0.4	4.43 ± 1.43
7h	3-CF ₃ –C ₆ H ₄ –	C ₁₈ H ₁₃ F ₃ N ₄	1.28 ± 0.18	4.86 ± 0.4	4.66 ± 1.43
7i	3-Cl–C ₆ H ₄ –	C ₁₇ H ₁₃ ClN ₄	1.78 ± 0.35	4.88 ± 0.4	4.25 ± 1.43
7j	3-COOH–C ₆ H ₄ –	C ₁₈ H ₁₄ N ₄ O ₂	3.47 ± 0.55	5.28 ± 0.4	3.36 ± 1.43
7k	3-F–C ₆ H ₄ –	C ₁₇ H ₁₃ F N ₄	24.9 ± 0.27	4.88 ± 0.4	3.71 ± 1.43
7l	3-CN–C ₆ H ₄ –	C ₁₈ H ₁₃ N ₅	27.0 ± 2.2	4.83 ± 0.4	3.12 ± 1.43
7m	3-NO ₂ –C ₆ H ₄ –	C ₁₇ H ₁₃ N ₅ O ₂	40.0 ± 3.9	4.57 ± 0.4	3.21 ± 1.43
7n	3-(C ₆ H ₅ O)–	C ₁₅ H ₁₂ N ₄ O	74.1 ± 3.1	5.21 ± 0.4	2.99 ± 1.43
EAPB0203	C ₆ H ₅ –(CH ₂) ₂ –	C ₁₉ H ₁₈ N ₄	1.57 ± 0.56	5.61 ± 0.4	4.91 ± 1.42
Imiquimod			70.3 ± 4.3		
Fotemustine			173 ± 24		

^{*}pK_a and log *P* values are calculated using the ACDlabs software.
^a IC₅₀: concentration of the compound (μM) producing 50% cell growth inhibition after 96 h of drug exposure, as determined by the MIT assay. Each experiment was run at least three times, and the results are presented as average values ± standard deviation.

(compounds **7b**, **7c**). No significant differences were observed between the pK_a and log *P* calculated values (Table 1) of this series of compounds which are all substituted by an amino group.

In summary, a new series of imidazo[1,2-*a*]quinoxaline derivatives were synthesized with short reaction times by using microwave assistance and catalysts. These compounds were evaluated for their antitumor activities against human melanoma. These new analogues demonstrated interesting antitumor activities effect on A375 cell lines more potent than imiquimod and fotemustine. We found promising lead compounds (**7b**, **7c**, **7e**, **7f**, **7g**), **EAPB0203** analogues, which exhibited high activities up to 9 times more potent for compound **7b** (**EAPB0503**) than **EAPB0203** (lead compound) on human melanoma cell lines. **EAPB0503** can be considered as a new lead compound of the imidazo[1,2-*a*]quinoxaline series. Further studies are now in progress for the optimization of these compounds. On the other hand, in vivo studies will be complemented and documented to depict the antitumor activities of this new lead (**EAPB0503**) in female Swiss mice xenografted with human melanoma cell lines.

4. Experimental

4.1. Chemistry

All solvents and reagents were obtained from commercial sources and used without further purification unless indicated otherwise. ¹H and ¹³C NMR spectra were recorded using a Bruker AC 300 spectrometer. Chemical shifts are reported in parts per million (ppm) from the tetramethylsilane resonance in the indicated solvent. Coupling constants are reported in Hertz (Hz), spectral splitting partners are designed as follow: singlet (s); doublet (d); triplet (t); multiplet (m). Column chromatography was performed on Merck silica gel 60 (200–400 mesh). Elemental analysis was carried out at the Microanalytical Central Department (Montpellier, France). The microwave organic synthesis was assisted by the Biotage initiator (France). Melting points of solid compounds were measured by the Biocote

apparatus. pK_a and log *P* values are calculated using the ACDLabs software (Toronto, Canada).

