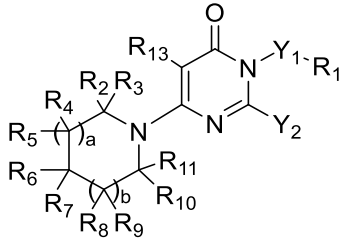
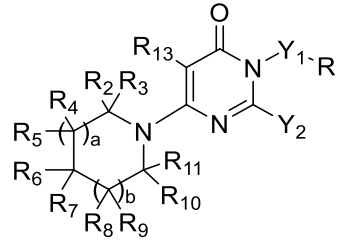


	Espacio reservado para el adhesivo de radicación
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DIRECCIÓN DE NUEVAS CREACIONES
Procedimiento acelerado de exámenes de patente (PPH)

1	NÚMERO DE SOLICITUD ANTE LA SIC NC2021/0003074		
2	GLOBAL - PPH ☐ PAÍS	3	PCT – PPH ☐ PAÍS
4	PROSUR ☐ PAÍS	5	Alianza del Pacífico (AdP) ☐ PAÍS
6	OFICINA DE PRIMERA PRESENTACIÓN (OPP) ☐	7	OFICINA DE EXAMEN ANTERIOR (OEA) ☒
8	NÚMERO DE SOLICITUD O PUBLICACIÓN DE LA SOLICITUD (OPP) ☐ NÚMERO DE SOLICITUD EN OFICINA DE EXAMEN ANTERIOR (OEA): ☒ NÚMERO DE SOLICITUD PCT: ☐		1. 16/536,923
9	☐ REPRESENTANTE LEGAL ☒ APODERADO		
APELLIDOS DAZA MONTALVO		NOMBRES CAROLINA MERCEDES	IDENTIFICACIÓN C.C. 66783790 T.P. 141563 CSJ
DIRECCIÓN	Carrera 11 No. 86-53 Piso 3		No. TELÉFONO 6181088
CIUDAD	Bogotá		CORREO ELECTRÓNICO notificaciones@clarke modet.com.co
PAÍS	Colombia		No. RADICACIÓN O PROTOCOLO DE PODER GENERAL
10	CONDICIONES PARA ACCEDER A ESTE PROCESO:		
10.1. DATOS DE PRIORIDAD ☒ SI ☐ NO			
(33) PAÍS DE ORIGEN 1. ESTADOS UNIDOS DE AMÉRICA 2. ESTADOS UNIDOS DE AMÉRICA		CÓDIGO PAÍS US US	(31) NÚMERO 62/717,588 62/773,921 (32) FECHA (AAAA/MM/DD) 2018/08/10 2018/11/30
10.2. DATOS SOLICITUD INTERNACIONAL PCT ☒ SI ☐ NO			
Solicitud Internacional No. PCT/US2019/045903		Fecha 2019/08/09	
Publicación Internacional No. WO/2020/033828		Fecha 2020/02/13	
* El solicitante presenta una copia de la(s) reivindicación(es) patentable(s) u otorgable(s) de la solicitud de la Oficina de Primera Presentación que profirió el examen de patentabilidad, junto con una traducción al castellano. ☒ SI ☐ NO			
* El solicitante presenta los resultados de búsqueda de la Oficina de Examen Anterior, junto con una traducción al castellano. ☒ SI ☐ NO			
REQUISITOS PARA EL RECONOCIMIENTO DE LOS RESULTADOS DE EXÁMENES DE PATENTABILIDAD EFECTUADOS A SOLICITUDES EXTRANJERAS			
Para que la Superintendencia de Industria y Comercio pueda reconocer los resultados de exámenes de patentabilidad producidos por otras Oficinas de Propiedad Industrial se debe cumplir con las siguientes condiciones:			
(a) Al momento de solicitar el examen de patentabilidad, si el solicitante pide que se reconozcan exámenes de patentabilidad de otras oficinas extranjeras, deberá aportar junto con el pago de la tasa correspondiente, los siguientes anexos:			
(i) Copia de las resoluciones, fallos o decisiones que han sido relevantes para la determinación de la patentabilidad de la(s) solicitud(es) en la Oficina que haya proferido el examen de patentabilidad que contienen reivindicaciones patentables u otorgables que son la base de la petición, y si es del caso, su traducción al castellano. ☒ SI ☐ NO			
(ii) Copia de todas las reivindicaciones determinadas como patentables u otorgables en la Oficina que profirió el examen de patentabilidad y, si es del caso, su traducción al castellano. ☒ SI ☐ NO			
(iii) Copia de los antecedentes, información o documentos citados por el examinador de la Oficina de Primera Presentación del examen de patentabilidad, en las resoluciones, fallos o decisiones correspondientes, incluyendo la literatura no patente. Los documentos de patente deberán presentarse siempre y cuando no estén disponibles para la Superintendencia de Industria y Comercio mediante el simple acceso a bases de datos gratuitas. ☒ SI ☐ NO			
(iv) Una tabla de correspondencias de las reivindicaciones que han sido determinadas como patentables u otorgables en la solicitud de la Oficina que haya proferido el examen de patentabilidad. ☒ SI ☐ NO			
11	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)		

10 (iv)	TABLA DE CORRESPONDENCIA DE REIVINDICACIONES		
Reivindicaciones en la solicitud en Colombia (NOTA. LAS REIVINDICACIONES ACÁ INDICADAS CORRESPONDEN A AQUELLAS MODIFICADAS EN MODIFICACIÓN VOLUNTARIA PRESENTADA EL 10 DE DICIEMBRE DEL 2021)		Reivindicaciones otorgables en la Solicitud de Estados Unidos No. 16/536,923 que corresponde a la Patente US No. 11,104,675	Explicación sobre la suficiente correspondencia ó Comentarios que explican la correspondencia
1. un compuesto representado por la Fórmula I  (I) o una salde este, donde: el subíndice a es 0 o 1 el subíndice b es 0 o 1; Y₁ es un enlace directo o CR₁₇R₁₈; Y₂ se selecciona del grupo que consiste en alquiloC₁₋₄, amino, alquiloC₁₋₄C(O)O-, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; R₁ se selecciona del grupo que consiste en ariloC₆₋₁₀, cycloalquiloC₃₋₈, cicloalqueniloC₃₋₈, y un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos como vértices		2. un compuesto representado por la Fórmula I  (I) o una salde este, donde: el subíndice a es 0 o 1 el subíndice b es 0 o 1; Y₁ es un enlace directo o CR₁₇R₁₈; Y₂ se selecciona del grupo que consiste en alquiloC₁₋₄, amino, alquiloC₁₋₄C(O)O-, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; R₁ se selecciona del grupo que consiste en ariloC₆₋₁₀, cycloalquiloC₃₋₈, cicloalqueniloC₃₋₈, y un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos como vértices	<i>Igual</i>

del anillo que se seleccionan independientemente de N, C(O), O y S; dicho arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan independientemente del grupo que consiste en halo, hidroxí, amino, alquilaminoC₁₋₄, di(alquiloC₁₋₄)amino, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄, cicloalquiloC₃₋₈, cicloalquenoC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄; R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈; R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxí, cicloalquiloC₃₋₈, halo, y alquilaminoC₁₋₄;	del anillo que se seleccionan independientemente de N, C(O), O y S; dicho arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan independientemente del grupo que consiste en halo, hidroxí, amino, alquilaminoC₁₋₄, di(alquiloC₁₋₄)amino, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄, cicloalquiloC₃₋₈, cicloalquenoC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄; R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈; R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxí, cicloalquiloC₃₋₈, halo, y alquilaminoC₁₋₄;	
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R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄; R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxí, o se selecciona del grupo que consiste en alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m; el subíndice m es 0, 1 o 2; dicho anillo saturado o insaturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos seleccionados independientemente del grupo que	R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄; R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxí, o se selecciona del grupo que consiste en alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m; el subíndice m es 0, 1 o 2; dicho anillo saturado o insaturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos seleccionados independientemente del grupo que	
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consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se eligen de N, O y S; dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos; R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(C H₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P,	consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se eligen de N, O y S; dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos; R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(C H₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P,	
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heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH, -NH₂, -OR₂₁, halo, ciano, y oxo; R₁₄, R₁₅ y R₁₆ se seleccionan cada uno independientemente del grupo que consiste en hidrógeno, alquilo C₁₋₄, cicloalquilo C₃₋₈, arilo C₆₋₁₀ y heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del grupo que consiste en amido, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, haloalcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄;	heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH, -NH₂, -OR₂₁, halo, ciano, y oxo; R₁₄, R₁₅ y R₁₆ se seleccionan cada uno independientemente del grupo que consiste en hidrógeno, alquilo C₁₋₄, cicloalquilo C₃₋₈, arilo C₆₋₁₀ y heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del grupo que consiste en amido, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, haloalcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄;	
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cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y CF₃; cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₆, haloalquiloC₁₋₆, hidroxialquiloC₁₋₆, alqueniloC₂₋₆, alquiniloC₂₋₆ y cicloalquiloC₃₋₆; y cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆ y cicloalquilo C₃₋₆.	cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y CF₃; cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₆, haloalquiloC₁₋₆, hidroxialquiloC₁₋₆, alqueniloC₂₋₆, alquiniloC₂₋₆ y cicloalquiloC₃₋₆; y cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆ y cicloalquilo C₃₋₆.	
3. El compuesto de la reivindicación 1, donde: el subíndice a es 0 o 1 el subíndice b es 0 o 1; Y₁ es un enlace directo o CR₁₇R₁₈; Y₂ se selecciona del grupo que consiste en alquiloC₁₋₄, amino, alquiloC₁₋₄C(O)O-,	4. El compuesto de la reivindicación 1, donde: el subíndice a es 0 o 1 el subíndice b es 0 o 1; Y₁ es un enlace directo o CR₁₇R₁₈; Y₂ se selecciona del grupo que consiste en alquiloC₁₋₄, amino, alquiloC₁₋₄C(O)O-,	<i>Igual</i>

alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; R₁ se selecciona del grupo que consiste en ariloC₆₋₁₀, cycloalquiloC₃₋₈, cicloalqueniloC₃₋₈, y un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S; dicho arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan independientemente del grupo que consiste en halo, hidroxilo, amino, alquilaminoC₁₋₄, di(alquiloC₁₋₄)amino, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄, cicloalquiloC₃₋₈, cicloalqueniloC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄; R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈;	alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; R₁ se selecciona del grupo que consiste en ariloC₆₋₁₀, cycloalquiloC₃₋₈, cicloalqueniloC₃₋₈, y un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S; dicho arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan independientemente del grupo que consiste en halo, hidroxilo, amino, alquilaminoC₁₋₄, di(alquiloC₁₋₄)amino, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄, cicloalquiloC₃₋₈, cicloalqueniloC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄; R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈;	
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R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxí, cicloalquiloC₃₋₈, halo, y alquilaminoC₁₋₄; R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄; R₇ se selecciona del grupo que consiste en hidrógeno, halo e hidroxí, o se selecciona del grupo que consiste en amido, alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o	R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxí, cicloalquiloC₃₋₈, halo, y alquilaminoC₁₋₄; R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄; R₇ se selecciona del grupo que consiste en hidrógeno, halo e hidroxí, o se selecciona del grupo que consiste en amido, alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxí, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o	
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grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m; el subíndice m es 0, 1 o 2; dicho anillo saturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxilo, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se seleccionan de N, O y S; dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos; R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉,	grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m; el subíndice m es 0, 1 o 2; dicho anillo saturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxilo, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄; dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se seleccionan de N, O y S; dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos; R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉,	
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-NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(C H₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P, heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH, -NH₂, -OR₂₁, halo, ciano, y oxo; cada R₁₄, R₁₅ y R₁₆ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄, cicloalquiloC₃₋₈, ariloC₆₋₁₀ y heteroarilo de 5 a 10 miembros, cualquiera de los cuales se sustituye opcionalmente por uno o más	-NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(C H₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P, heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH, -NH₂, -OR₂₁, halo, ciano, y oxo; cada R₁₄, R₁₅ y R₁₆ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄, cicloalquiloC₃₋₈, ariloC₆₋₁₀ y heteroarilo de 5 a 10 miembros, cualquiera de los cuales se sustituye opcionalmente por uno o más	
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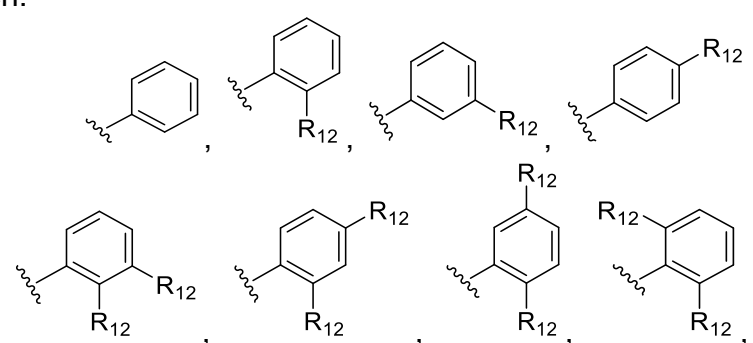
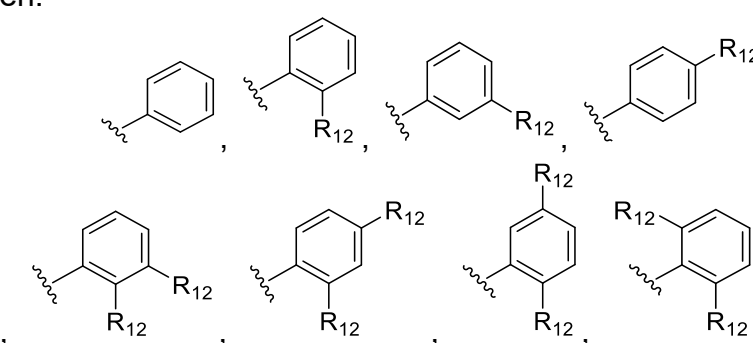
grupos que se seleccionan independientemente del grupo que consiste en halo, hidroxilo, ciano, alquiloC₁₋₄; cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y CF₃; cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₆, haloalquiloC₁₋₆, hidroxialquiloC₁₋₆, alquenoC₂₋₆, alquinoC₂₋₆ y cicloalquiloC₃₋₆; y cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alqueno C₂₋₆, alquino C₂₋₆ y cicloalquilo C₃₋₆.	grupos que se seleccionan independientemente del grupo que consiste en halo, hidroxilo, ciano, alquiloC₁₋₄; cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y CF₃; cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₆, haloalquiloC₁₋₆, hidroxialquiloC₁₋₆, alquenoC₂₋₆, alquinoC₂₋₆ y cicloalquiloC₃₋₆; y cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alqueno C₂₋₆, alquino C₂₋₆ y cicloalquilo C₃₋₆.	
5. El compuesto de la reivindicación 1, donde los subíndices a y b son cada uno 1.	6. El compuesto de la reivindicación 1, donde los subíndices a y b son cada uno 1.	<i>Igual</i>

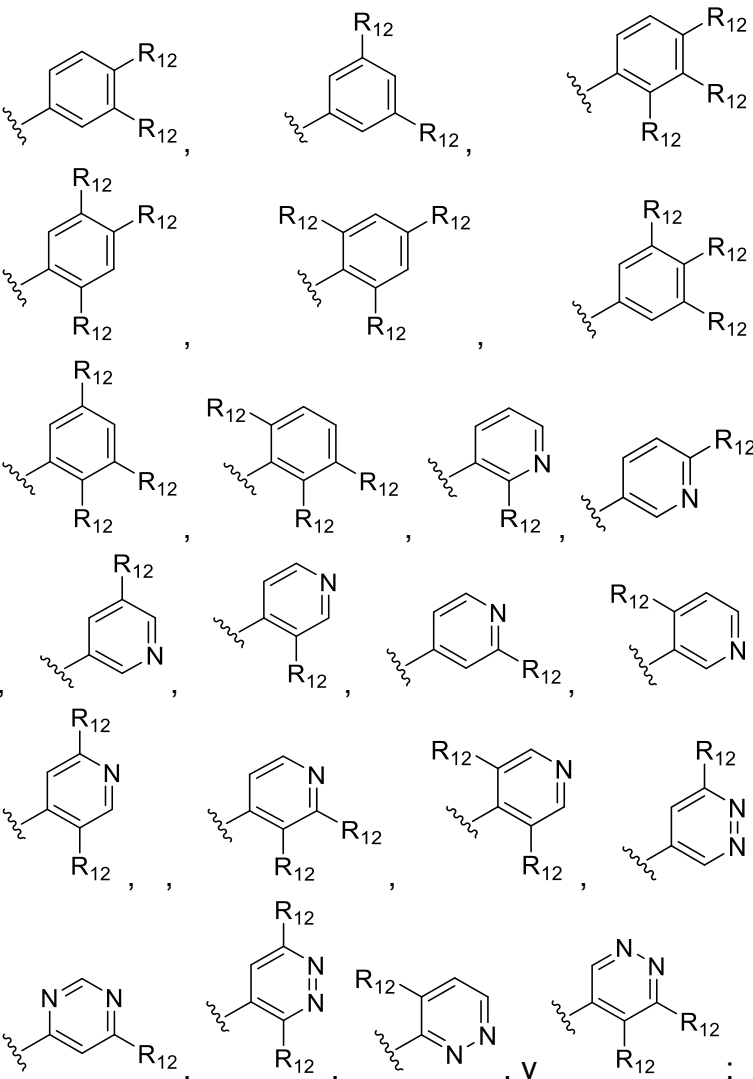
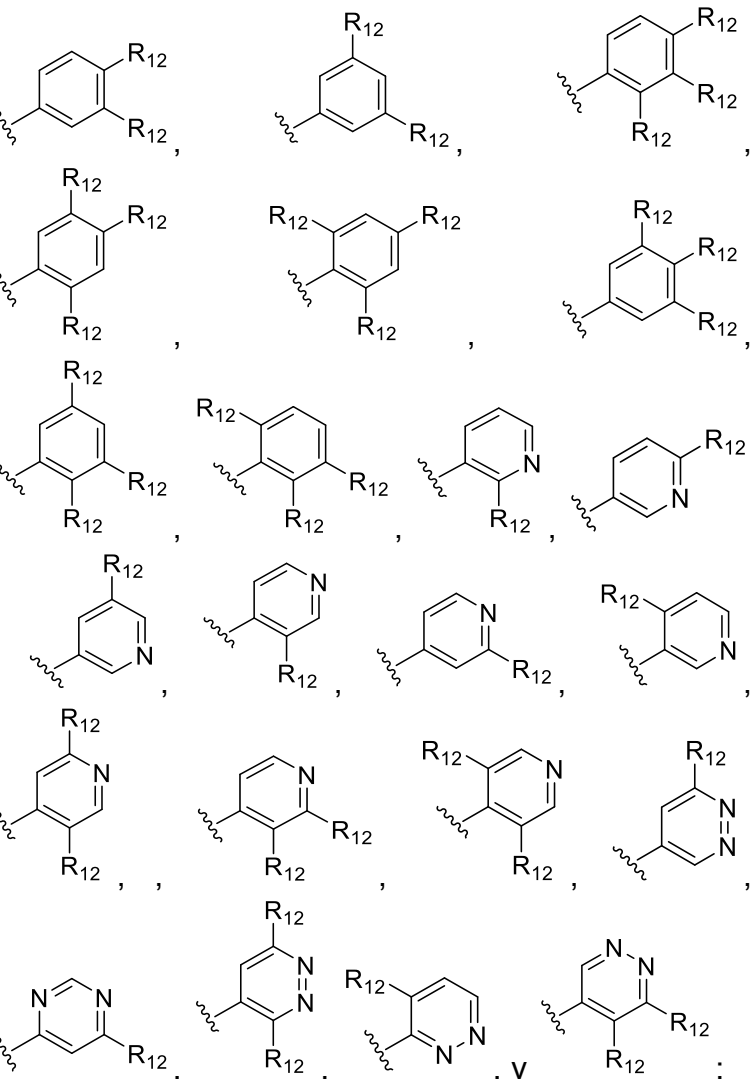
7. El compuesto de la reivindicación 1, donde Y ₁ es un enlace directo.	8. El compuesto de la reivindicación 1, donde Y ₁ es un enlace directo.	<i>Igual</i>
9. El compuesto de la reivindicación 1, donde Y ₂ es alquilo C ₁₋₄ .	10. El compuesto de la reivindicación 1, donde Y ₂ es alquilo C ₁₋₄ .	<i>Igual</i>
11. El compuesto de la reivindicación 9, donde Y ₂ es metilo.	12. El compuesto de la reivindicación 9, donde Y ₂ es metilo.	<i>Igual</i>
13. El compuesto de la reivindicación 1, donde R ₁₃ se selecciona del grupo que consiste en hidrógeno, halo, alquilo C ₁₋₆ , y haloalquilo C ₁₋₆ .	14. El compuesto de la reivindicación 1, donde R ₁₃ se selecciona del grupo que consiste en hidrógeno, halo, alquilo C ₁₋₆ , y haloalquilo C ₁₋₆ .	<i>Igual</i>
8. El compuesto de la reivindicación 7, donde R ₁₃ es hidrógeno, Cl, Br, metilo, o CF ₃ .	8. El compuesto de la reivindicación 7, donde R ₁₃ es hidrógeno, Cl, Br, metilo, o CF ₃ .	<i>Igual</i>

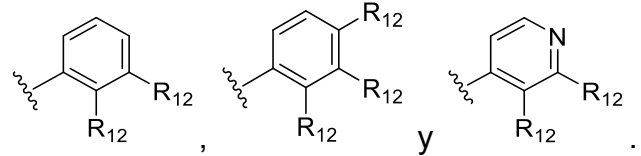
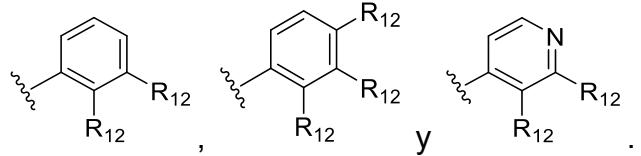
9. El compuesto de la reivindicación 1, donde R₁ es fenilo o un grupo heteroarilo de 5 a 6 miembros que tiene de 1 a 4 heteroátomos como vértices del anillo seleccionados independientemente entre N, O y S; y está opcionalmente sustituido con 1, 2 o 3 grupos R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄, y OR₁₄.	9. El compuesto de la reivindicación 1, donde R₁ es fenilo o un grupo heteroarilo de 5 a 6 miembros que tiene de 1 a 4 heteroátomos como vértices del anillo seleccionados independientemente entre N, O y S; y está opcionalmente sustituido con 1, 2 o 3 grupos R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄, y OR₁₄.	<i>Igual</i>
15. El compuesto de la reivindicación 1, donde R₂, R₃, R₄, R₅, R₈, R₉, R₁₀ y R₁₁ son hidrógeno.	16. El compuesto de la reivindicación 1, donde R₂, R₃, R₄, R₅, R₈, R₉, R₁₀ y R₁₁ son hidrógeno.	<i>Igual</i>
17. El compuesto de la reivindicación 1, donde: R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilamino C₁₋₄; y R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxilo, o se selecciona del grupo que consiste en alquilo C₁₋₄, hidroxialquilo C₁₋₄, cicloalquilo C₃₋₆, fenilo, y heteroarilo	18. El compuesto de la reivindicación 1, donde: R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilamino C₁₋₄; y R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxilo, o se selecciona del grupo que consiste en alquilo C₁₋₄, hidroxialquilo C₁₋₄, cicloalquilo C₃₋₆, fenilo, y heteroarilo	<i>Igual</i>

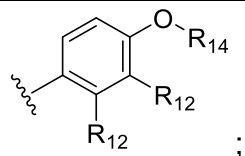
de 5 o 6 miembros, cualquiera de los cuales está opcionalmente sustituido con uno o dos grupos seleccionados del grupo que consiste en amino, halo, hidroxilo, ciano, trifluorometilo, trifluorometoxi, alquilo C ₁₋₄ , y alcoxi C ₁₋₄ alkoxy.	de 5 o 6 miembros, cualquiera de los cuales está opcionalmente sustituido con uno o dos grupos seleccionados del grupo que consiste en amino, halo, hidroxilo, ciano, trifluorometilo, trifluorometoxi, alquilo C ₁₋₄ , y alcoxi C ₁₋₄ alkoxy.	
19. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₆ es amino o aminometilo; y R₇ se selecciona del grupo que consiste en hidroxilo, alquilo C₁₋₄, e hidroxialquilo C₁₋₄.	20. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₆ es amino o aminometilo; y R₇ se selecciona del grupo que consiste en hidroxilo, alquilo C₁₋₄, e hidroxialquilo C₁₋₄.	<i>Igual</i>
21. El compuesto de la reivindicación 1, donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado o no saturado de 3 a 7 miembros que tiene de 1 a 3 heteroátomos o grupos como vértices del anillo seleccionados independientemente entre N, C(O), O y S(O)_m, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que	22. El compuesto de la reivindicación 1, donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado o no saturado de 3 a 7 miembros que tiene de 1 a 3 heteroátomos o grupos como vértices del anillo seleccionados independientemente entre N, C(O), O y S(O)_m, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que	<i>Igual</i>

consiste en amino, halo, hidroxí, alquilo C ₁₋₄ , alcoxi C ₁₋₄ , alquilamino C ₁₋₄ y aminoalquilo C ₁₋₄ .	consiste en amino, halo, hidroxí, alquilo C ₁₋₄ , alcoxi C ₁₋₄ , alquilamino C ₁₋₄ y aminoalquilo C ₁₋₄ .	
23. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado de 4 a 6 miembros que tiene de 1 a 3 heteroátomos como vértices del anillo seleccionados independientemente de N y O, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxí, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄y aminoalquilo C₁₋₄.	24. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado de 4 a 6 miembros que tiene de 1 a 3 heteroátomos como vértices del anillo seleccionados independientemente de N y O, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxí, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄y aminoalquilo C₁₋₄.	<i>Igual</i>
25. El compuesto de la reivindicación 1, donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo de cicloalquilo de 3 a 7 miembros que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxí, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.	26. El compuesto de la reivindicación 1, donde R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo de cicloalquilo de 3 a 7 miembros que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxí, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.	<i>Igual</i>

27. El compuesto de la reivindicación 1, donde R₁ se fenilo o piridilo cada uno de los cuales se sustituye con 1 a 3 R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxi, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄ y OR₁₄.	28. El compuesto de la reivindicación 1, donde R₁ se fenilo o piridilo cada uno de los cuales se sustituye con 1 a 3 R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxi, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄ y OR₁₄.	<i>Igual</i>
29. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₁ se selecciona del grupo que consiste en: 	30. El compuesto de la reivindicación ¡Error! No se encuentra el origen de la referencia., donde R₁ se selecciona del grupo que consiste en: 	<i>Igual</i>

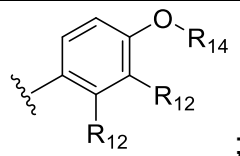
 cada R_{12} se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C_{1-4}, di(alquilo C_{1-4})amino, alquilo C_{1-4}, alcoxi C_{1-4}, haloalquilo C_{1-4},	 cada R_{12} se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C_{1-4}, di(alquilo C_{1-4})amino, alquilo C_{1-4}, alcoxi C_{1-4}, haloalquilo C_{1-4},	
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aminoalquilo C ₁₋₄ , y OR ₁₄ .	aminoalquilo C ₁₋₄ , y OR ₁₄ .	
18. El compuesto de la reivindicación 17, donde R₁ se selecciona del grupo que consiste en: 	18. El compuesto de la reivindicación 17, donde R₁ se selecciona del grupo que consiste en: 	<i>Igual</i>
19. El compuesto de la reivindicación 1, donde R₁₄ se selecciona del grupo que consiste en arilo C₆₋₁₀ y un heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del grupo que consiste en alquilamido C₁₋₄, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, haloalcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.	19. El compuesto de la reivindicación 1, donde R₁₄ se selecciona del grupo que consiste en arilo C₆₋₁₀ y un heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del grupo que consiste en alquilamido C₁₋₄, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, haloalcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.	<i>Igual</i>
31. El compuesto de la reivindicación 1, donde R₁ es	32. El compuesto de la reivindicación 1, donde R₁ es	<i>Igual</i>



cada R₁₂ se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, y aminoalquilo C₁₋₄, y.

R₁₄ es fenilo o un heteroarilo de 5 a 6 miembros que tiene de 1 a 4 heteroátomos como vértices del anillo seleccionados independientemente de N, O y S, cada uno de los cuales está opcionalmente sustituido por uno o dos grupos seleccionados independientemente del grupo que consiste en alquilamido C₁₋₄, halo, hidroxilo, ciano y alquilo C₁₋₄.



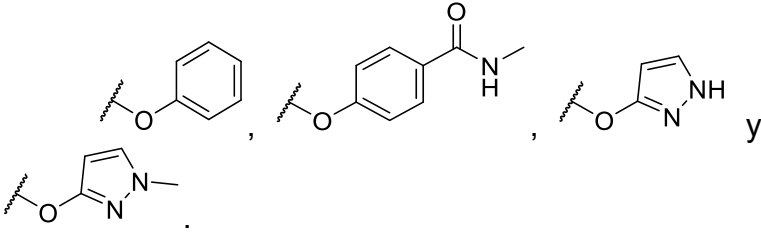
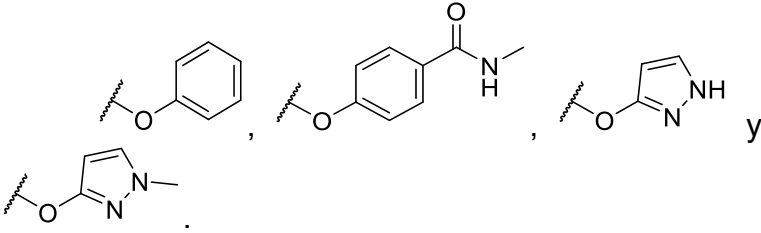
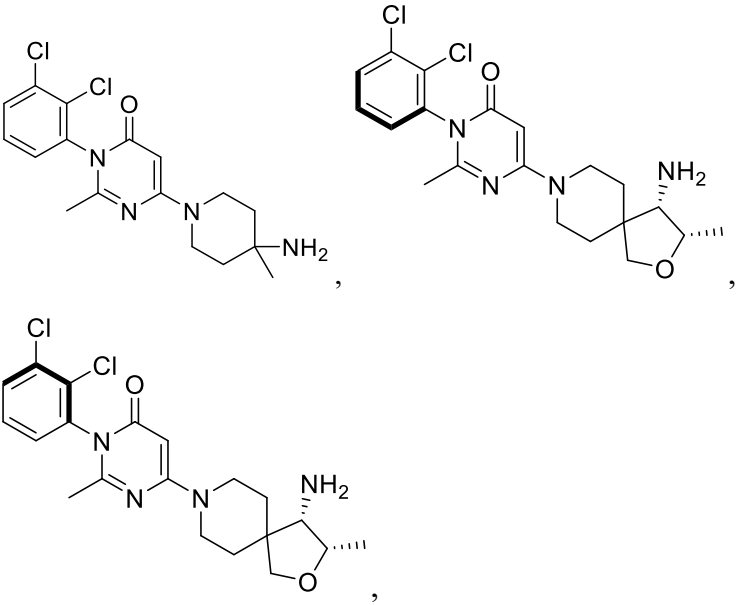
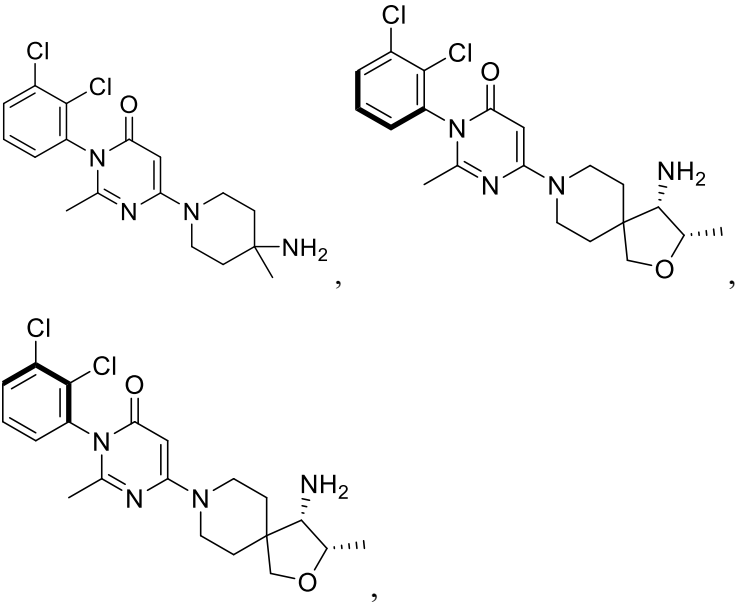
cada R₁₂ se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, y aminoalquilo C₁₋₄, y.

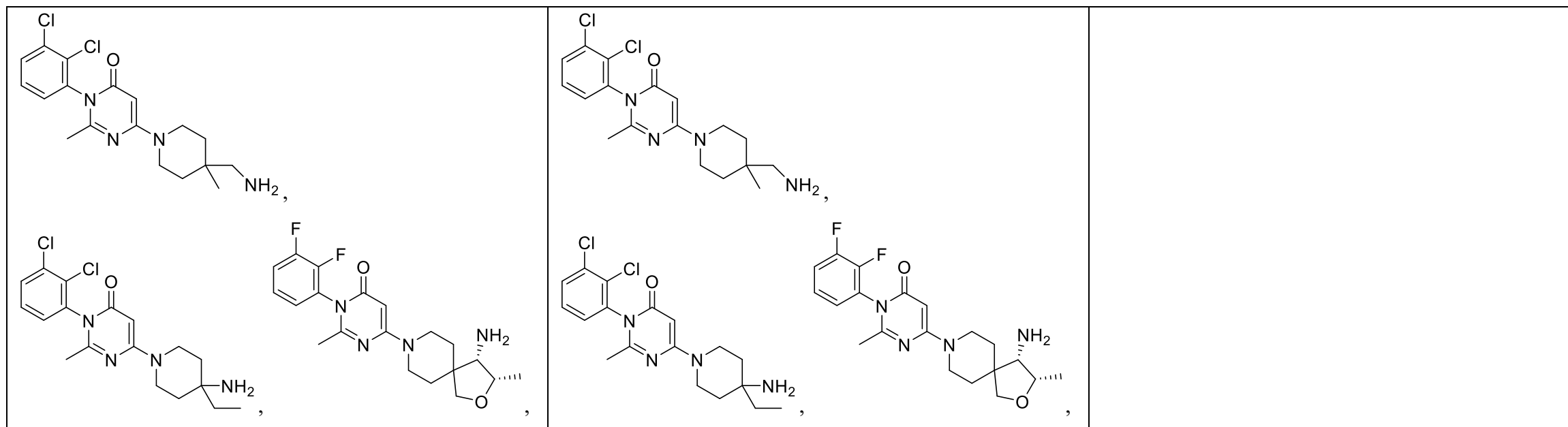
R₁₄ es fenilo o un heteroarilo de 5 a 6 miembros que tiene de 1 a 4 heteroátomos como vértices del anillo seleccionados independientemente de N, O y S, cada uno de los cuales está opcionalmente sustituido por uno o dos grupos seleccionados independientemente del grupo que consiste en alquilamido C₁₋₄, halo, hidroxilo, ciano y alquilo C₁₋₄.

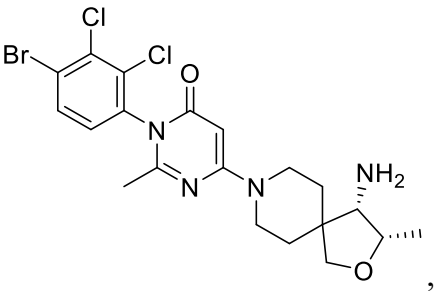
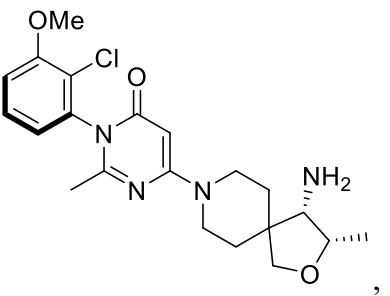
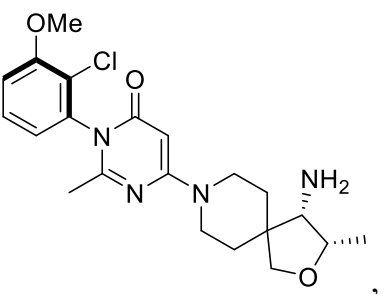
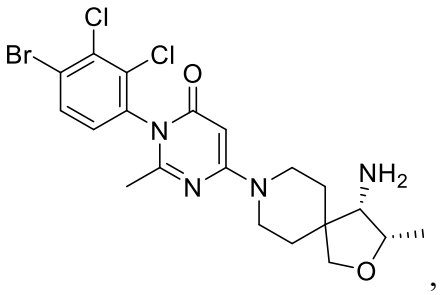
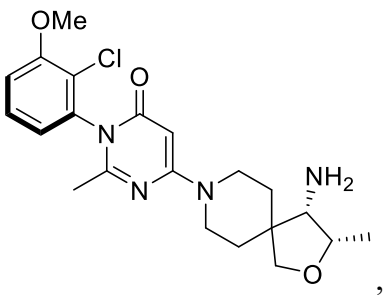
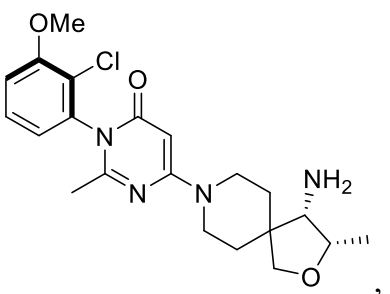
33. El compuesto de la reivindicación 1, donde cada R₁₂ se selecciona de forma independiente del grupo que consiste en F, Cl, Br, CH₃, OCH₃, CF₃,

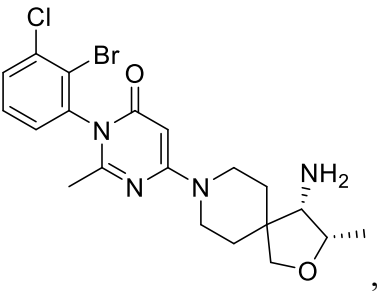
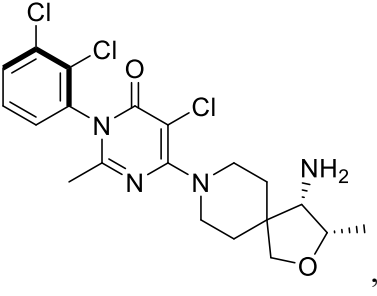
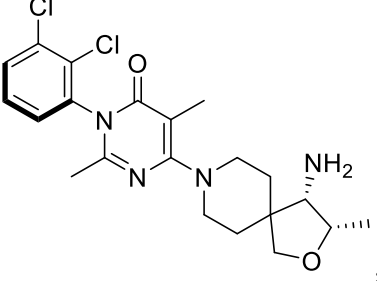
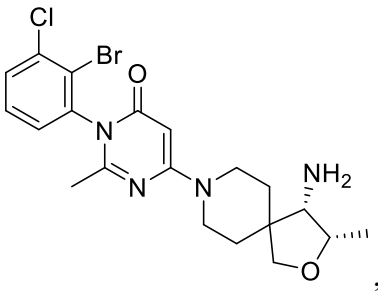
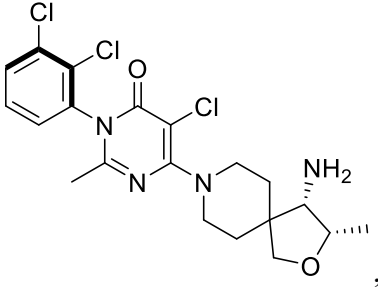
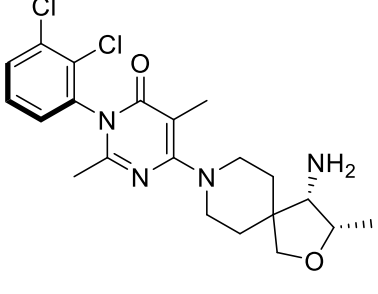
34. El compuesto de la reivindicación 1, donde cada R₁₂ se selecciona de forma independiente del grupo que consiste en F, Cl, Br, CH₃, OCH₃, CF₃,