4.1.1. Diimidazo[1,2-*a*]piperazine-5,10-dione (**1**)

The imidazole-2-carboxylic acid (5 g, 44.6 mmol) in suspension in thionyl chloride (75 mL) was heated to reflux, under agitation for 18 h. The reaction mixture was cooled, then filtered, washed with toluene, and dried under high vacuum to obtain a yellow solid (yield, 90–95%). M.p.: 164 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 8.85 (s, 2H); 8.2 (s, 2H). ¹³C NMR (300 MHz, DMSO-*d*₆) δ: 145.85, 144.95, 125.20, 120.15. Anal. calcd for C₈H₄N₄O₂: C, 51.07; H, 2.14; N, 29.78. Found: C, 51.15; H, 2.21; N, 29.46.

4.1.2. (2-Fluoroaniline)-1H-imidazole-4-carboxamide (**2**)

To 2-fluoroaniline (1.91 mL, 19.77 mmol) in THF (13 mL), cooled in a –10 °C bath, (45.2 mL, 45.2 mmol, 1.0 M) sodium bis-(trimethylsilyl)amide in THF was added. The mixture was stirred for 1 h, and a suspension of **1** (1.77 g, 9.4 mmol) in THF (20 mL) was added and allowed to warm to room temperature. The mixture was stirred for 2 h, and acetic acid was added to pH 7. The reaction mixture was concentrated in vacuum followed by the addition of water and saturated NaHCO₃. The solid was collected by filtration, washed with water and cyclohexane, and dried under high vacuum to give **2** as a beige solid (yield, 40%). M.p.: 250 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 8.12 (dd, 1H), 7.92 (dd, 1H), 6.92 (t, 1H), 7.13 (t, 1H), 7.07 (s, 2H). ¹⁹F NMR: 126. ¹³C NMR (300 MHz, DMSO-*d*₆) δ: 151.69, 150.92, 150.52, 130.14, 128.79, 126.96, 123.97, 121.03, 119.53, 115.03. Anal. calcd for C₁₀H₈FN₃O: C, 58.54; H, 3.93; N, 20.48C, 64.37; H, 6.13; N, 16.09. Found: C, 58.33; H, 3.55; N, 20.14.

4.1.3. Imidazo[1,2-*a*]quinoxaline-6-(5H)-one (**3**)

A mixture of **2** (2 g, 9.75 mmol) and sodium hydride (0.3 g, 1.17 mmol) in dimethylacetamide (80 mL) was heated to reflux for 6 h. The reaction mixture was then concentrated in vacuum followed by the addition of water and saturated ammonium chloride. The solid was collected by filtration, washed with water, and dried to give **3** (yield, 80%). M.p.: 322 °C. ¹H NMR (300 MHz, DMSO-*d*₆)

δ : 9.4 (s, 1H), 8.2 (d, 1H), 7.8 (d, 1H), 7.31 (d, 1H), 7.1–7.3 (m, 3H). ^{13}C NMR (300 MHz, DMSO- d_6) δ : 149.26, 139.11, 137.89, 132.43, 126.43, 123.94, 123.02, 119.40, 118.17, 115.42. Anal. calcd for $\text{C}_{10}\text{H}_7\text{N}_3\text{O}$: C, 64.86; H, 3.81; N, 22.69. Found: C, 64.44; H, 4.03; N, 22.98.

4.1.4. 6-Chloroimidazo[1,2-a]quinoxaline (4)

To **3** (1.42 g, 7.7 mmol), phosphorus oxychloride (10 mL) and *N,N*-diethylaniline (1.6 mL) were added and the mixture was heated to reflux for 2 h. The reaction mixture was concentrated under vacuum, and the residue was cooled in an ice bath. Water was added to the residue and neutralized with saturated NaHCO_3 . The solid was collected by filtration and purified by recrystallisation from methanol to yield **4** as a white solid (yield, 80%). M.p.: 188 °C. ^1H NMR (300 MHz, DMSO- d_6) δ : 8.5 (s, 1H), 8.06 (d, 1H), 7.56 (s, 1H), 7.35 (m, 1H), 7.25 (m, 2H). ^{13}C NMR (300 MHz, DMSO- d_6) δ : 135.82, 132.19, 131.75, 130.4, 129.95, 127.12, 125.03, 115.67, 113.4. Anal. calcd for $\text{C}_{10}\text{H}_6\text{N}_3\text{Cl}$: C, 58.98; H, 2.97; N, 20.64. Found: C, 59.12; H, 2.76; N, 20.45.

4.1.5. *N*-Methylimidazo[1,2-a]quinoxalin-4-amine (5)

Methylamine of a 40% (w/v) aqueous solution, was added dropwise to a stirred solution of **4** (0.110 g, 0.54 mmol) in absolute EtOH (10 mL) at room temperature. After 40 h, another portion of methylamine (0.15 mL, 1.74 mmol) of a 40% (w/v) aqueous solution was added and stirring was maintained for additional 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (10 mL). The organic fraction was successively washed with 5% NaHCO_3 (15 mL) and water (15 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with C_6H_{12} –EtOAc (70:30, v/v) as eluent to yield a white solid (yield, 93%). M.p.: 180 °C. ^1H NMR (300 MHz, DMSO- d_6) δ : 7.92 (s, 1H), 7.75 (dd, 1H), 7.65 (dd, 1H), 7.52 (s, 1H), 7.4 (t, 1H), 7.25 (t, 1H), 6.15 (s, 1H), 3.25 (d, 3H). ^{13}C NMR (300 MHz, DMSO- d_6) δ : 142.25, 139.17, 131.99, 129.4, 128.17, 127.2, 125.14, 124.34, 114.14, 113.89, 29.20. Anal. calcd for $\text{C}_{11}\text{H}_{10}\text{N}_4$: C, 66.65; H, 5.08; N, 28.26. Found: C, 66.26; H, 5.53; N, 28.23.

4.1.6. 1-Bromo-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (6)

A solution of **5** (1.5 g, 7.5 mmol) and *N*-bromosuccinimide (1.5 g, 7.5 mmol) in CHCl_3 (200 mL) was heated under reflux for 2 h. The resulting solution was cooled, washed with 5% sodium hydrogen carbonate (50 mL), dried and concentrated. The residue was purified by recrystallisation from chloroform to a white solid (1.18 g, 67%). M.p.: 202 °C. ^1H NMR (300 MHz, DMSO- d_6) δ : 9.10 (s, 1H), 7.75 (dd, 1H), 7.65 (dd, 1H), 7.4 (t, 1H), 7.25 (t, 1H), 6.15 (s, 1H), 3.25 (d, 3H). ^{13}C NMR (300 MHz, DMSO- d_6) δ : 141.97, 140.54, 138.85, 128.96, 127.78, 127.09, 126.03, 125.13, 113.86, 101.4, 29.20. Anal. calcd for $\text{C}_{11}\text{H}_9\text{N}_4\text{Br}$: C, 47.68; H, 3.27; N, 20.22. Found: C, 47.33; H, 3.53; N, 20.53.

4.2. General procedure for the Suzuki reaction

To a mixture of **6** (300 mg, 1.08 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (63 mg, 0.05 mmol) in DME (15 mL) was added the corresponding aryl boronic acid followed by the addition of sodium carbonate (234 mg) in water (5 mL). The reaction was irradiated in a sealed tube at 140 °C for 20 min using a Biotage Initiator microwave synthesizer. The reaction mixture was poured on water and then extracted with dichloromethane (2 $\times$ 40 mL). The combined organic extracts were washed with water (40 mL), dried and concentrated to dryness under vacuo. The crude product was purified by column chromatography (silica gel eluting with dichloromethane).

4.2.1. *N*-Methyl-1-phenylimidazo[1,2-a]quinoxalin-4-amine (7a)

Phenylboronic acid (260 mg, 2.13 mmol). White solid (90%). M.p.: 186 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.15 (s, 1H), 7.99 (dd, 1H), 7.52 (m, 8H), 5.7 (s, 1H), 3.01 (d, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 142.86, 139.37, 131.13, 130.74, 129.67, 129.02, 126.77, 125.75, 124.90, 124.78, 124.47, 121.12, 114.96, 29.20. Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4$: C, 74.43; H, 5.14; N, 20.42. Found: C, 74.15; H, 5.46; N, 20.09.