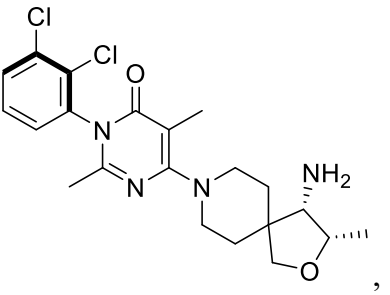
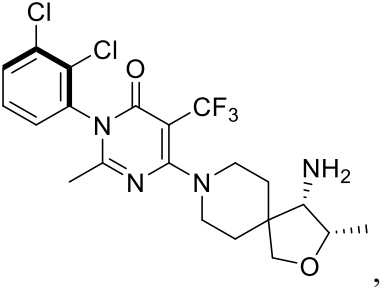
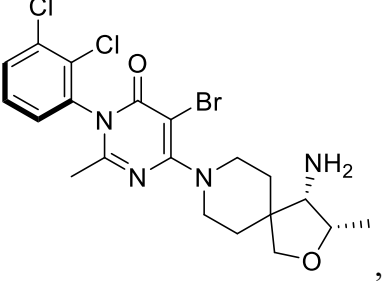
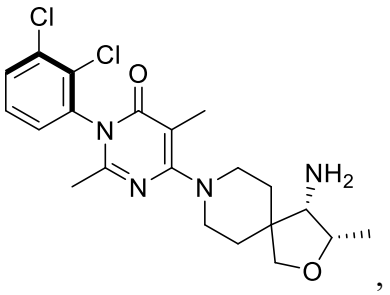
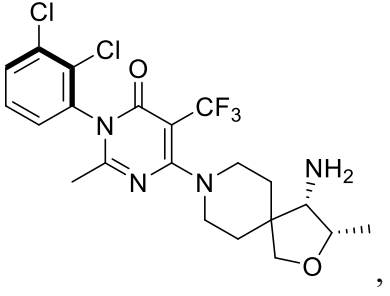
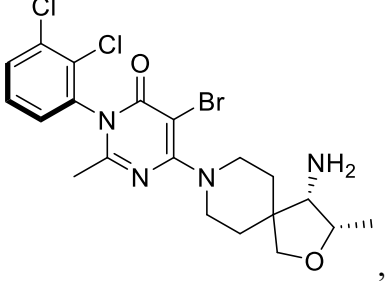
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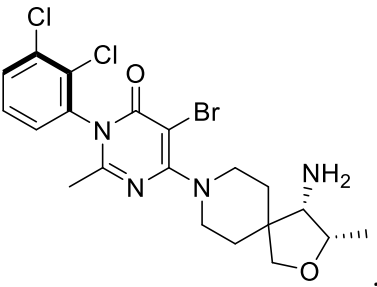
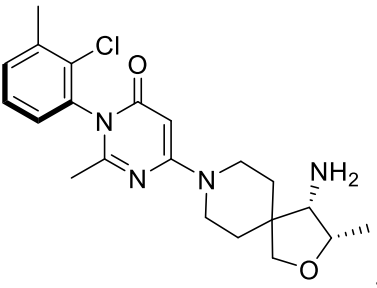
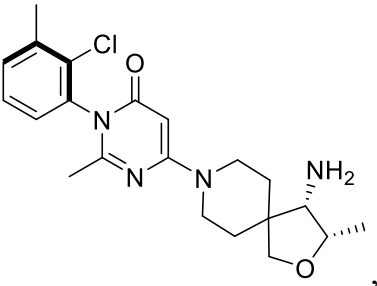
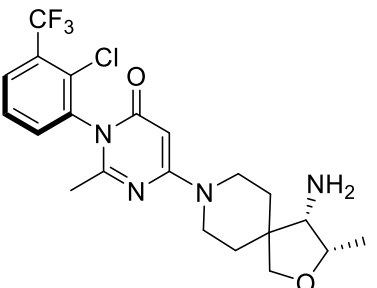
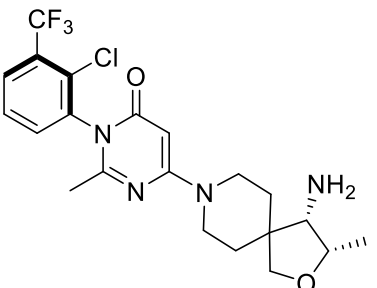
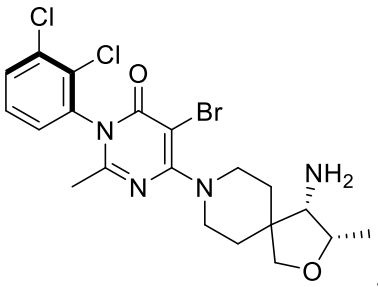
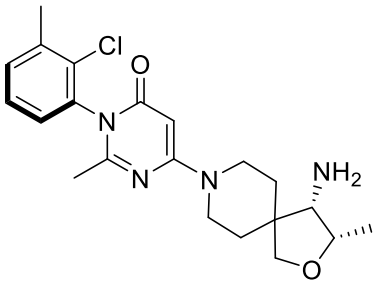
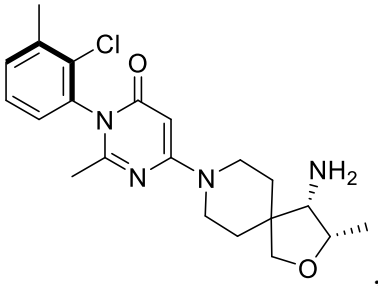
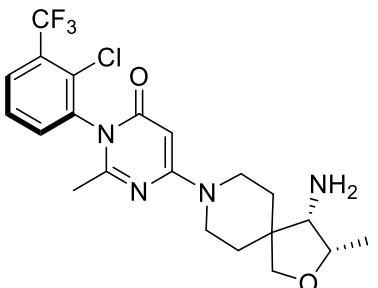
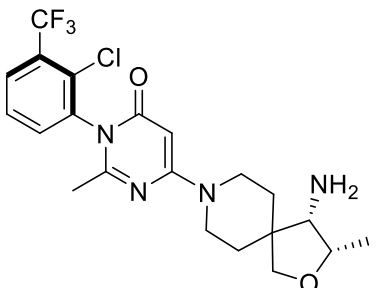
		
35. El compuesto de la reivindicación 1, que tiene una estructura seleccionada del grupo que consiste en: 	36. El compuesto de la reivindicación 1, que tiene una estructura seleccionada del grupo que consiste en: 	<i>Iguals</i>

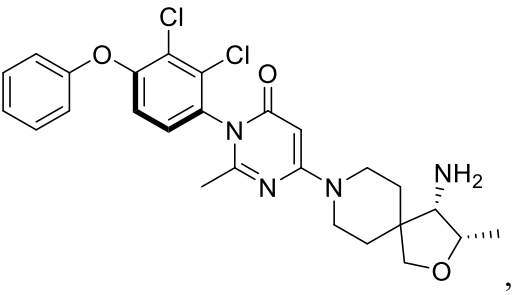
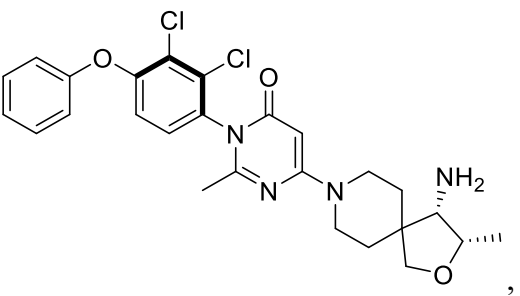
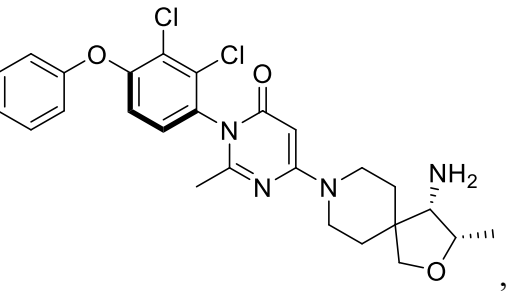
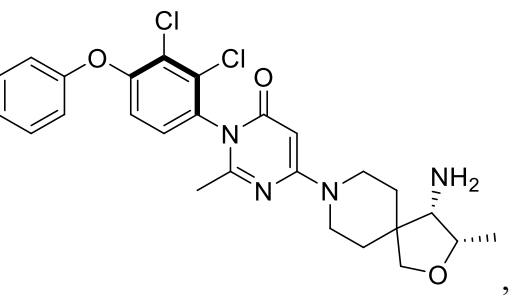


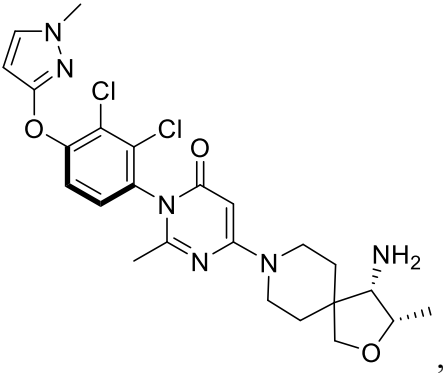
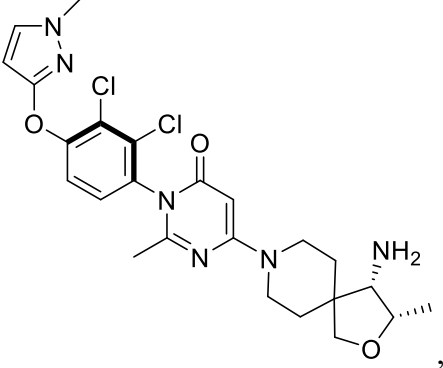
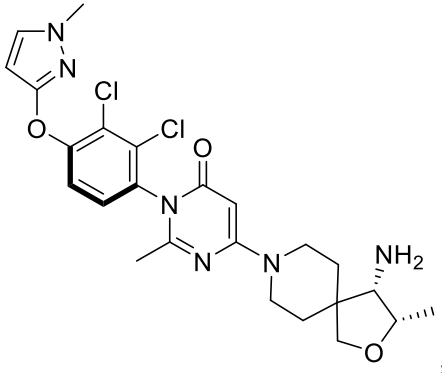
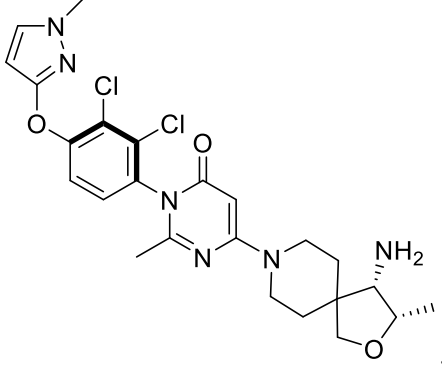
  	  	
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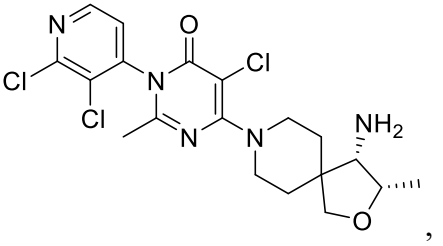
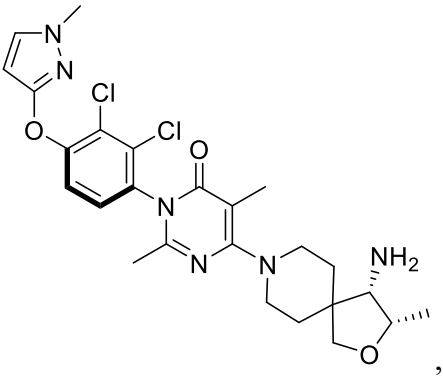
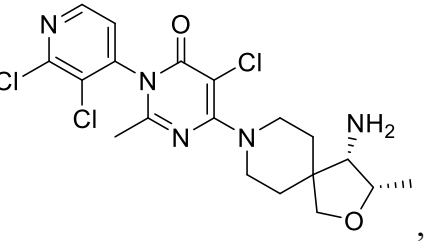
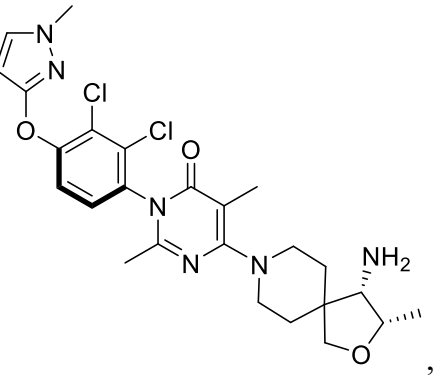
  	  	
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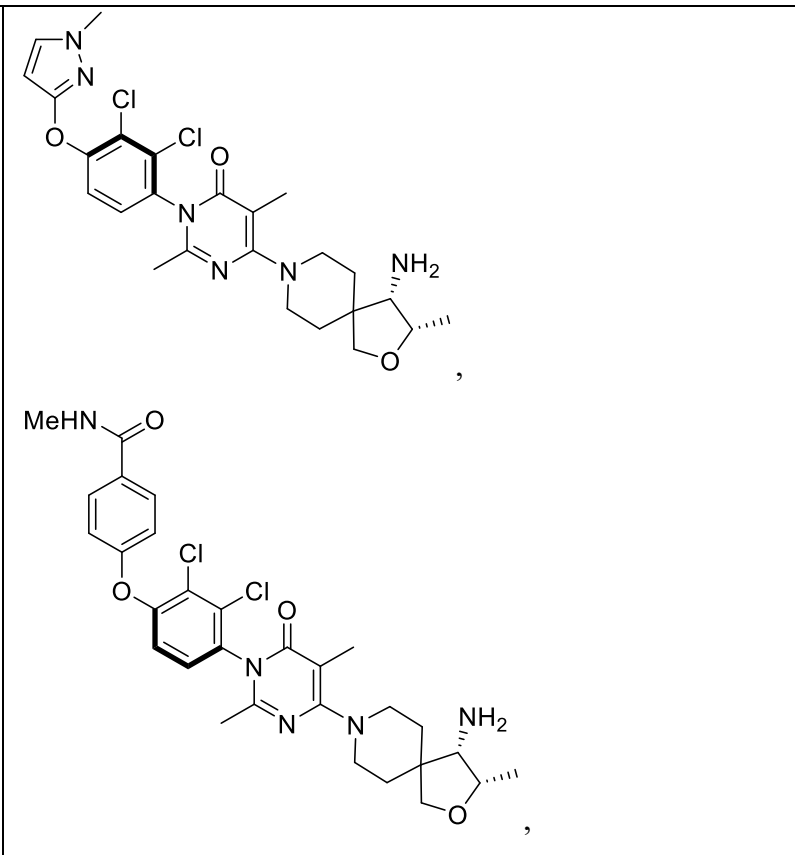
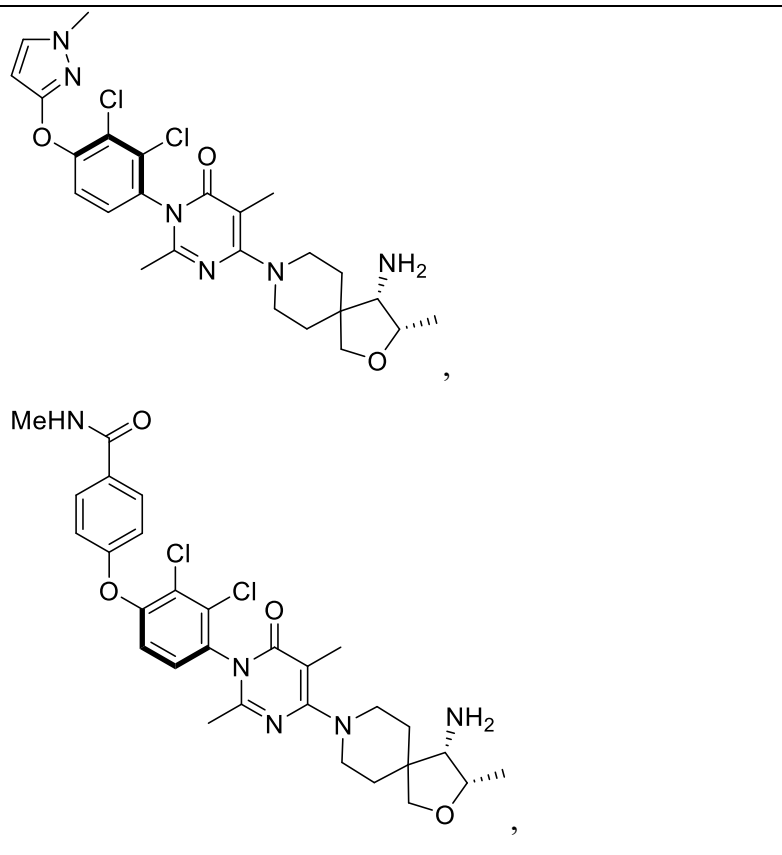
  	  	
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MeHN-C(=O)-c1ccc(Oc2cc(Cl)c(Cl)c3c2n(C)c4c(=O)n(C)c(N5CCN(CC5)C6COC6)c43)cc1 c1cc(Cl)c(Cl)c2c1c3c(=O)n(C)c(N4CCN(CC4)C5COC5)c32 c1cc(Cl)c(Cl)c2c1c3c(=O)n(C)c(N4CCN(CC4)C5COC5)c32	MeHN-C(=O)-c1ccc(Oc2cc(Cl)c(Cl)c3c2n(C)c4c(=O)n(C)c(N5CCN(CC5)C6COC6)c43)cc1 c1cc(Cl)c(Cl)c2c1c3c(=O)n(C)c(N4CCN(CC4)C5COC5)c32 c1cc(Cl)c(Cl)c2c1c3c(=O)n(C)c(N4CCN(CC4)C5COC5)c32	
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37. Una composición farmacéutica que comprende un compuesto de la reivindicación 1, junto con un portador farmacéuticamente aceptable.	38. Una composición farmacéutica que comprende un compuesto de la reivindicación 1, junto con un portador farmacéuticamente aceptable.	<i>Igual</i>
	24. Un método de tratamiento de una enfermedad mediada por PTPN11 que comprende la administración de una cantidad terapéuticamente eficaz de un compuesto de la reivindicación 1, a un paciente que lo necesita, en el que la enfermedad mediada por PTPN11 es un síndrome de Noonan, un síndrome LEOPARD o un cáncer seleccionado del grupo que consiste en cáncer de mama, cáncer de colon, cáncer de pulmón, cáncer de esófago, cáncer gástrico, leucemia y melanoma.	<i>En la modificación voluntaria presentada el 10 de diciembre de 2021, se eliminó esta reivindicación por tratarse de un método de tratamiento, lo cual es materia no patentable.</i>



US011104675B2

(12) **United States Patent**
Jones et al.

(10) **Patent No.:** **US 11,104,675 B2**
(45) **Date of Patent:** **Aug. 31, 2021**

- (54) **PTPN11 INHIBITORS**
- (71) Applicant: **NAVIRE PHARMA, INC.**, Palo Alto, CA (US)
- (72) Inventors: **Philip Jones**, Houston, TX (US); **Barbara Czako**, Bellaire, TX (US); **Christopher L. Carroll**, Houston, TX (US); **Pijus Mandal**, Sugarland, TX (US); **Jason Cross**, Pearland, TX (US)
- (73) Assignee: **NAVIRE PHARMA, INC.**, Palo Alto, CA (US)
- (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.
- (21) Appl. No.: **16/536,923**
- (22) Filed: **Aug. 9, 2019**
- (65) **Prior Publication Data**
US 2020/0048249 A1 Feb. 13, 2020
- Related U.S. Application Data**
- (60) Provisional application No. 62/717,588, filed on Aug. 10, 2018, provisional application No. 62/773,921, filed on Nov. 30, 2018.

- (51) **Int. Cl.**
C07D 471/04 (2006.01)
A61P 35/00 (2006.01)
C07D 491/107 (2006.01)
- (52) **U.S. Cl.**
CPC **C07D 471/04** (2013.01); **A61P 35/00** (2018.01); **C07D 491/107** (2013.01)
- (58) **Field of Classification Search**
CPC C07D 471/04
See application file for complete search history.

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Primary Examiner — Paul V Ward

(74) *Attorney, Agent, or Firm* — Mintz, Levin, Cohn, Ferris, Glovsky & Popeo, P.C.

(57) **ABSTRACT**

The present invention relates to compounds which are useful as inhibitors of PTPN11 for the treatment or prevention of cancer and other PTP-mediated diseases. Disclosed herein are new compounds and compounds based on pyrazolopyrazines and their application as pharmaceuticals for the treatment of disease.