4.2.2. 1-(3-Methoxyphenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7b) (EAPB0503)

3-Methoxyphenylboronic acid (329 mg, 2.16 mmol). White solid (90%). M.p.: 168 °C. ^1H NMR (300 MHz, CDCl_3) δ : 9.04 (s, 1H), 8 (dd, 1H), 7.82 (m, 2H), 7.4 (m, 3H), 6.99 (d, 1H), 6.86 (s, 1H), 5.7 (s, 1H), 3.83 (s, 3H), 2.88 (d, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 160.98, 142.86, 139.57, 131.13, 130.56, 129.5, 129.02, 128.4, 127.39, 124.9, 124.40, 122.10, 118.02, 114.96, 111.87, 108.08, 55.30, 29.20. Anal. calcd for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}$: C, 71.04; H, 5.30; N, 18.41. Found: C, 71.40; H, 5.66; N, 18.09.

4.2.3. 1-(4-Methoxyphenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7c)

4-Methoxyphenylboronic acid (329 mg, 2.16 mmol). White solid (95%). M.p.: 198 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.14 (s, 1H), 8.00 (d, 1H), 7.67 (m, 3H), 7.5 (m, 2H), 7.05 (m, 2H), 5.77 (s, 1H), 3.82 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 158.05, 142.86, 139.57, 131.13, 129.61, 129.02, 125.96, 125.68, 124.47, 124.02, 121.12, 114.96, 113.35, 57.83, 29.20. Anal. calcd for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}$: C, 71.04; H, 5.30; N, 18.41. Found: C, 71.18; H, 5.58; N, 18.13.

4.2.4. 1-(2-Methoxyphenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7d)

2-Methoxyphenylboronic acid (329 mg, 2.16 mmol). Beige solid (94%). M.p.: 180 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.1 (d, 1H), 7.60 (m, 3H), 7.48 (t, 1H), 7.30 (m, 4H), 5.74 (s, 1H), 3.85 (s, 3H), 3.00 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 153.81, 142.74, 139.57, 131.64, 131.13, 129.02, 128.44, 127.58, 124.45, 124.28, 121.37, 118.02, 114.86, 11.98, 55.32, 29.20. Anal. calcd for $\text{C}_{18}\text{H}_{16}\text{N}_4\text{O}$: C, 71.04; H, 5.30; N, 18.41. Found: C, 70.98; H, 5.12; N, 18.22.

4.2.5. 1-(3-Ethoxyphenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7e)

3-Ethoxyphenylboronic acid (360 mg, 2.17 mmol). White solid (52%). M.p.: 162 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.34 (s, 1H), 8.02 (d, 1H), 7.83 (d, 1H), 7.67 (t, 1H), 7.5 (m, 2H), 6.96 (d, 1H), 6.85 (s, 1H), 5.74 (s, 1H), 3.89 (m, 2H), 2.89 (s, 3H), 1.38 (t, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 161.36, 142.86, 139.57, 131.13, 130.22, 129.5, 129.02, 128.41, 127.05, 124.9, 122.1, 118.02, 114.96, 113.77, 108.12, 63.8, 29.2, 15.2. Anal. calcd for $\text{C}_{19}\text{H}_{18}\text{N}_4\text{O}$: C, 71.68; H, 5.70; N, 17.60. Found: C, 71.43; H, 5.56; N, 17.39.

4.2.6. 1-(3-Hydroxyphenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7f)

3-Hydroxyphenylboronic acid (329 mg, 2.16 mmol). White solid (85%). M.p.: 278 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.33 (s, 1H), 7.99 (d, 1H), 7.79 (d, 1H), 7.70 (m, 2H), 7.67 (t, 1H), 7.50 (m, 3H), 6.857 (m, 2H), 3.02 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 160.07, 142.86, 139.57, 131.46, 131.13, 130.35, 129.63, 129.02, 127.64, 126.78, 125.39, 124.9, 124.40, 122.83, 120.71, 114.86, 114.54, 29.02. Anal. calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}$: C, 70.33; H, 4.86; N, 19.30. Found: C, 70.49; H, 5.19; N, 19.09.

4.2.7. 1-(3-Bromophenyl)-*N*-methylimidazo[1,2-a]quinoxalin-4-amine (7g)

3-Bromophenylboronic acid (435 mg, 2.16 mmol). Yellow solid (78%). M.p.: 144 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.18 (s, 1H), 7.90

(m, 2H), 7.67 (t, 1H), 7.50 (m, 2H), 7.45 (m, 3H), 5.74 (s, 1H), 2.98 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 142.86, 139.57, 132.86, 131.13, 130.53, 129.63, 129.03, 127.64, 126.78, 125.39, 124.90, 124.30, 122.93, 120.71, 114.96, 29.20. Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{BrN}_4$: C, 57.81; H, 3.71; N, 15.86. Found: C, 58.05; H, 3.55; N, 15.99.

4.2.8. 1-(3-Trifluoromethylphenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7h)

3-Trifluoromethylphenylboronic acid (411 mg, 2.16 mmol). White solid (98%). M.p.: 210 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.14 (s, 1H), 8.10 (s, 1H), 8.02 (m, 2H), 7.85 (d, 1H), 7.60 (m, 2H), 7.48 (t, 1H), 5.74 (s, 1H), 2.96 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 142.86, 139.57, 132.62, 132.30, 132.02, 131.60, 131.13, 129.02, 128.86, 124.90, 124.47, 122.51, 121.59, 120.40, 114.86, 114.54, 29.02. Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{N}_4\text{F}_3$: C, 63.16; H, 3.83; N, 16.65. Found: C, 63.03; H, 3.76; N, 16.74.

4.2.9. 1-(3-Chlorophenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7i)

3-Chlorophenylboronic acid (339 mg, 2.16 mmol). Yellow solid (63%). M.p.: 188 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.48 (s, 1H), 8.02 (d, 1H), 7.67 (t, 1H), 7.58 (m, 2H), 7.49 (m, 3H), 7.10 (s, 1H), 5.74 (s, 1H), 2.81 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 142.86, 139.57, 135.86, 131.13, 130.50, 130.16, 129.76, 129.36, 129.02, 126.68, 124.9, 124.46, 123.39, 122.96, 121.74, 114.96, 29.15. Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{ClN}_4$: C, 66.13; H, 4.24; N, 11.48. Found: C, 66.35; H, 4.01; N, 11.25.

4.2.10. 1-(3-Carboxyphenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7j)

3-Carboxyphenylboronic acid (357 mg, 2.16 mmol). Orange solid (87%). M.p.: 130 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.41 (d, 1H), 8.26 (s, 1H), 8.01 (m, 2H), 7.80 (m, 4H), 7.58 (m, 2H), 7.47 (t, 1H), 2.95 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 167.35, 142.86, 139.54, 131.88, 131.13, 130.34, 129.02, 128.76, 127.1, 126.72, 124.9, 124.47, 122.51, 122.38, 114.96, 29.20. Anal. calcd for $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_2$: C, 67.92; H, 4.43; N, 17.60. Found: C, 67.72; H, 4.35; N, 17.63.

4.2.11. 1-(3-Fluorophenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7k)

3-Fluorophenylboronic acid (303 mg, 2.16 mmol). Yellow solid (66%). M.p.: 220 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.22 (s, 1H), 7.98 (d, 1H), 7.59 (m, 3H), 7.48 (m, 2H), 7.22 (s, 1H), 7.03 (d, 1H), 5.74 (s, 1H), 2.96 (s, 1H). ^{13}C NMR (300 MHz, CDCl_3) δ : 163.95, 142.86, 139.57, 132.57, 132.15, 131.13, 129.02, 128.63, 126.77, 124.90, 124.47, 121.59, 115.29, 114.96, 111.90, 111.36, 29.20. Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{FN}_4$: C, 69.85; H, 4.48; N, 19.17. Found: C, 69.79; H, 4.48; N, 18.97.

4.2.12. 1-(3-Cyanophenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7l)

3-Cyanophenylboronic acid (351 mg, 2.16 mmol). Beige solid (84%). M.p.: 249 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.41 (s, 1H), 8.13 (d, 1H), 8.00 (d, 1H), 7.77 (d, 1H), 7.64 (m, 3H), 7.51 (m, 2H), 5.77 (s, 1H), 2.76 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 142.86, 139.57, 132.25, 131.64, 131.13, 130.04, 129.02, 128.70, 128.67, 127.96, 127.41, 124.90, 124.47, 119.70, 114.96, 113.80, 29.20. Anal. calcd for $\text{C}_{18}\text{H}_{13}\text{N}_5$: C, 72.23; H, 4.38; N, 23.40. Found: C, 72.01; H, 4.71; N, 23.25.