24 Claims, 2 Drawing Sheets

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FIG. 1

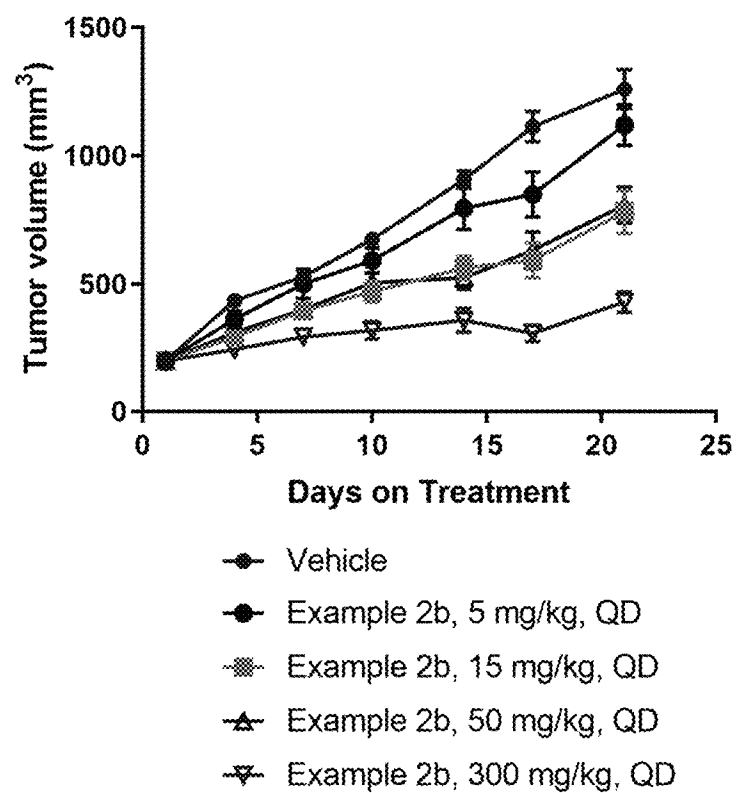
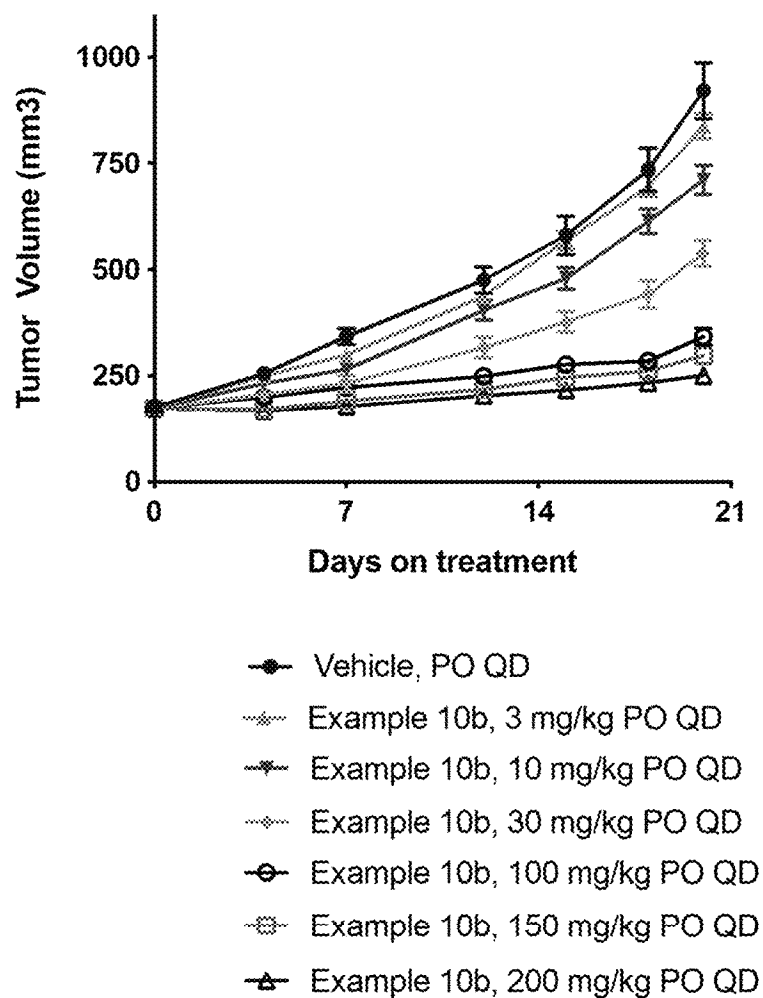


FIG. 2



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PTPN11 INHIBITORS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/717,588, filed Aug. 10, 2018 and U.S. Provisional Application No. 62/773,921, filed Nov. 30, 2018, each of which is incorporated herein by reference in its entirety and for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

NOT APPLICABLE

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON A COMPACT DISK

NOT APPLICABLE

BACKGROUND OF THE INVENTION

Disclosed herein are new compounds and compounds based on pyrimidinones and their application as pharmaceuticals for the treatment of disease. Methods of inhibition of PTPN11 (SHP2) activity in a human or animal subject are also provided for the treatment diseases such as cancer, including leukemia and melanoma, and cancers of the breast, lung, and colon.

Tyrosyl phosphorylation regulates human cellular processes from cell differentiation to growth and apoptosis, and others. Tyrosyl phosphorylation is regulated by protein-tyrosine kinases (PTK) and protein-tyrosine phosphatases (PTP). The breakdown of regulation governed by PTK and PTP activity is thought to lead to cancer. PTK inhibitors have been developed as potential cancer therapeutic agents. Recent studies disclose a role for PTPs in cellular regulation as well. (A J Barr et al. *Cell* 2009, 136, 352-363. J N Andersen et al *Mol. Cell. Biol.* 2001, 21, 7117-7136).

Protein-tyrosine phosphatase non-receptor type 11 (PTPN11, also known as Src Homology-2 phosphatase (SHP2)) is a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene. This PTP contains two tandem Src homology-2 (SH2) domains, which function as phospho-tyrosine binding domains, a catalytic domain, and a C-terminal tail. In the basal state the protein typically exists in an inactive, self-inhibited conformation with the N-terminal SH2 domain blocking the active site. When stimulated by signal transduction mediated by cytokines and growth factor binding of phosphorylated proteins to the SH2 domains the auto-inhibition is relieved, this makes the active site available for dephosphorylation of PTPN11 substrates (M G Mohl, B G Neel, *Curr. Opin. Genetics Dev.* 2007, 17, 23-30. K S Grossmann, *Adv. Cancer Res.* 2010, 106, 53-89. W. Q. Huang et. al. *Curr. Cancer Drug Targets* 2014, 14, 567-588. C. Gordon et. al. *Cancer Metastasis Rev.* 2008, 27, 179-192).

Germ-line and somatic mutations in PTPN11 have been reported in several human diseases resulting in gain-of-function in the catalytic activity, including Noonan Syndrome and Leopard Syndrome; as well as multiple cancers such as juvenile myelomonocytic leukemia, neuroblastoma, myelodysplastic syndrome, B cell acute lymphoblastic leu-

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kemia/lymphoma, melanoma, acute myeloid leukemia and cancers of the breast, lung and colon (M G Mohl, B G Neel, *Curr. Opin. Genetics Dev.* 2007, 17, 23-30). Recent studies have demonstrated that single PTPN11 mutations are able to induce Noonan syndrome, JMML-like myeloproliferative disease and acute leukemia in mice. These mutations disrupt the auto-inhibition between the N-SH2 domains and the catalytic site allowing constitutive access of substrates to the catalytic site of the enzyme (E. Darian et al, *Proteins*, 2011, 79, 1573-1588. Z H Yu et al, *JBC*, 2013, 288, 10472, W Qiu et al *BMC Struct. Biol.* 2014, 14, 10).

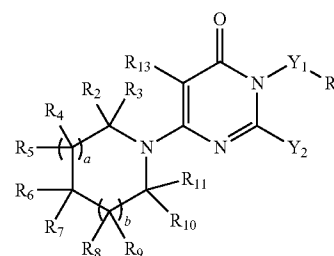
PTPN11 is widely expressed in most tissues and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions that includes proliferation, differentiation, cell cycle maintenance, EMT transition, mitogenic activation, metabolic control, transcription regulation, and cell migration, through multiple signaling pathways including the Ras-MAPK, the JAK-STAT or the PI3K-AKT pathways (Tajan, M. et. al. *Eur. J. Medical Genetics*, 2015, 58, 509-525. Prahallad, A. et. al. *Cell Reports*, 2015, 12, 1978-1985).

Additionally there is growing evidence that PTPN11/SHP2 is implicated in immune evasion during tumorigenesis, and hence a SHP2 inhibitor could stimulate the immune response in cancer patients (*Cancer Res.* 2015 Feb. 1; 75(3):508-18. T Yokosuka T, *J Exp Med.* 2012, 209(6), 1201. S Amarnath *Sci Transl Med.* 2011, 3, 111ra120. T Okazaki, *PNAS* 2001, 98:24, 13866-71).

Novel compounds and pharmaceutical compositions, certain of which have been found to inhibit PTPN11 (SHP2) have been discovered, together with methods of synthesizing and using the compounds including methods for the treatment of PTP-mediated diseases in a patient by administering the compounds.

BRIEF SUMMARY OF THE INVENTION

In certain embodiments of the present invention, compounds are represented by Formula I:



(I)

or a salt or tautomer thereof, wherein the subscripts a and b, Y₁, Y₂, and R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₃ are as provided herein.

In certain embodiments, the present invention provides a pharmaceutical composition including a compound having Formula I, together with a pharmaceutically acceptable carrier.

In certain embodiments, the present invention provides methods of inhibition of PTPN11 (SHP2) activity in a human or animal subject for the treatment diseases such as cancer, including leukemia and melanoma, and cancers of the breast, lung, and colon.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a dose-dependent tumor growth inhibition by 6-(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]de-

can-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 2b) in the KYSE520 xenograft model.

FIG. 2 shows a dose-dependent tumor growth inhibition by 6-(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10b) in the KYSE520 xenograft model.

DETAILED DESCRIPTION OF THE INVENTION

I. General

Certain compounds of Formula I disclosed herein may possess useful PTPN11 inhibiting activity, and may be used in the treatment or prophylaxis of a disease or condition in which PTPN11 plays an active role. Thus, in broad aspect, certain embodiments also provide pharmaceutical compositions comprising one or more compounds disclosed herein together with a pharmaceutically acceptable carrier, as well as methods of making and using the compounds and compositions. Certain embodiments provide methods for inhibiting PTPN11. Other embodiments provide methods for treating a PTPN11-mediated disorder in a patient in need of such treatment, comprising administering to said patient a therapeutically effective amount of a compound or composition according to the present invention. Also provided is the use of certain compounds disclosed herein for use in the manufacture of a medicament for the treatment of a disease or condition ameliorated by the inhibition of PTPN11. In particular, the PTPN11-mediated disease is cancer.

II. Definitions

As used herein, the terms below have the meanings indicated.

When ranges of values are disclosed, and the notation “from n₁ . . . to n₂” or “between n₁ . . . and n₂” is used, where n₁ and n₂ are the numbers, then unless otherwise specified, this notation is intended to include the numbers themselves and the range between them. This range may be integral or continuous between and including the end values. By way of example, the range “from 2 to 6 carbons” is intended to include two, three, four, five, and six carbons, since carbons come in integer units. Compare, by way of example, the range “from 1 to 3 μM (micromolar),” which is intended to include 1 μM, 3 μM, and everything in between to any number of significant figures (e.g., 1.255 μM, 2.1 μM, 2.9999 μM, etc.).

The term “about,” as used herein, is intended to qualify the numerical values which it modifies, denoting such a value as variable within a margin of error. When no particular margin of error, such as a standard deviation to a mean value given in a chart or table of data, is recited, the term “about” should be understood to mean that range which would encompass the recited value and the range which would be included by rounding up or down to that figure as well, taking into account significant figures.

The term “acyl,” as used herein, alone or in combination, refers to a carbonyl attached to an alkenyl, alkyl, aryl, cycloalkyl, heteroaryl, heterocycle, or any other moiety were the atom attached to the carbonyl is carbon. An “acetyl” group refers to a —C(O)CH₃ group. An “alkylcarbonyl” or “alkanoyl” group refers to an alkyl group attached to the parent molecular moiety through a carbonyl group.

Examples of such groups include methylcarbonyl and ethylcarbonyl. Examples of acyl groups include formyl, alkanoyl and aroyl.

The term “alkenyl,” as used herein, alone or in combination, refers to a straight-chain or branched-chain hydrocarbon radical having one or more double bonds and containing from 2 to 20 carbon atoms. In certain embodiments, said alkenyl will comprise from 2 to 6 carbon atoms. The term “alkenylene” refers to a carbon-carbon double bond system attached at two or more positions such as ethenylene [(—CH=CH—), (—C::C—)]. Examples of suitable alkenyl radicals include ethenyl, propenyl, 2-methylpropenyl, 1,4-butadienyl and the like. Unless otherwise specified, the term “alkenyl” may include “alkenylene” groups.

The term “alkynyl” refers to either a straight chain or branched hydrocarbon having at least 2 carbon atoms and at least one triple bond and having the number of carbon atom indicated (i.e., C₂₋₆ means to two to six carbons). Alkynyl can include any number of carbons, such as C₂, C₂₋₃, C₂₋₄, C₂₋₅, C₂₋₆, C₂₋₇, C₂₋₈, C₂₋₉, C₂₋₁₀, C₃, C₃₋₄, C₃₋₅, C₃₋₆, C₄, C₅, C₄₋₆, C₅, C₅₋₆, and C₆. Examples of alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butenyl, 2-butenyl, butadiynyl, 1-pentynyl, 2-pentynyl, isopentenyl, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatrienyl.

The term “alkoxy,” as used herein, alone or in combination, refers to an alkyl ether radical, wherein the term alkyl is as defined below. Examples of suitable alkyl ether radicals include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, and the like.

The term “alkyl,” as used herein, alone or in combination, refers to a straight-chain or branched-chain alkyl radical containing from 1 to 20 carbon atoms. In certain embodiments, said alkyl will comprise from 1 to 10 carbon atoms. In further embodiments, said alkyl will comprise from 1 to 8 carbon atoms. Alkyl groups are optionally substituted as defined herein. Examples of alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, iso-amyl, hexyl, octyl, nonyl and the like. The term “alkylene,” as used herein, alone or in combination, refers to a saturated aliphatic group derived from a straight or branched chain saturated hydrocarbon attached at two or more positions, such as methylene (—CH₂—). Unless otherwise specified, the term “alkyl” may include “alkylene” groups.

The term “alkylamino,” as used herein, alone or in combination, refers to an alkyl group attached to the parent molecular moiety through an amino group. Suitable alkylamino groups may be mono- or dialkylated, forming groups such as, for example, N-methylamino, N-ethylamino, N,N-dimethylamino, N,N-ethylmethylamino and the like.

The term “alkylthio,” as used herein, alone or in combination, refers to an alkyl thioether (R—S—) radical wherein the term alkyl is as defined above and wherein the sulfur may be singly or doubly oxidized. Examples of suitable alkyl thioether radicals include methylthio, ethylthio, n-propylthio, isopropylthio, n-butylthio, iso-butylthio, sec-butylthio, tert-butylthio, methanesulfonyl, ethanesulfinyl, and the like.

The terms “amido” and “carbamoyl,” as used herein, alone or in combination, refer to an amino group as described below attached to the parent molecular moiety through a carbonyl group, or vice versa. The “amido” group as used herein includes a “C-amido” and “N-amido” groups. The term “C-amido” as used herein, alone or in combination, refers to a —C(O)N(RR') group with R and R' as

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defined herein or as defined by the specifically enumerated “R” groups designated. In some embodiments, the “amido” group includes —C(O)NH_2 , C_{1-4} alkylamido, and $\text{di(C}_{1-4}\text{alkyl)amido}$. The term “ C_{1-4} alkylamido”, as used herein, refers to $\text{—C(O)NH(C}_{1-4}\text{alkyl)}$, wherein C_{1-4} alkyl is as defined herein. The term “N-amido” as used herein, alone or in combination, refers to a RC(O)N(R')— group, with R and R' as defined herein or as defined by the specifically enumerated “R” groups designated. The term “acylamino” as used herein, alone or in combination, embraces an acyl group attached to the parent moiety through an amino group. An example of an “acylamino” group is acetylamino ($\text{CH}_3\text{C(O)NH—}$).

The term “amino,” as used herein, alone or in combination, refers to —NRR' , wherein R and R' are independently selected from hydrogen, alkyl, acyl, heteroalkyl, aryl, cycloalkyl, heteroaryl, and heterocycloalkyl, any of which may themselves be optionally substituted. Additionally, R and R' may combine to form heterocycloalkyl, either of which is optionally substituted.

The term “aryl,” as used herein, alone or in combination, means a carbocyclic aromatic system containing one, two or three rings wherein such polycyclic ring systems are fused together. The term “aryl” embraces aromatic groups such as phenyl, naphthyl, anthracenyl, and phenanthryl.

The term “arylalkenyl” or “aralkenyl,” as used herein, alone or in combination, refers to an aryl group attached to the parent molecular moiety through an alkenyl group.

The term “arylalkoxy” or “aralkoxy,” as used herein, alone or in combination, refers to an aryl group attached to the parent molecular moiety through an alkoxy group.

The term “aryllalkyl” or “aralkyl,” as used herein, alone or in combination, refers to an aryl group attached to the parent molecular moiety through an alkyl group.

The term aryloxy as used herein, alone or in combination, refers to an aryl group attached to the parent molecular moiety through an oxy.

The term “carbamate,” as used herein, alone or in combination, refers to an ester of carbamic acid (—NHCOO—) which may be attached to the parent molecular moiety from either the nitrogen or acid end, and which is optionally substituted as defined herein.

The term “O-carbamyl” as used herein, alone or in combination, refers to a —OC(O)NRR' , group—with R and R' as defined herein.

The term “N-carbamyl” as used herein, alone or in combination, refers to a ROC(O)NR'— group, with R and R' as defined herein.

The term “carbonyl,” as used herein, when alone includes formyl [—C(O)H] and in combination is a —C(O)— group.

The term “carboxyl” or “carboxy,” as used herein, refers to —C(O)OH or the corresponding “carboxylate” anion, such as is in a carboxylic acid salt. An “O-carboxy” group refers to a RC(O)O— group, where R is as defined herein. A “C-carboxy” group refers to a —C(O)OR groups where R is as defined herein.

The term “cyano,” as used herein, alone or in combination, refers to —CN .

The term “cycloalkyl,” or, alternatively, “carbocycle,” as used herein, alone or in combination, refers to a saturated or partially saturated monocyclic, bicyclic or tricyclic alkyl group wherein each cyclic moiety contains from 3 to 12 carbon atom ring members and which may optionally be a benzo fused ring system which is optionally substituted as defined herein. The term “cycloalkenyl” refers to a cycloalkyl group having one or two double bonds. In certain embodiments, said cycloalkyl (or cycloalkenyl) will com-

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prise from 5 to 7 carbon atoms. Examples of such groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, tetrahydronaphthyl, indanyl, octahydronaphthyl, 2,3-dihydro-1H-indenyl, adamantyl and the like. “Bicyclic” and “tricyclic” as used herein are intended to include both fused ring systems, such as decahydronaphthalene, octahydronaphthalene as well as the multicyclic (multicentered) saturated or partially unsaturated type. The latter type of isomer is exemplified in general by, bicyclo[1,1,1]pentane, camphor, adamantane, and bicyclo[3,2,1]octane.

The term “ester,” as used herein, alone or in combination, refers to a carboxy group bridging two moieties linked at carbon atoms.

The term “ether,” as used herein, alone or in combination, refers to an oxy group bridging two moieties linked at carbon atoms.

The term “halo,” or “halogen,” as used herein, alone or in combination, refers to fluorine, chlorine, bromine, or iodine.

The term “haloalkoxy,” as used herein, alone or in combination, refers to a haloalkyl group attached to the parent molecular moiety through an oxygen atom.

The term “haloalkyl,” as used herein, alone or in combination, refers to an alkyl radical having the meaning as defined above wherein one or more hydrogens are replaced with a halogen. Specifically embraced are monohaloalkyl, dihaloalkyl and polyhaloalkyl radicals. A monohaloalkyl radical, for one example, may have an iodo, bromo, chloro, or fluoro atom within the radical. Dihalo and polyhaloalkyl radicals may have two or more of the same halo atoms or a combination of different halo radicals. Examples of haloalkyl radicals include fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl and dichloropropyl. “Haloalkylene” refers to a haloalkyl group attached at two or more positions. Examples include fluoromethylene (—CFH—), difluoromethylene ($\text{—CF}_2\text{—}$), chloromethylene (—CHCl—) and the like.

The term “heteroalkyl,” as used herein, alone or in combination, refers to a stable straight or branched chain, or combinations thereof, fully saturated or containing from 1 to 3 degrees of unsaturation, consisting of the stated number of carbon atoms and from one to three heteroatoms selected from N, O, and S, and wherein the N and S atoms may optionally be oxidized and the N heteroatom may optionally be quaternized. The heteroatom(s) may be placed at any interior position of the heteroalkyl group. Up to two heteroatoms may be consecutive, such as, for example, $\text{—CH}_2\text{—NH—OCH}_3$.

The term “heteroaryl,” as used herein, alone or in combination, refers to a 3 to 15 membered unsaturated heteromonocyclic ring, or a fused monocyclic, bicyclic, or tricyclic ring system in which at least one of the fused rings is aromatic, which contains at least one atom selected from N, O, and S. In certain embodiments, said heteroaryl will comprise from 1 to 4 heteroatoms as ring members. In further embodiments, said heteroaryl will comprise from 1 to 2 heteroatoms as ring members. In certain embodiments, said heteroaryl will comprise from 5 to 7 atoms. The term also embraces fused polycyclic groups wherein heterocyclic rings are fused with aryl rings, wherein heteroaryl rings are fused with other heteroaryl rings, wherein heteroaryl rings are fused with heterocycloalkyl rings, or wherein heteroaryl rings are fused with cycloalkyl rings. Examples of heteroaryl groups include pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, tri-

azolyl, pyranyl, furyl, thienyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, thiadiazolyl, isothiazolyl, indolyl, isoindolyl, indolizynyl, benzimidazolyl, quinolyl, isoquinolyl, quinoxalynyl, quinazolinyl, indazolyl, benzotriazolyl, benzodioxolyl, benzopyranyl, benzoxazolyl, benzoxadiazolyl, benzothiazolyl, benzothiadiazolyl, benzofuryl, benzothienyl, chromonyl, coumarinyl, benzopyranyl, tetrahydroquinolynyl, tetrazolopyridazinyl, tetrahydroisoquinolynyl, thienopyridinyl, furopyridinyl, pyrrolopyridinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, dibenzofuranyl, acridinyl, phenanthridinyl, xanthenyl and the like.

The terms “heterocycloalkyl” and, interchangeably, “heterocycle,” as used herein, alone or in combination, each refer to a saturated, partially unsaturated, or fully unsaturated (but nonaromatic) monocyclic, bicyclic, or tricyclic heterocyclic group containing at least one heteroatom as a ring member, wherein each said heteroatom may be independently selected from nitrogen, oxygen, and sulfur. In certain embodiments, said heterocycloalkyl will comprise from 1 to 4 heteroatoms as ring members. In further embodiments, said heterocycloalkyl will comprise from 1 to 2 heteroatoms as ring members. In certain embodiments, said heterocycloalkyl will comprise from 3 to 8 ring members in each ring. In further embodiments, said heterocycloalkyl will comprise from 3 to 7 ring members in each ring. In yet further embodiments, said heterocycloalkyl will comprise from 5 to 6 ring members in each ring. “Heterocycloalkyl” and “heterocycle” are intended to include sulfones, sulfoxides, N-oxides of tertiary nitrogen ring members, and carbocyclic fused and benzo fused ring systems; additionally, both terms also include systems where a heterocycle ring is fused to an aryl group, as defined herein, or an additional heterocycle group. Examples of heterocycle groups include aziridinyl, azetidynyl, 1,3-benzodioxolyl, dihydroisoindolyl, dihydroisoquinolynyl, dihydrocinnolynyl, dihydrobenzodioxinyl, dihydro[1,3]oxazolo[4,5-b]pyridinyl, benzothiazolyl, dihydroindolyl, dihydropyridinyl, 1,3-dioxanyl, 1,4-dioxanyl, 1,3-dioxolanyl, isoindolynyl, morpholynyl, piperazinyl, pyrrolidinyl, tetrahydropyridinyl, piperidinyl, thiomorpholynyl, and the like. The heterocycle groups are optionally substituted unless specifically prohibited.

The term “hydrazinyl” as used herein, alone or in combination, refers to two amino groups joined by a single bond, i.e., —N—N— .

The term “hydroxy,” as used herein, alone or in combination, refers to —OH .

The term “hydroxyalkyl,” as used herein, alone or in combination, refers to a hydroxy group attached to the parent molecular moiety through an alkyl group.

The term “iminohydroxy,” as used herein, alone or in combination, refers to =N(OH) and =N—O— .

The term “lower amino,” as used herein, alone or in combination, refers to —NRR' , wherein R and R' are independently selected from hydrogen and lower alkyl, either of which is optionally substituted.

The term “mercaptal” as used herein, alone or in combination, refers to an RS— group, where R is as defined herein.

The term “nitro,” as used herein, alone or in combination, refers to NO_2 .

The terms “oxy” or “oxa,” as used herein, alone or in combination, refer to —O— .

The term “oxo,” as used herein, alone or in combination, refers to =O .

The term “perhaloalkoxy” refers to an alkoxy group where all of the hydrogen atoms are replaced by halogen atoms.

The term “perhaloalkyl” as used herein, alone or in combination, refers to an alkyl group where all of the hydrogen atoms are replaced by halogen atoms.

The term “ring,” or equivalently, “cycle,” as used herein, in reference to a chemical structure or portion thereof, means a group in which every atom is a member of a common cyclic structure. A ring can be saturated or unsaturated, including aromatic, unless otherwise provided, and may have between 3 and 9 members. If the ring is a heterocycle, it may contain between 1 and 4 heteroatoms or heteroatom-comprising groups selected from B, N, O, S, C(O), S(O)_m. Unless specifically prohibited, a ring is optionally substituted.

The terms “sulfonate,” “sulfonic acid,” and “sulfonic,” as used herein, alone or in combination, refer to the $\text{—SO}_3\text{H}$ group and its anion as the sulfonic acid is used in salt formation.

The term “sulfanyl,” as used herein, alone or in combination, refers to —S— .

The term “sulfinyl,” as used herein, alone or in combination, refers to —S(O)— .

The term “sulfonyl,” as used herein, alone or in combination, refers to $\text{—S(O)}_2\text{—}$.

The term “N-sulfonamido” refers to a $\text{RS(=O)}_2\text{NR'—}$ group with R and R' as defined herein.

The term “S-sulfonamido” refers to a $\text{—S(=O)}_2\text{NRR'}$ group, with R and R' as defined herein.

The term “tautomer,” as used herein, alone or in combination, refers to one of two or more isomers that rapidly interconvert. Generally, this interconversion is sufficiently fast so that an individual tautomer is not isolated in the absence of another tautomer. The ratio of the amount of tautomers can be dependent on solvent composition, ionic strength, and pH, as well as other solution parameters. The ratio of the amount of tautomers can be different in a particular solution and in the microenvironment of a biomolecular binding site in said solution. Examples of tautomers that are well known in the art include keto/enol, enamine/imine, and lactam/lactim tautomers. Examples of tautomers that are well known in the art also include 2-hydroxypyridine/2(1H)-pyridone and 2-aminopyridine/2(1H)-iminopyridone tautomers.

The terms “thia” and “thio,” as used herein, alone or in combination, refer to a —S— group or an ether wherein the oxygen is replaced with sulfur. The oxidized derivatives of the thio group, namely sulfinyl and sulfonyl, are included in the definition of thia and thio.

The term “thiol,” as used herein, alone or in combination, refers to an —SH group.

The term “thiocarbonyl,” as used herein, when alone includes thioformyl —C(S)H and in combination is a —C(S)— group.

The term “N-thiocarbamyl” refers to an ROC(S)NR' group, with R and R' as defined herein.

The term “O-thiocarbamyl” refers to a —OC(S)NRR' group with R and R' as defined herein.

The term “thiocyanato” refers to a —CNS group.

Any definition herein may be used in combination with any other definition to describe a composite structural group. By convention, the trailing element of any such definition is that which attaches to the parent moiety. For example, the composite group alkylamido would represent an alkyl group attached to the parent molecule through an amido group, and

the term alkoxyalkyl would represent an alkoxy group attached to the parent molecule through an alkyl group.

When a group is defined to be “null,” what is meant is that said group is absent.

The term “optionally substituted” means the antecedent group may be substituted or unsubstituted. When substituted, the substituents of an “optionally substituted” group may include, without limitation, one or more substituents independently selected from the following groups or a particular designated set of groups, alone or in combination: lower alkyl, lower alkenyl, lower alkynyl, lower alkanoyl, lower heteroalkyl, lower haloalkyl, lower haloalkenyl, lower haloalkynyl, lower perhaloalkyl, lower perhaloalkoxy, phenyl, aryl, aryloxy, lower alkoxy, lower haloalkoxy, oxo, lower acyloxy, carbonyl, carboxyl, lower alkylcarbonyl, lower carboxyester, lower carboxamido, cyano, hydrogen, halogen, hydroxy, amino, lower alkylamino, arylamino, amido, nitro, thiol, lower alkylthio, lower haloalkylthio, lower perhaloalkylthio, arylthio, sulfonate, sulfonic acid, trisubstituted silyl, N_3 , SH, SCH_3 , $C(O)CH_3$, CO_2CH_3 , CO_2H , pyridinyl, thiophene, furanyl, lower carbamate, and lower urea. Where structurally feasible, two substituents may be joined together to form a fused five-, six-, or seven-membered carbocyclic or heterocyclic ring consisting of zero to three heteroatoms, for example forming methylenedioxy or ethylenedioxy. An optionally substituted group may be unsubstituted (e.g., $-CH_2CH_3$), fully substituted (e.g., $-CF_2CF_3$), monosubstituted (e.g., $-CH_2CH_2F$) or substituted at a level anywhere in-between fully substituted and monosubstituted (e.g., $-CH_2CF_3$). Where substituents are recited without qualification as to substitution, both substituted and unsubstituted forms are encompassed. Where a substituent is qualified as “substituted,” the substituted form is specifically intended. Additionally, different sets of optional substituents to a particular moiety may be defined as needed; in these cases, the optional substitution will be as defined, often immediately following the phrase, “optionally substituted with.”

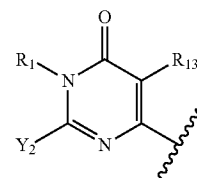
The term R or the term R', appearing by itself and without a number designation, unless otherwise defined, refers to a moiety selected from hydrogen, alkyl, cycloalkyl, heteroalkyl, aryl, heteroaryl and heterocycloalkyl, any of which is optionally substituted. Such R and R' groups should be understood to be optionally substituted as defined herein. Whether an R group has a number designation or not, every R group, including R, R' and R'' where $n=(1, 2, 3, \dots n)$, every substituent, and every term should be understood to be independent of every other in terms of selection from a group. Should any variable, substituent, or term (e.g. aryl, heterocycle, R, etc.) occur more than one time in a formula or generic structure, its definition at each occurrence is independent of the definition at every other occurrence. Those of skill in the art will further recognize that certain groups may be attached to a parent molecule or may occupy a position in a chain of elements from either end as written. For example, an unsymmetrical group such as $-C(O)N(R)-$ may be attached to the parent moiety at either the carbon or the nitrogen.

The term “bond” refers to a covalent linkage between two atoms, or two moieties when the atoms joined by the bond are considered to be part of larger substructure. A bond may be single, double, or triple unless otherwise specified. A dashed line between two atoms in a drawing of a molecule indicates that an additional bond may be present or absent at that position.

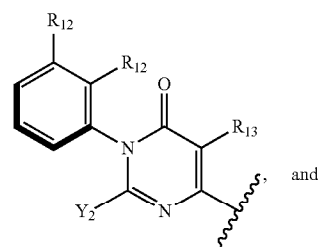
Asymmetric centers exist in the compounds disclosed herein. These centers are designated by the symbols “R” or

“S,” depending on the configuration of substituents around the chiral carbon atom. It should be understood that the invention encompasses all stereochemical isomeric forms, including diastereomeric, enantiomeric, and epimeric forms, as well as d-isomers and l-isomers, and mixtures thereof. Individual stereoisomers of compounds can be prepared synthetically from commercially available starting materials which contain chiral centers or by preparation of mixtures of enantiomeric products followed by separation such as conversion to a mixture of diastereomers followed by separation or recrystallization, chromatographic techniques, direct separation of enantiomers on chiral chromatographic columns, or any other appropriate method known in the art. Starting compounds of particular stereochemistry are either commercially available or can be made and resolved by techniques known in the art. Additionally, the compounds disclosed herein may exist as geometric isomers. The present invention includes all cis, trans, syn, anti, entgegen (E), and zusammen (Z) isomers as well as the appropriate mixtures thereof. Additionally, compounds may exist as tautomers; all tautomeric isomers are provided by this invention. Additionally, the compounds disclosed herein can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. In general, the solvated forms are considered equivalent to the unsolvated forms.

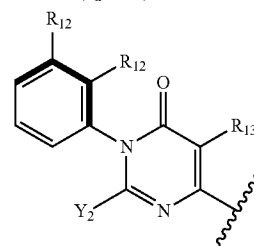
Conformational isomers exist in the compounds disclosed herein. When R_1 is aryl or heteroaryl in the formula:



the aryl or heteroaryl group can orient in different conformations in relation to the pyrimidinone moiety, as represented by:



(S_a form)



(R_a form)

These forms are designated by the symbols "S_a" or "R_a", depending on the conformation of the aryl or heteroaryl group in relation to the pyrimidinone moiety. Examples of "S_a" and "R_a" forms can be found in Examples 1-20.

The term "disease" as used herein is intended to be generally synonymous, and is used interchangeably with, the terms "disorder," "syndrome," and "condition" (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

The term "combination therapy" means the administration of two or more therapeutic agents to treat a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients or in multiple, separate capsules for each active ingredient. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

"PTPN11 inhibitor" is used herein to refer to a compound that exhibits an IC₅₀ with respect to PTPN11 activity of no more than about 100 μM and more typically not more than about 50 μM, as measured in the PTPN11 assay described generally herein. "IC₅₀" is that concentration of inhibitor which reduces the activity of an enzyme (e.g., PTPN11) to half-maximal level. Certain compounds disclosed herein have been discovered to exhibit inhibition against PTPN11. In certain embodiments, compounds will exhibit an IC₅₀ with respect to PTPN11 of no more than about 50 μM; in further embodiments, compounds will exhibit an IC₅₀ with respect to PTPN11 of no more than about 10 μM; in yet further embodiments, compounds will exhibit an IC₅₀ with respect to PTPN11 of not more than about 1 μM; in yet further embodiments, compounds will exhibit an IC₅₀ with respect to PTPN11 of not more than about 200 μM, as measured in the PTPN11 assay described herein.

The phrase "therapeutically effective" is intended to qualify the amount of active ingredients used in the treatment of a disease or disorder or on the effecting of a clinical endpoint.

The term "therapeutically acceptable" refers to those compounds (or salts, prodrugs, tautomers, zwitterionic forms, etc.) which are suitable for use in contact with the tissues of patients without undue toxicity, irritation, and allergic response, are commensurate with a reasonable benefit/risk ratio, and are effective for their intended use.

As used herein, reference to "treatment" of a patient is intended to include prophylaxis. Treatment may also be preemptive in nature, i.e., it may include prevention of disease. Prevention of a disease may involve complete protection from disease, for example as in the case of prevention of infection with a pathogen, or may involve prevention of disease progression. For example, prevention of a disease may not mean complete foreclosure of any effect related to the diseases at any level, but instead may mean prevention of the symptoms of a disease to a clinically significant or detectable level. Prevention of diseases may also mean prevention of progression of a disease to a later stage of the disease.

The term "patient" is generally synonymous with the term "subject" and includes all mammals including humans. Examples of patients include humans, livestock such as

cows, goats, sheep, pigs, and rabbits, and companion animals such as dogs, cats, rabbits, and horses. Preferably, the patient is a human.

The term "prodrug" refers to a compound that is made more active in vivo. Certain compounds disclosed herein may also exist as prodrugs. Prodrugs of the compounds described herein are structurally modified forms of the compound that readily undergo chemical changes under physiological conditions to provide the compound. Additionally, prodrugs can be converted to the compound by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to a compound when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent. Prodrugs are often useful because, in some situations, they may be easier to administer than the compound, or parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. A wide variety of prodrug derivatives are known in the art, such as those that rely on hydrolytic cleavage or oxidative activation of the prodrug. An example, without limitation, of a prodrug would be a compound which is administered as an ester (the "prodrug"), but then is metabolically hydrolyzed to the carboxylic acid, the active entity. Additional examples include peptidyl derivatives of a compound.

The compounds disclosed herein can exist as therapeutically acceptable salts. The present invention includes compounds listed above in the form of salts, including acid addition salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts will normally be pharmaceutically acceptable. However, salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Basic addition salts may also be formed and be pharmaceutically acceptable.

The term "therapeutically acceptable salt," as used herein, represents salts or zwitterionic forms of the compounds disclosed herein which are water or oil-soluble or dispersible and therapeutically acceptable as defined herein. The salts can be prepared during the final isolation and purification of the compounds or separately by reacting the appropriate compound in the form of the free base with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, L-ascorbate, aspartate, benzoate, benzenesulfonate (besylate), bisulfate, butyrate, camphorate, camphorsulfonate, citrate, digluconate, formate, fumarate, gentisate, glutarate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hippurate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate (isethionate), lactate, maleate, malonate, DL-mandelate, mesitylenesulfonate, methanesulfonate, naphthalenesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphonate, picrate, pivalate, propionate, pyroglutamate, succinate, sulfonate, tartrate, L-tartrate, trichloroacetate, trifluoroacetate, phosphate, glutamate, bicarbonate, para-toluenesulfonate (p-tosylate), and undecanoate. Also, basic groups in the compounds disclosed herein can be quaternized with methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides; dimethyl, diethyl, dibutyl, and diamyl sulfates; decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides; and benzyl and phenethyl bromides. Examples of acids which can be employed to form therapeutically acceptable addition salts include inorganic acids such as hydrochloric, hydrobromic, sulfuric, and phosphoric, and organic acids such as oxalic, maleic, succinic, and citric. Salts can also be formed

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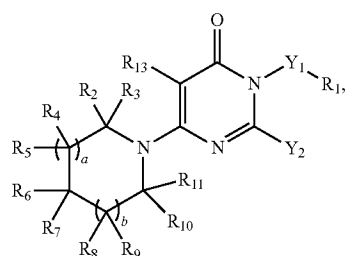
by coordination of the compounds with an alkali metal or alkaline earth ion. Hence, the present invention contemplates sodium, potassium, magnesium, and calcium salts of the compounds disclosed herein, and the like.

Basic addition salts can be prepared during the final isolation and purification of the compounds by reacting a carboxy group with a suitable base such as the hydroxide, carbonate, or bicarbonate of a metal cation or with ammonia or an organic primary, secondary, or tertiary amine. The cations of therapeutically acceptable salts include lithium, sodium, potassium, calcium, magnesium, and aluminum, as well as nontoxic quaternary amine cations such as ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributylamine, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, N,N-dibenzylphenethylamine, 1-phenamine, and N,N-dibenzylethylenediamine. Other representative organic amines useful for the formation of base addition salts include ethylenediamine, ethanolamine, diethanolamine, piperidine, and piperazine.