4.2.13. 1-(3-Nitrophenyl)-N-methylimidazo[1,2-a]quinoxalin-4-amine (7m)

3-Nitrophenylboronic acid (399 mg, 2.16 mmol). Yellow solid (95%). M.p.: 236 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.21 (s, 1H), 8.01 (d, 1H), 7.70 (m, 2H), 7.58 (d, 1H), 7.40 (m, 2H), 7.17 (m, 2H), 5.17 (s, 1H), 3.01 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 147.62, 142.86,

139.57, 131.19, 131.13, 129.02, 127.32, 127.16, 126.99, 126.43, 124.90, 124.66, 124.40, 122.75, 120.76, 114.96, 29.20. Anal. calcd for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{O}_2$: C, 63.94; H, 4.10; N, 21.93. Found: C, 64.13; H, 3.89; N, 21.66.

4.2.14. 1-(3-Furan-N-methylimidazo[1,2-a]quinoxalin-4-amine (7n)

3-Furanboronic acid (243 mg, 2.16 mmol). White solid (96%). M.p.: 190 °C. ^1H NMR (300 MHz, CDCl_3) δ : 8.24 (s, 1H), 7.90 (m, 3H), 7.83 (s, 1H), 7.62 (t, 1H), 7.50 (m, 2H), 7.12 (m, 2H), 6.98 (s, 1H), 5.74 (s, 1H), 4.85 (d, 1H), 2.85 (s, 3H). ^{13}C NMR (300 MHz, CDCl_3) δ : 141.50, 137.73, 135.60, 130.13, 128.79, 128.02, 124.24, 123.70, 117.85, 116.09, 113.61, 107.72, 94.65, 29.20. Anal. calcd for $\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}$: C, 68.17; H, 4.58; N, 21.20. Found: C, 68.20; H, 4.56; N, 21.17.

4.3. Cell lines and culture techniques

Melanoma (A375) human cancer cell lines were obtained from American Type Culture Collection (Rockville, Md., USA). Cells were cultured in RPMI medium containing RPMI-1640 (Gibco Laboratories, France), 10% heat-inactivated (56 °C) foetal bovine serum (FBS) (Polylabo, Paris, France), 2 mM L-glutamine, 100 IU/ml penicillin G sodium, 100 $\mu\text{g}/\text{ml}$ streptomycin sulphate, and 0.25 $\mu\text{g}/\text{ml}$ amphotericin B. Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C.

4.4. In vitro cytotoxicity assay

Previously to the experiments, the number of cells by well, the doubling time and the MTT concentration have been optimized. In all the experiments, A375 cells were seeded at a final concentration of 5000 cells/well in 96-well microtiter plates and allowed to attach overnight. After 20–24 h incubation, the medium was aspirated carefully from the plates using a sterile Pasteur pipette, and cells were exposed (i) to vehicle controls (1% DMSO/culture medium and culture medium alone), (ii) fotemustine and imiquimod at concentrations of 10^{-4} – 10^{-10} μM , diluted in the culture medium, and (iii) to the synthesized compounds (10^{-4} – 10^{-10} μM) dissolved in a mixture 1% DMSO/culture medium (v/v). After 96 h of incubation, 10 μl of MTT solution in PBS (5 mg/ml, phosphate-buffer saline pH 7.3) was added to each well, and the wells were incubated at 37 °C for 4 h. This colorimetric assay is based on the ability of live and metabolically unimpaired tumor-cell targets to reduce MTT to a blue formazan product. At the end of the incubation period, the supernatant was carefully aspirated, then, 100 μl of a mixture of isopropyl alcohol and 1 M hydrochloric acid (96/4, v/v) was added to each well. After 10 min of incubation and vigorous shaking to solubilize formazan crystals, the optical density was measured at 570 nm in a microculture plate reader (Dynatech MR 5000, France). For each assay, at least three experiments were performed in triplicate.

The individual cell line growth curves confirmed that all A375 lines in control medium remained in the log phase of cell growth 96 h after plating. Cell survival was expressed as percent of vehicle control. The IC_{50} values defined as the concentrations of drugs which produced 50% cell growth inhibition; 50% reduction of absorbance, were estimated from the sigmoidal dose–response curves.

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