A salt of a compound can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

III. Compounds

In one aspect, the present invention provides a compound represented by Formula I:



or a salt, ester, or prodrug thereof, wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y_1 is a direct bond or $CR_{17}R_{18}$;

Y_2 is selected from the group consisting of C_{1-4} alkyl, amino, C_{1-4} alkylC(O)O—, C_{1-4} alkylamino and C_{1-4} aminoalkyl;

R_1 is selected from the group consisting of C_{6-10} aryl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R_1 is optionally substituted with 1 to 5 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, $NR_{15}C(O)R_{14}$, $NR_{15}C(O)OR_{14}$, $NR_{14}C(O)NR_{15}R_{16}$, $NR_{15}S(O)R_{14}$, $NR_{15}S(O)OR_{14}$, $C(O)NR_{15}R_{16}$, $S(O)NR_{15}R_{16}$, $S(O)_2NR_{15}R_{16}$, $C(O)R_{14}$, $C(O)OR_{14}$, OR_{14} , SR_{14} , $S(O)R_{14}$, and $S(O)_2R_{14}$;

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R_2 , R_3 , R_{10} , and R_{11} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, and C_{3-8} cycloalkyl;

R_4 , R_5 , R_8 , and R_9 are each independently selected from the group consisting of hydrogen, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, amino, hydroxy, C_{3-8} cycloalkyl, halo, and C_{1-4} alkylamino;

R_6 is selected from the group consisting of amino, C_{1-4} aminoalkyl, and C_{1-4} alkylamino;

R_7 is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{3-6} cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino, and C_{1-4} aminoalkyl;

or R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated or unsaturated ring formed by R_6 and R_7 is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl;

any two groups of R_2 , R_3 , R_4 , R_5 , R_7 , R_8 , R_9 , R_{10} and R_{11} can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices elected from N, O and S; any two groups of R_2 , R_4 , R_6 , R_8 and R_{10} can form a direct bond, or a 1 or 2 atom carbon bridge;

R_{13} is selected from the group consisting of hydrogen, halo, cyano, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} dihydroxyalkyl, —NH—NHR₁₉, —NHR₁₉, —OR₁₉, —NHC(O)R₁₉, —NHC(O)NHR₁₉, —NHS(O)₂NHR₁₉, —NHS(O)₂R₁₉, —C(O)OR₁₉, —C(O)NR₁₉R₂₀, —C(O)NH(CH₂)_qOH, —C(O)NH(CH₂)_qR₂₁, —C(O)R₂₁, —NH₂, —OH, —S(O)₂NR₁₉R₂₀, C_{3-8} cycloalkyl, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C_{1-4} alkyl, —OH, —NH₂, —OR₂₁, halo, cyano, and oxo;

R_{14} , R_{15} and R_{16} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, C_{3-8} cycloalkyl, C_{6-10} aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of amido, amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl;

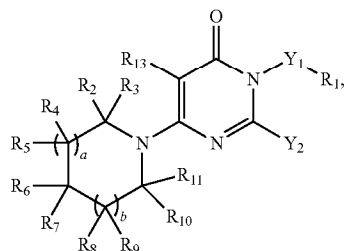
R_{17} and R_{18} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, and CF₃;

R_{19} and R_{20} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and C_{3-6} cycloalkyl; and

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each R_{21} is independently selected from the group consisting of hydrogen, $-\text{OH}$, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{1-5}\text{haloalkyl}$, $\text{C}_{1-6}\text{hydroxyalkyl}$, $\text{C}_{1-6}\text{alkenyl}$, $\text{C}_{1-6}\text{alkynyl}$ and $\text{C}_{3-6}\text{cycloalkyl}$.

In some embodiments, the present invention provides a compound represented by Formula I:



or a salt, ester, or prodrug thereof, wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y_1 is a direct bond or $\text{CR}_{17}\text{R}_{18}$;

Y_2 is selected from the group consisting of $\text{C}_{1-4}\text{alkyl}$, amino, $\text{C}_{1-4}\text{alkylC(O)O}-$, $\text{C}_{1-4}\text{alkylamino}$ and $\text{C}_{1-4}\text{aminoalkyl}$;

R_1 is selected from the group consisting of $\text{C}_{6-10}\text{aryl}$, $\text{C}_{3-8}\text{cycloalkyl}$, $\text{C}_{3-8}\text{cycloalkenyl}$, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R_1 is optionally substituted with 1 to 5 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, $\text{C}_{1-4}\text{alkylamino}$, $\text{di}(\text{C}_{1-4}\text{alkyl})\text{amino}$, cyano, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{1-4}\text{alkoxy}$, $\text{C}_{1-4}\text{hydroxyalkyl}$, $\text{C}_{1-4}\text{haloalkyl}$, $\text{C}_{1-4}\text{aminoalkyl}$, $\text{C}_{3-8}\text{cycloalkyl}$, $\text{C}_{3-8}\text{cycloalkenyl}$, $\text{NR}_{15}\text{C(O)R}_{14}$, $\text{NR}_{15}\text{C(O)OR}_{14}$, $\text{NR}_{14}\text{C(O)NR}_{15}\text{R}_{16}$, $\text{NR}_{15}\text{S(O)R}_{14}$, $\text{NR}_{15}\text{S(O)}_2\text{R}_{14}$, $\text{C(O)NR}_{15}\text{R}_{16}$, $\text{S(O)NR}_{15}\text{R}_{16}$, $\text{S(O)}_2\text{NR}_{15}\text{R}_{16}$, C(O)R_{14} , C(O)OR_{14} , OR_{14} , SR_{14} , S(O)R_{14} , and $\text{S(O)}_2\text{R}_{14}$;

R_2 , R_3 , R_{10} , and R_{11} are each independently selected from the group consisting of hydrogen, $\text{C}_{1-4}\text{alkyl}$, and $\text{C}_{3-8}\text{cycloalkyl}$;

R_4 , R_5 , R_8 , and R_9 are each independently selected from the group consisting of hydrogen, cyano, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{1-4}\text{alkoxy}$, amino, hydroxy, $\text{C}_{3-8}\text{cycloalkyl}$, halo, and $\text{C}_{1-4}\text{alkylamino}$;

R_6 is selected from the group consisting of amino, $\text{C}_{1-4}\text{aminoalkyl}$, and $\text{C}_{1-4}\text{alkylamino}$;

R_7 is selected from the group consisting of hydrogen, halo, and hydroxy, or is selected from the group consisting of amido, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{1-4}\text{hydroxyalkyl}$, $\text{C}_{3-6}\text{cycloalkyl}$, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{1-4}\text{alkoxy}$, $\text{C}_{1-4}\text{haloalkyl}$, $\text{C}_{1-4}\text{haloalkoxy}$, $\text{C}_{1-4}\text{alkylamino}$, and $\text{C}_{1-4}\text{aminoalkyl}$;

or R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated ring formed by R_6 and R_7 is unsubstituted or substituted with 1 to 3 groups independently selected

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from the group consisting of amino, halo, hydroxy, cyano, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{1-4}\text{alkoxy}$, $\text{C}_{1-4}\text{haloalkyl}$, $\text{C}_{1-4}\text{haloalkoxy}$, $\text{C}_{1-4}\text{alkylamino}$ and $\text{C}_{1-4}\text{aminoalkyl}$;

any two groups of R_2 , R_3 , R_4 , R_5 , R_7 , R_8 , R_9 , R_{10} and R_{11} can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices selected from N, O and S;

any two groups of R_2 , R_4 , R_6 , R_8 and R_{10} can form a direct bond, or a 1 or 2 atom carbon bridge;

R_{13} is selected from the group consisting of hydrogen, halo, cyano, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{1-6}\text{haloalkyl}$, $\text{C}_{1-6}\text{hydroxyalkyl}$, $\text{C}_{1-6}\text{dihydroxyalkyl}$, $-\text{NH}-\text{NHR}_{19}$, $-\text{NHR}_{19}$, $-\text{OR}_{19}$, $-\text{NHC(O)R}_{19}$, $-\text{NHC(O)NHR}_{19}$, $-\text{NHS(O)}_2\text{NHR}_{19}$, $-\text{NHS(O)}_2\text{R}_{19}$, $-\text{C(O)OR}_{19}$, $-\text{C(O)NR}_{19}\text{R}_{20}$, $-\text{C(O)NH(CH}_2)_q\text{OH}$, $-\text{C(O)NH(CH}_2)_q\text{R}_{21}$, $-\text{C(O)R}_{21}$, $-\text{NH}_2$, $-\text{OH}$, $-\text{S(O)}_2\text{NR}_{19}\text{R}_{20}$, $\text{C}_{3-8}\text{cycloalkyl}$, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of $\text{C}_{1-4}\text{alkyl}$, $-\text{OH}$, $-\text{NH}_2$, $-\text{OR}_{21}$, halo, cyano, and oxo;

R_{14} , R_{15} and R_{16} are each independently selected from the group consisting of hydrogen, $\text{C}_{1-4}\text{alkyl}$, $\text{C}_{3-8}\text{cycloalkyl}$, $\text{C}_{6-10}\text{aryl}$ and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and $\text{C}_{1-4}\text{alkyl}$;

R_{17} and R_{18} are each independently selected from the group consisting of hydrogen, $\text{C}_{1-4}\text{alkyl}$, and CF_3 ;

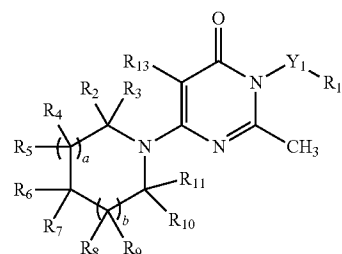
R_{19} and R_{20} are each independently selected from the group consisting of hydrogen, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{1-6}\text{haloalkyl}$, $\text{C}_{1-6}\text{hydroxyalkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$ and $\text{C}_{3-6}\text{cycloalkyl}$; and

each R_{21} is independently selected from the group consisting of hydrogen, $-\text{OH}$, $\text{C}_{1-6}\text{alkyl}$, $\text{C}_{1-6}\text{haloalkyl}$, $\text{C}_{1-6}\text{hydroxyalkyl}$, $\text{C}_{2-6}\text{alkenyl}$, $\text{C}_{2-6}\text{alkynyl}$ and $\text{C}_{3-6}\text{cycloalkyl}$.

In some embodiments of Formula I, Y_1 is a direct bond. In some embodiments, Y_1 is $\text{CR}_{17}\text{R}_{18}$. In some embodiments, R_{17} and R_{18} are each independently selected from the group consisting of hydrogen, $\text{C}_{1-4}\text{alkyl}$, and CF_3 . In some embodiments, R_{17} and R_{18} are each independently hydrogen or $\text{C}_{1-4}\text{alkyl}$. In some embodiments, Y_1 is $-\text{CH}_2$.

In some embodiments of Formula I, Y_2 is $\text{C}_{1-4}\text{alkyl}$. In some embodiments, Y_2 is methyl.

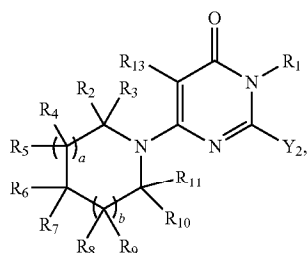
In some embodiments, the compound is represented by Formula Ia:



wherein the subscripts a and b, Y_1 , R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , R_{11} , and R_{13} are as defined and described herein.

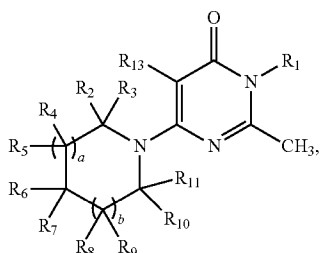
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In some embodiments, the compound is represented by Formula Ib:



wherein the subscripts a and b, Y₂, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₃ are as defined and described herein.

In some embodiments, the compound is represented by Formula Ic:



wherein the subscripts a and b, R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₁, and R₁₃ are as defined and described herein.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, subscripts a and b are each 1.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, C₃₋₈cycloalkyl, 3- or 6-membered heterocyclyl having 1-3 heteroatoms as ring vertices selected from N, O and S; wherein heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C₁₋₄alkyl, —OH, —NH₂, —OR₂₁, halo, cyano and oxo. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, and C₁₋₆haloalkyl. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₄alkyl, and C₁₋₄haloalkyl. In some embodiments, R₁₃ is selected from the group consisting of —CH₂OH, CF₂OH, and —CHFOH. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, Cl, Br, methyl, and CF₃. In some embodiments, R₁₃ is hydrogen. In some embodiments, R₁₃ is Cl. In some embodiments, R₁₃ is Br. In some embodiments, R₁₃ is methyl. In some embodiments, R₁₃ is CF₃.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁ is selected from the group consisting of C₆₋₁₀aryl and a 5- to 9-membered heteroaryl group having 1 to 4 heteroatoms groups as ring vertices independently selected from N, C(O), O, and S; and is optionally substituted with

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1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)N/R₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁ is selected from the group consisting of C₆₋₁₀aryl and a 5- to 9-membered heteroaryl group having 1 to 4 heteroatoms groups as ring vertices independently selected from N, C(O), O, and S; and is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄; and R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of amido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

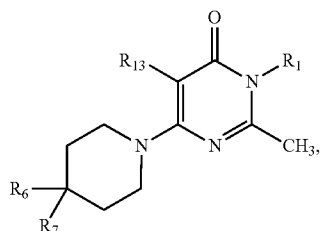
In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁ is selected from the group consisting of C₆₋₁₀aryl and a 5- to 9-membered heteroaryl group having 1 to 4 heteroatoms groups as ring vertices independently selected from N, C(O), O, and S; and is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄; and R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁ is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

In some embodiments of any one of Formulae I, Ia, Ib, and Ic, R₁ is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄; and R₁₄ is selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of amido, amino, halo,

In some embodiments, the compound is represented by Formula II:

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wherein R₁, R₆, R₇, and R₁₃ are as defined and described herein.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino; and R₇ is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to three substituents selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy. In some embodiments, R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino; and R₇ is selected from the group consisting of hydrogen, amido, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one or two substituents selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and methylamino. In some embodiments, R₆ is amino or C₁₋₄aminoalkyl. In certain embodiments, R₆ is amino, aminomethyl, or methylamino. In certain embodiments, R₆ is amino or aminomethyl. In certain embodiments, R₆ is amino. In certain embodiments, R₆ is aminomethyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one or two groups selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy. In some embodiments, R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl. In some embodiments, R₇ is hydroxy, C₁₋₄alkyl, or C₁₋₄hydroxyalkyl. In certain embodiments, R₇ is C₁₋₄alkyl. In certain embodiments, R₇ is methyl. In certain embodiments, R₇ is ethyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is C₁₋₄aminoalkyl; and R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to three groups independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is aminomethyl; and R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl.

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(II)

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is amino; and R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to three groups independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is amino; and R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ is amino; and R₇ is C₁₋₄hydroxyalkyl. In some embodiments, R₆ is amino; and R₇ is hydroxymethyl. In some embodiments, R₆ is amino; and R₇ is C₁₋₄alkyl. In certain embodiments, R₆ is amino; and R₇ is methyl. In some embodiments, R₆ is aminomethyl; and R₇ is C₁₋₄alkyl. In certain embodiments, R₆ is aminomethyl; and R₇ is methyl. In some embodiments, R₆ is aminomethyl; and R₇ is ethyl.

In any of the above embodiments, the amido of R₇ may specifically be C(O)NH₂.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are both attached form a 4- to 6-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are both attached form a 4- to 6-membered saturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are both attached form a 4- to 6-membered saturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₆ and R₇ together with the carbon atom to which they are

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected

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from N, O, and S; and is optionally substituted with 1, 2 or 3 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$; R_6 is amino or aminomethyl; and R_7 is selected from the group consisting of hydroxy, $C_{1-4}alkyl$, and $C_{1-4}hydroxyalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$; and R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}alkylamino$ and $C_{1-4}aminoalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$; and R_6 and R_7 together with the carbon atom to which they are both attached form a 4- to 6-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}alkylamino$ and $C_{1-4}aminoalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$; and R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered cycloalkyl ring that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}alkylamino$ and $C_{1-4}aminoalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of phenyl, pyridyl, pyrimidinyl, pyrazolyl, pyrazinyl, pyridazinyl, and 1,2,4-triazinyl; and is optionally substituted with 1, 2, or 3 R_{12} , wherein each R_{12} is as defined and described herein. In some embodiments, R_1 is selected from the group consisting of phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, and 1,2,4-triazinyl; and is optionally substituted with 1, 2, or 3 R_{12} , wherein each R_{12} is as defined and described herein. In some embodiments, R_1 is phenyl or pyridyl; and is optionally substituted with 1, 2, or 3 R_{12} , wherein each R_{12} is as defined and described herein.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, and 1,2,4-triazinyl; and is optionally substituted with 1, 2, or 3 R_{12} independently selected from the group consisting of halo,

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hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, $C_{1-4}aminoalkyl$, and OR₁₄.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, and 1,2,4-triazinyl; and is optionally substituted with 1, 2, or 3 R_{12} independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$.

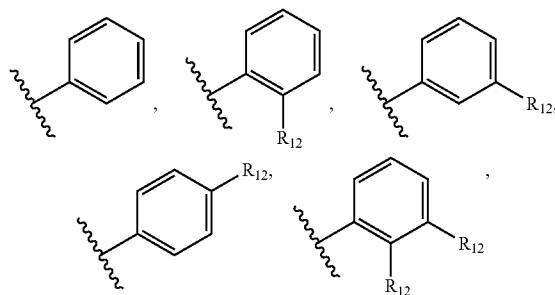
In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl or pyridyl, each of which is optionally substituted with 1, 2, or 3 R_{12} independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, $C_{1-4}aminoalkyl$, and OR₁₄.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl or pyridyl, each of which is optionally substituted with 1, 2, or 3 R_{12} independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is phenyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, $C_{1-4}aminoalkyl$, and OR₁₄. In some embodiments, R_1 is phenyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, $C_{1-4}alkyl$, $C_{1-4}haloalkyl$, and $C_{1-4}alkoxy$. In some embodiments, R_1 is phenyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, $C_{1-4}alkyl$, and $C_{1-4}alkoxy$.

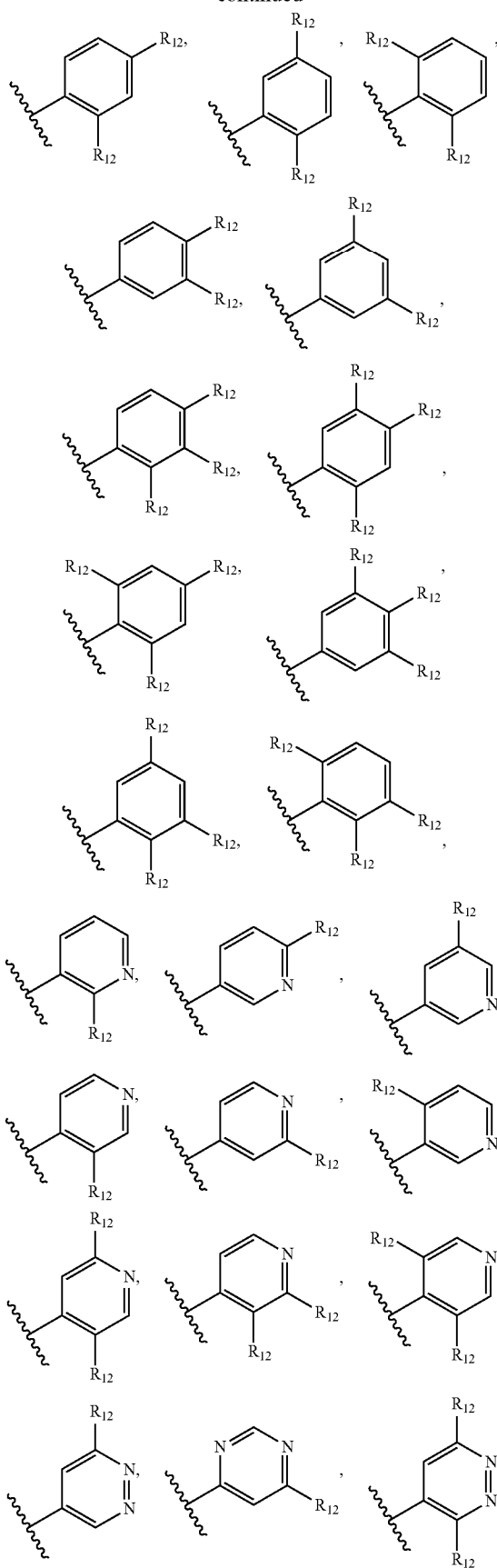
In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is pyridyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, amino, $C_{1-4}alkylamino$, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, $C_{1-4}aminoalkyl$, and OR₁₄. In some embodiments, R_1 is pyridyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, $C_{1-4}alkyl$, $C_{1-4}haloalkyl$, and $C_{1-4}alkoxy$. In some embodiments, R_1 is pyridyl and is optionally substituted with 1-3 R_{12} , each of which is independently selected from the group consisting of halo, hydroxy, $C_{1-4}alkyl$, and $C_{1-4}alkoxy$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of:



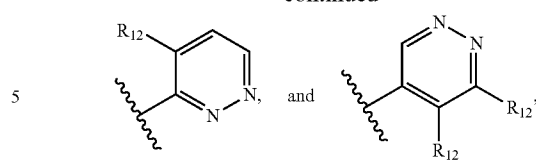
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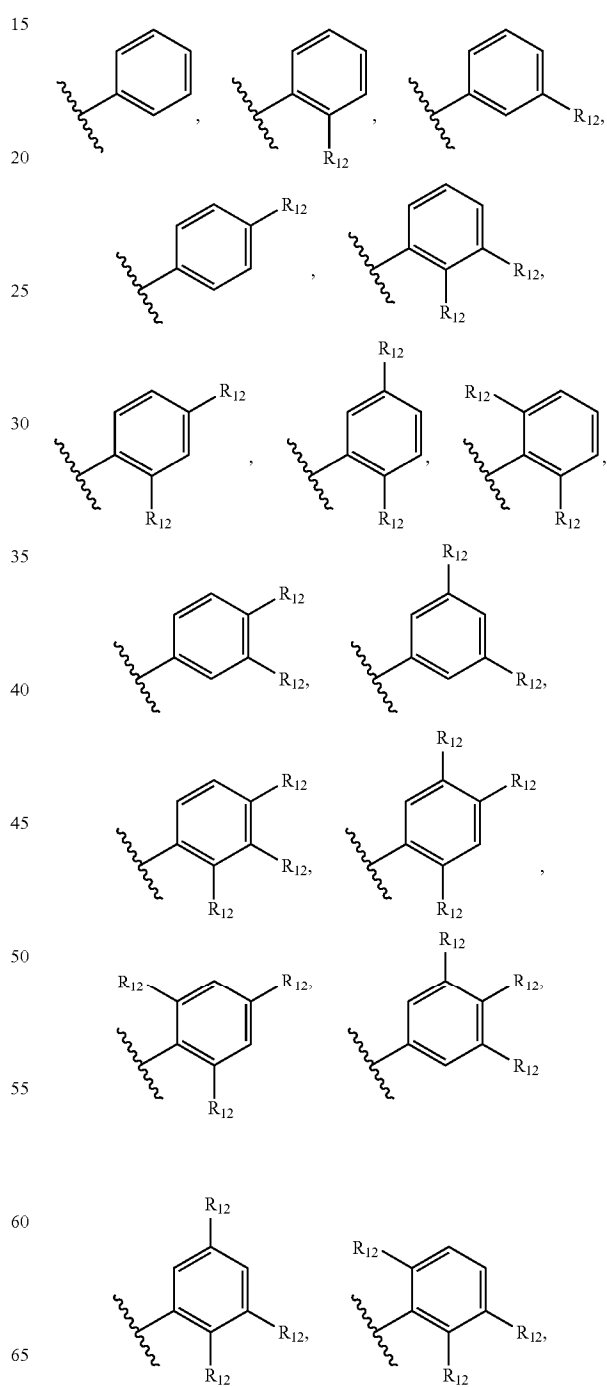
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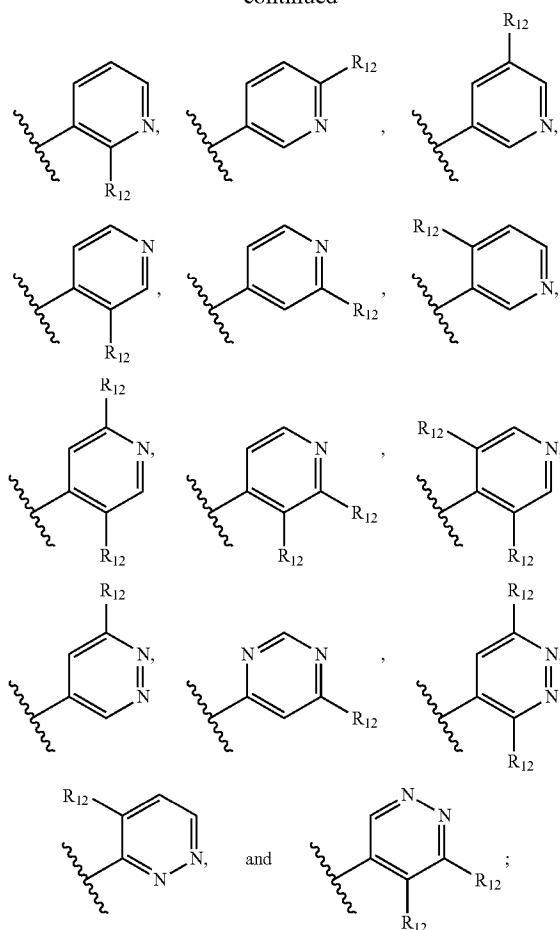
wherein each R_{12} is as defined and described herein.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of:



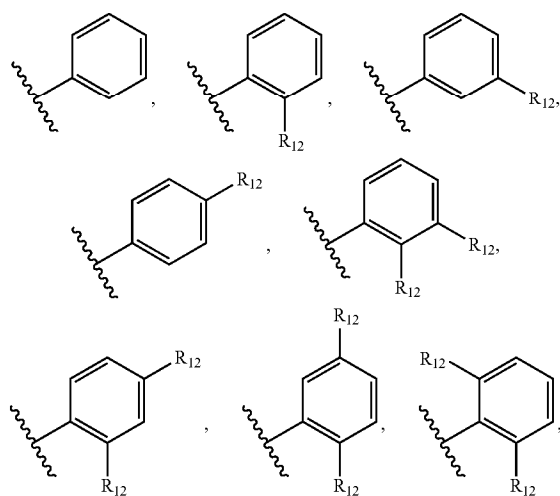
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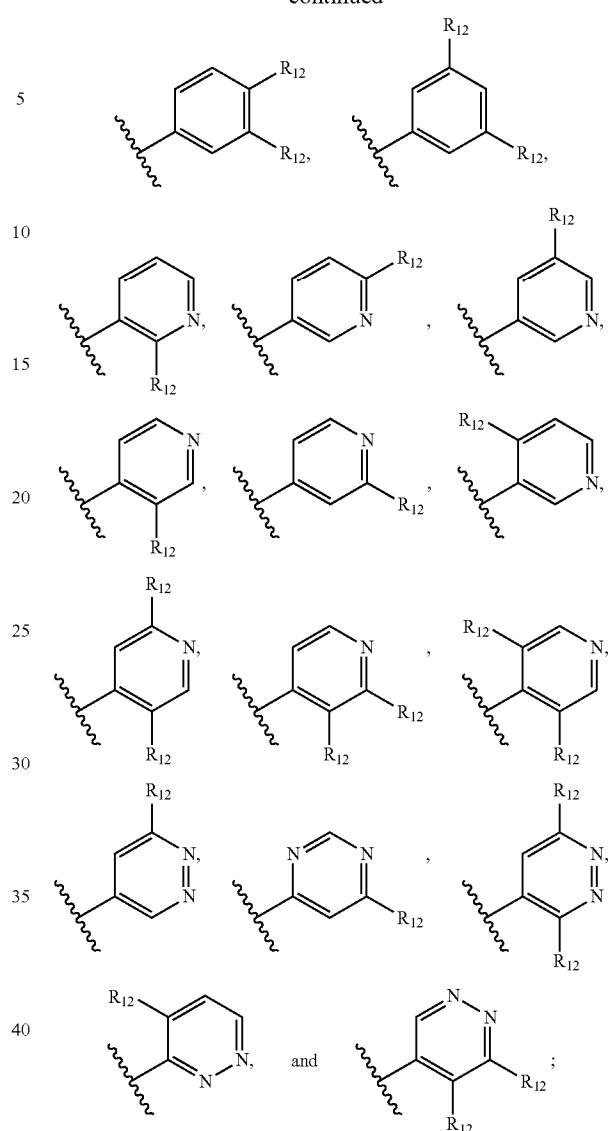


and each R_{12} is independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, $C_{1-4}aminoalkyl$, and OR_{14} , wherein R_{14} is as defined and described herein.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of:

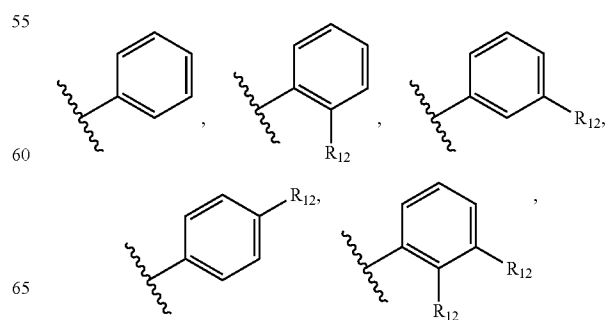
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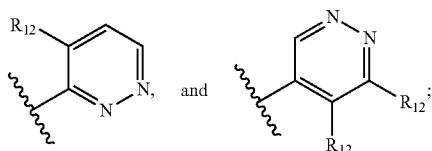
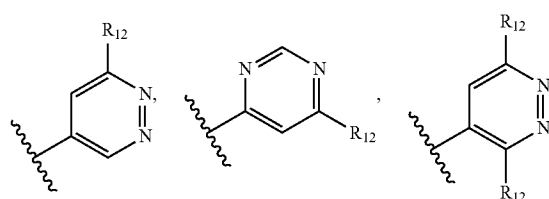
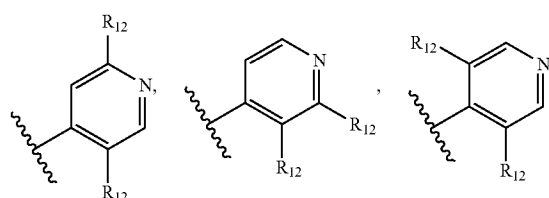
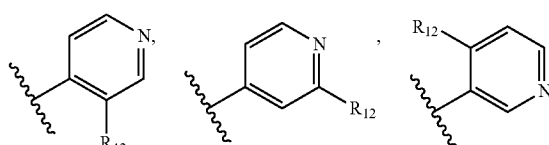
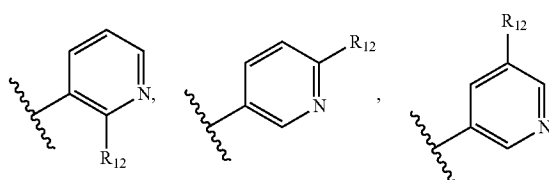
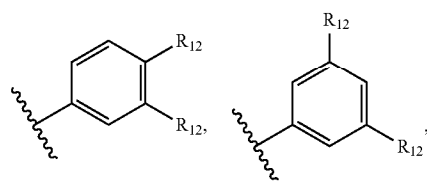
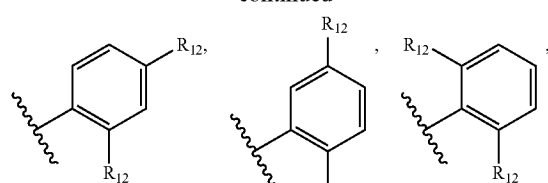
and each R_{12} is independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, $di(C_{1-4}alkyl)amino$, $C_{1-4}alkyl$, $C_{1-4}alkoxy$, $C_{1-4}haloalkyl$, and $C_{1-4}aminoalkyl$.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R_1 is selected from the group consisting of:



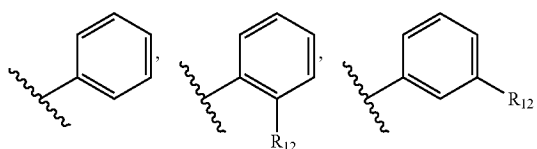
31

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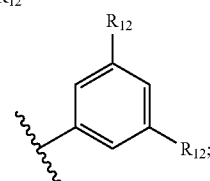
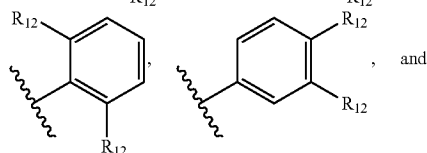
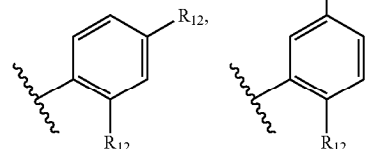
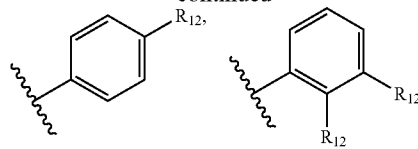
and each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is selected from the group consisting of:



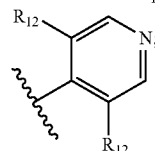
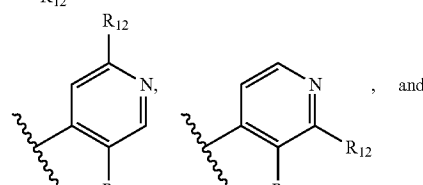
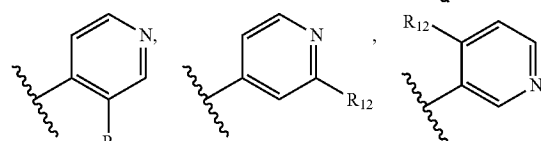
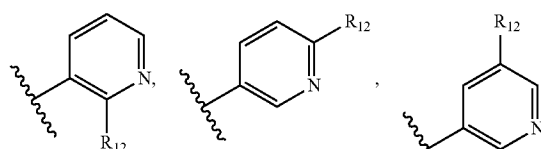
32

-continued



and each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is selected from the group consisting of:



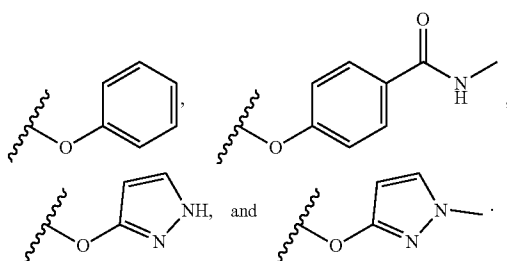
and each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy.

In some embodiments of any one of Formulae I, Ia-Ic, and II, each R₁₂ is independently selected from the group con-

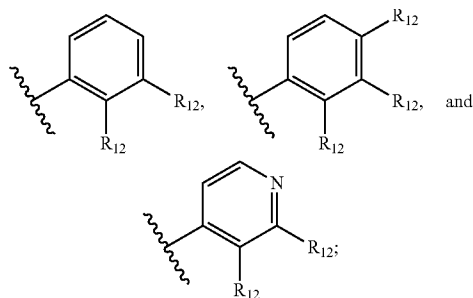
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sisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄. In some embodiments, each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄. In some embodiments, each R₁₁ is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy. In some embodiments, each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₄haloalkyl. In some embodiments, each R₁₂ independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃.

In some embodiments of any one of Formulae I, Ia-Ic, and II, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, CF₃,

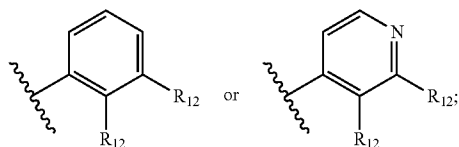


In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is selected from the group consisting of:



and each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄, wherein R₁₄ is as defined and described herein.

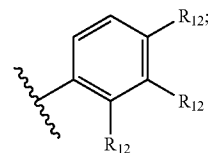
In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:



and each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₄haloalkyl. In some embodiments, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃. In some embodiments, each R₁₂ is Cl.

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In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:



and each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₄haloalkyl. In some embodiments, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃. In some embodiments, each R₁₂ is independently Cl or Br.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁₄ is independently selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of C₁₋₄alkylamido, amino, halo, hydroxy, cyano, C₁₋₄alkoxy, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl. In some embodiments, R₁₄ is independently selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is independently selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or two groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl.

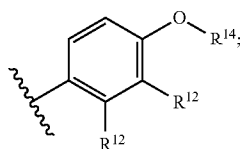
In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁₄ is independently phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or more groups independently selected from the group consisting of C₁₋₄alkylamido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl. In some embodiments, R₁₄ is independently phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is independently phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is phenyl, optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is phenyl, substituted with C₁₋₄alkylamido. In some embodiments, R₁₄ is phenyl substituted with —C(O)NHMe. In some embodiments, R₁₄ is phenyl. In some embodiments, R₁₄ is pyrazolyl, optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄al-

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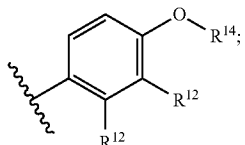
kylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is pyrazolyl substituted with C₁₋₄alkyl. In some embodiments, R₁₄ is pyrazolyl substituted with methyl. In some embodiments, R₁₄ is N-methylpyrazolyl. In some embodiments, R₁₄ is pyrazolyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:



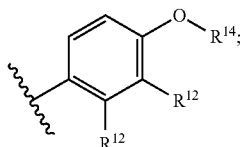
each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl) amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and C₁₋₄aminoalkyl; and R₁₄ is phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:



each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and R₁₄ is phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

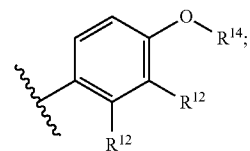
In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:



each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and R₁₄ is selected from the group consisting of phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

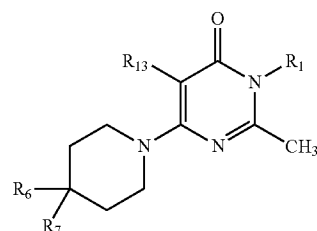
In some embodiments of any one of Formulae I, Ia-Ic, and II, R₁ is represented by:

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each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and R₁₄ is selected from the group consisting of phenyl, phenyl substituted with C₁₋₄alkylamido, pyrazolyl, and pyrazolyl substituted with C₁₋₄alkyl. In some embodiments, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃; and R₁₄ is selected from the group consisting of phenyl, MeNHC(O)-phenyl, pyrazolyl, and N-methylpyrazolyl. In some embodiments, each R₁₂ is Cl; and R₁₄ is selected from the group consisting of phenyl, MeNHC(O)-phenyl, pyrazolyl, and N-methylpyrazolyl.

In certain embodiments, the compound is represented by Formula II:



or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄;

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

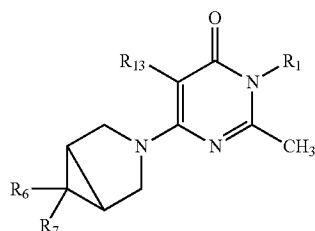
R₇ is selected from the group consisting of hydrogen, cyano, amido, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to three substituents independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl; and

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

In certain embodiments, the compound is represented by Formula III:

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or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄;

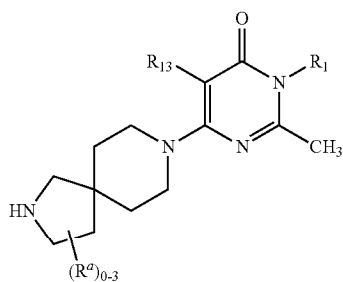
R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

R₇ is selected from the group consisting of hydrogen, cyano, amido, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to three substituents independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl; and

R₁₃ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

In certain embodiments, the compound is represented by Formula IV:



or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄;

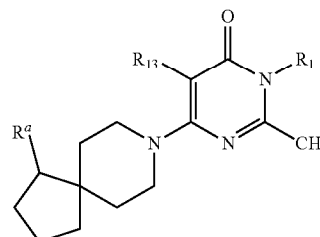
R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

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each IV is independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In certain embodiments, the compound is represented by Formula V:



or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

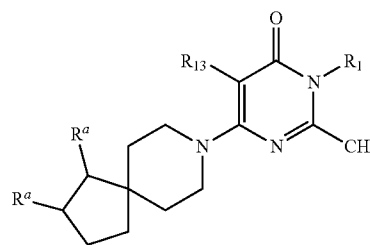
each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

R^a is selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In certain embodiments, the compound is represented by Formula VI:



or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

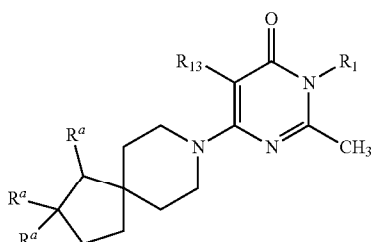
R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

each R^a is independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

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In certain embodiments, the compound is represented by Formula VII:



(VII) 5

or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

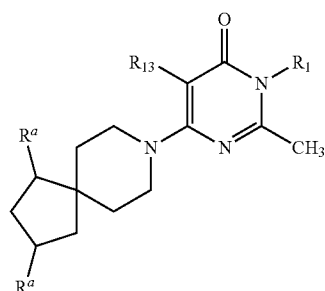
each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

each R^a is independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In certain embodiments, the compound is represented by Formula VIII:



(VIII) 5

or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

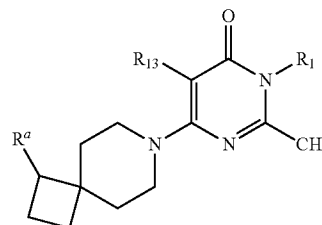
R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

each R^a is independently selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In certain embodiments, the compound is represented by Formula IX:

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(IX)



or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

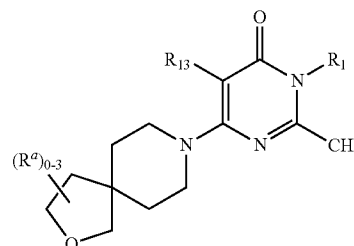
each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

R^a is selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

In certain embodiments, the compound is represented by Formula X:



(X) 35

or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and OR₁₄; and

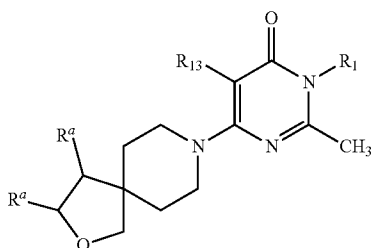
R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

each R^a is independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In certain embodiments, the compound is represented by Formula XI:

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or a salt, ester or prodrug thereof, wherein:

R₁ is phenyl or pyridyl, each of which is substituted with 0 to 3 R₁₂;

each R₁₂ is independently selected from the group consisting of halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and Or₁₄; and

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl;

R₁₄ is phenyl or pyrazolyl, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl; and

each R^a is independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

In some embodiment of any one of Formulae II-XI, R₁, R₆, R₇, R₁₂, R₁₃, and R₁₄ may have the meanings set forth in any one or more of the selected embodiments noted above.

In some embodiments of any one of Formulae II-XI, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, and C₃₋₈cycloalkyl. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, and C₁₋₆haloalkyl. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₄alkyl, and C₁₋₄haloalkyl. In some embodiments, R₁₃ is selected from the group consisting of hydrogen, Cl, Br, methyl, and CF₃. In some embodiments, R₁₃ is hydrogen. In some embodiments, R₁₃ is Cl. In some embodiments, R₁₃ is Br. In some embodiments, R₁₃ is methyl. In some embodiments, R₁₃ is CF₃.

In some embodiments of any one of Formulae II-XI, R₁ is phenyl or pyridyl, each of which is substituted with 1 to 3 R₁₂. In some embodiments, R₁ is phenyl or pyridyl, each of which is substituted with 2 or 3 R₁₂. In some embodiments, R₁ is phenyl substituted with 2 or 3 R₁₂. In some embodiments, R₁ is phenyl substituted with 2 R₁₂. In some embodiments, R₁ is phenyl substituted with 3 R₁₂. In some embodiments, R₁ is pyridyl substituted with 2 R₁₂.

In some embodiments of any one of Formulae II-XI, each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and Or₁₄. In some embodiments, each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, methylamino, dimethylamino, cyano, C₁₋₄alkyl, C₁₋₄haloalkyl, and C₁₋₄alkoxy. In some embodiments, each R₁₂ is independently selected from the group consisting of halo, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₄haloalkyl. In some embodiments, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃.

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(XI)

In some embodiments of any one of Formulae II-XI, each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, CF₃,

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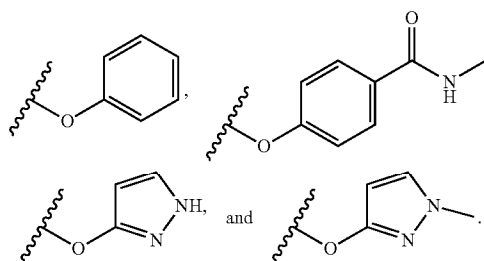
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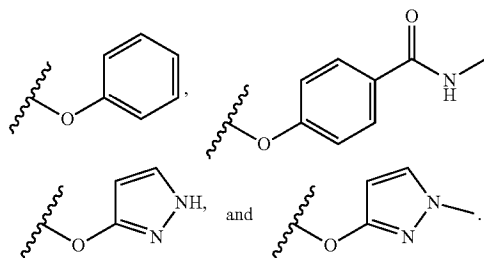
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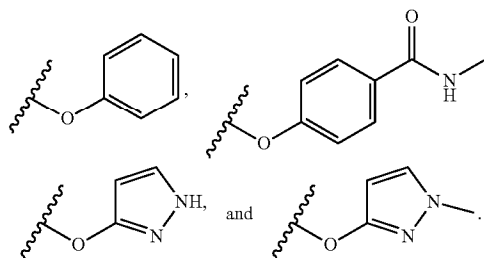


In some embodiments of any one of Formulae II-XI, R₁ is phenyl substituted with 2 R₁₂; and each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, and CF₃. In some embodiments, R₁ is phenyl substituted with 2 R₁₂; and each R₁₂ is Cl.

In some embodiments of any one of Formulae II-XI, R₁ is phenyl substituted with 3 R₁₂; and each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, CF₃,



In some embodiments of any one of Formulae II-XI, R₁ is phenyl substituted with 3 R₁₂; the first and second R₁₂ are each Cl; and the third R₁₂ is Br. In some embodiments, R₁ is phenyl substituted with 3 R₁₂; the first and second R₁₂ are each Cl; and the third R₁₂ is selected from the group consisting of:



In some embodiments of any one of Formulae II-XI, In some embodiments, R₁₄ is phenyl, optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is phenyl, substituted with C₁₋₄alkylamido. In some embodiments, R₁₄ is phenyl substituted with —C(O)NHMe. In some embodiments, R₁₄ is phenyl. In some embodiments, R₁₄ is pyrazolyl, optionally substituted by one or two groups independently selected

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from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl. In some embodiments, R₁₄ is pyrazolyl substituted with C₁₋₄alkyl. In some embodiments, R₁₄ is pyrazolyl substituted with methyl. In some embodiments, R₁₄ is N-methylpyrazolyl. In some embodiments, R₁₄ is pyrazolyl.

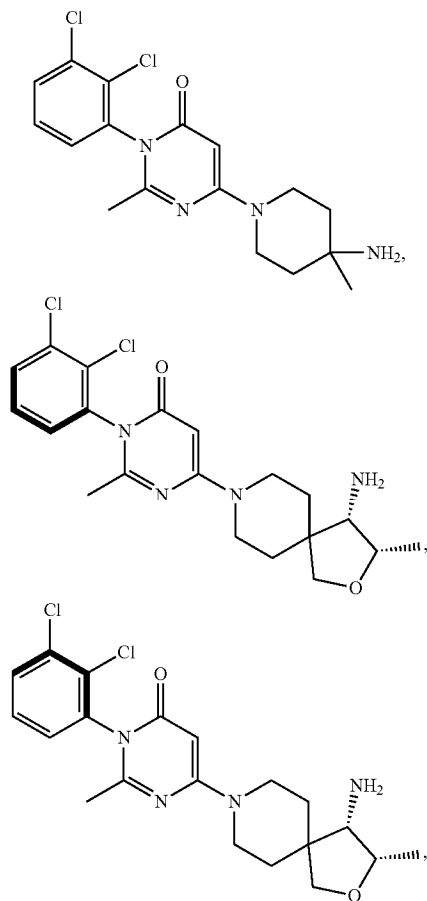
In some embodiments of Formula II or III, R₆ is amino or C₁₋₄aminoalkyl. In certain embodiments, R₆ is amino or aminomethyl. In certain embodiments, R₆ is amino. In certain embodiments, R₆ is aminomethyl.

In some embodiments of Formula II or III, R₇ is hydroxy, C₁₋₄alkyl, or C₁₋₄hydroxyalkyl. In certain embodiments, R₇ is C₁₋₄alkyl. In certain embodiments, R₇ is methyl. In certain embodiments, R₇ is ethyl.

In some embodiments of Formula II or III, R₆ is amino; and R₇ is C₁₋₄alkyl. In certain embodiments, R₆ is amino; and R₇ is methyl. In some embodiments, R₆ is amino; and R₇ is ethyl. In some embodiments, R₆ is aminomethyl; and R₇ is C₁₋₄alkyl. In certain embodiments, R₆ is aminomethyl; and R₇ is methyl.

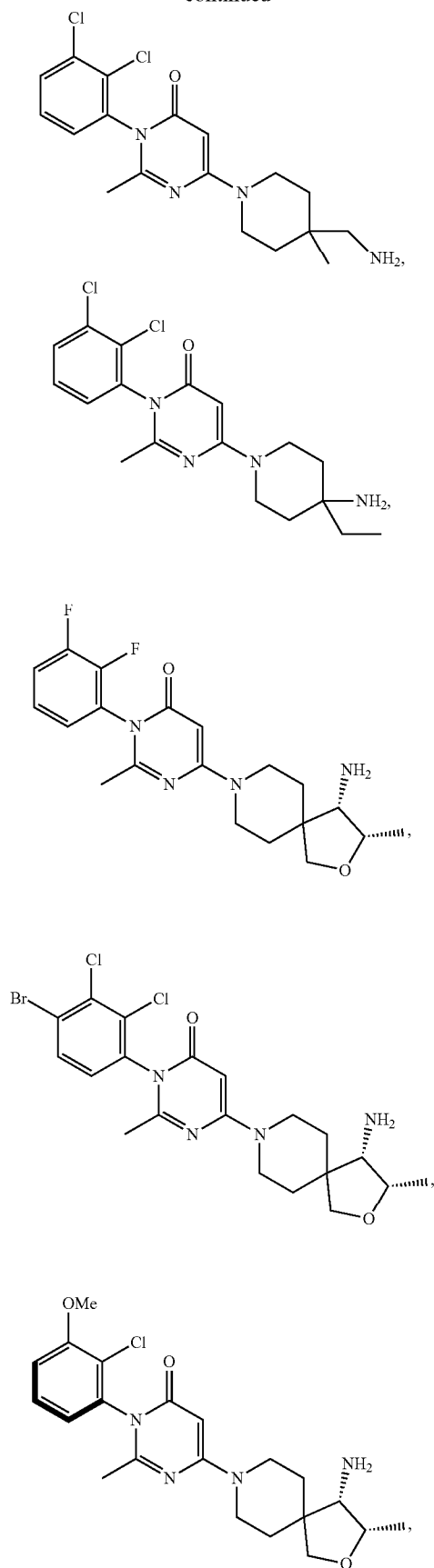
In some embodiments of any one of Formulae IV-XI, each IV is independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, and C₁₋₄haloalkyl. In some embodiments, each IV is independently amino or C₁₋₄alkyl. In some embodiments, each IV is independently amino or methyl.

In some embodiments, the compound is represented by the formula selected from the group consisting of:



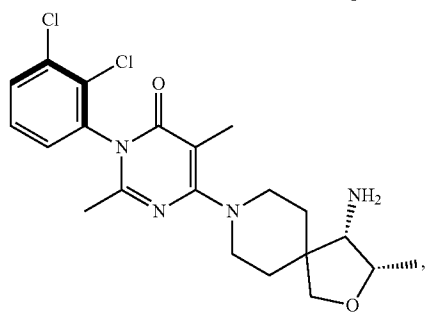
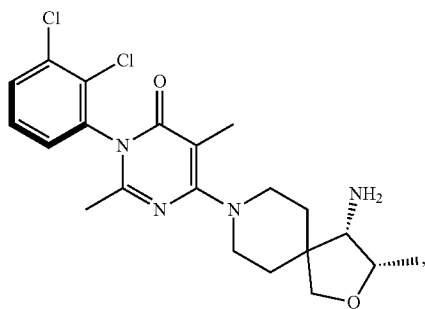
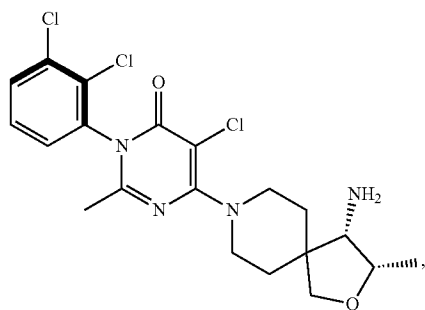
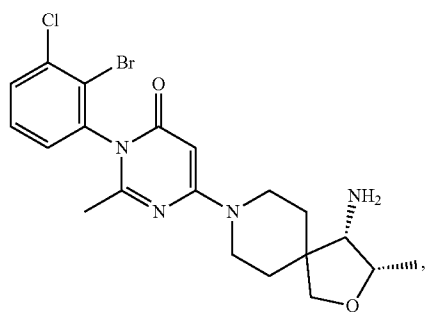
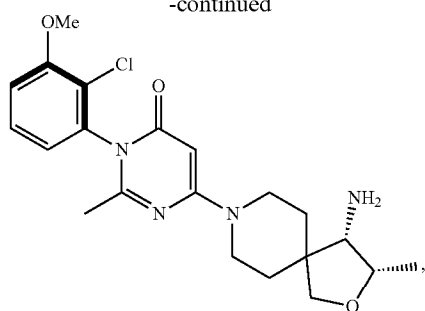
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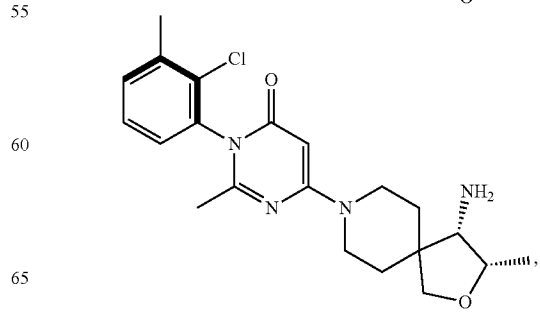
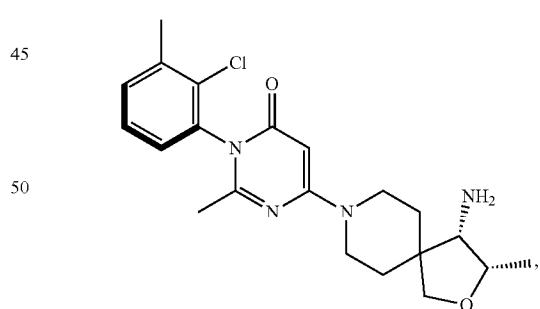
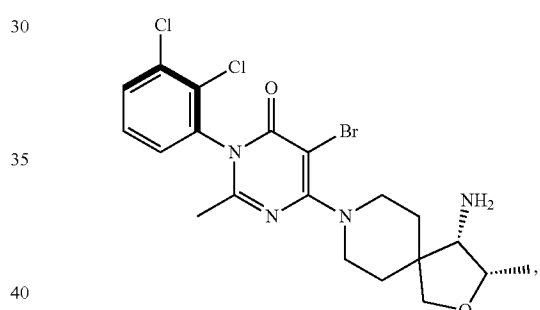
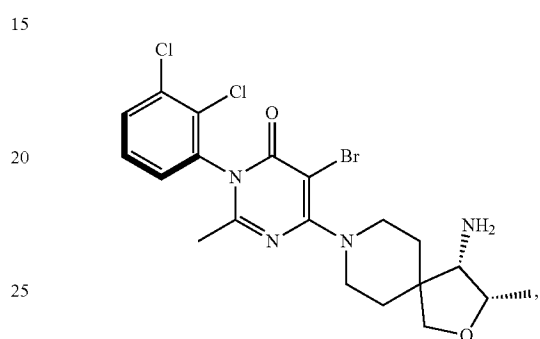
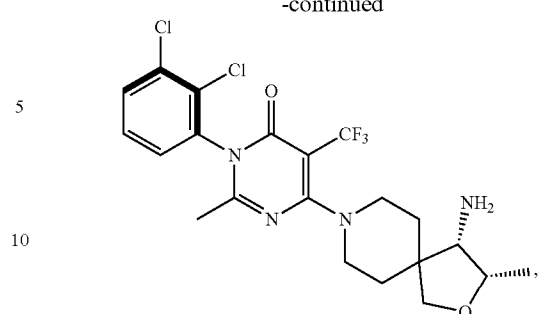


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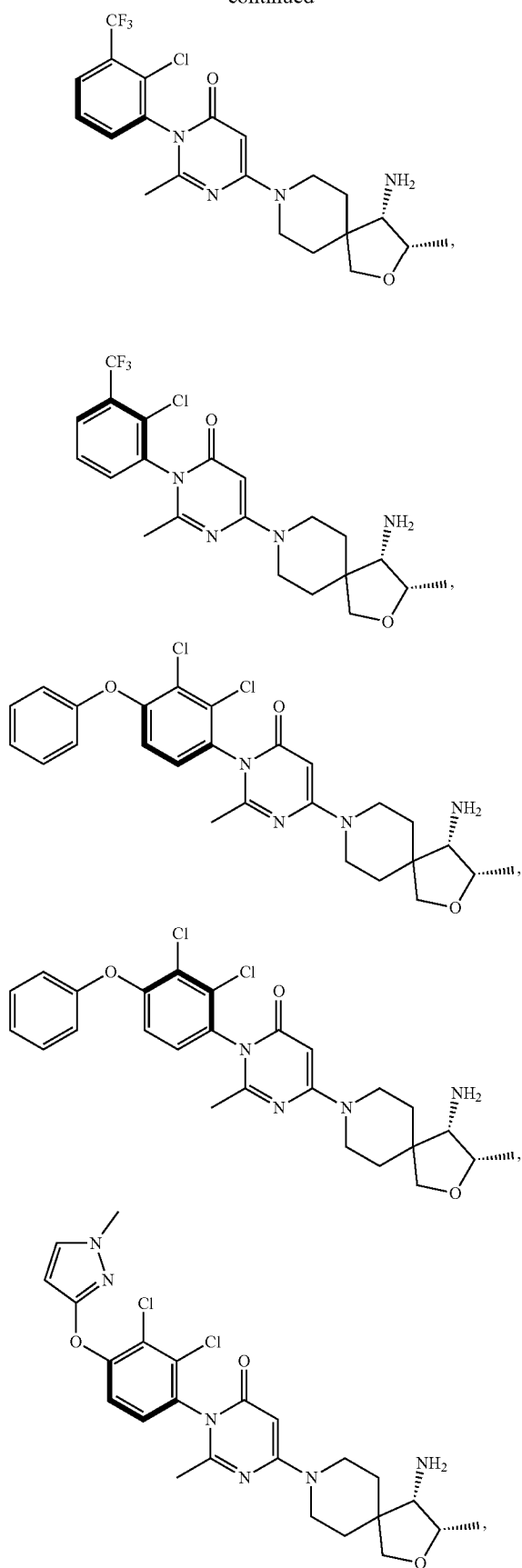
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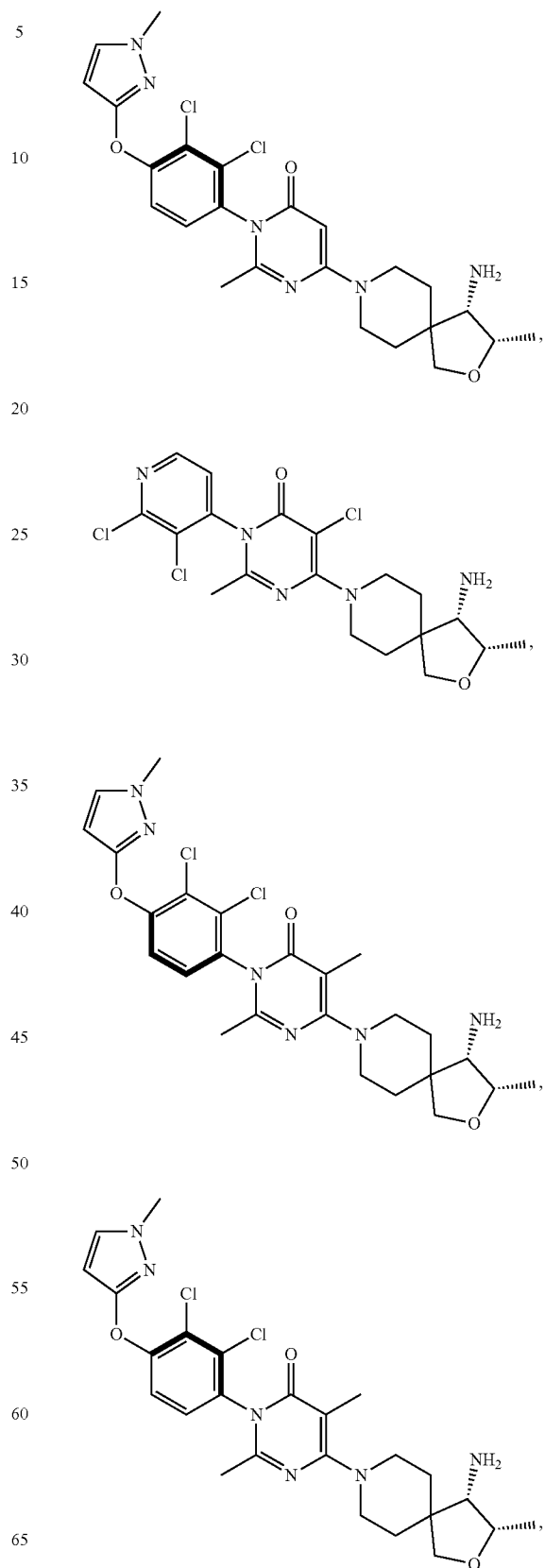
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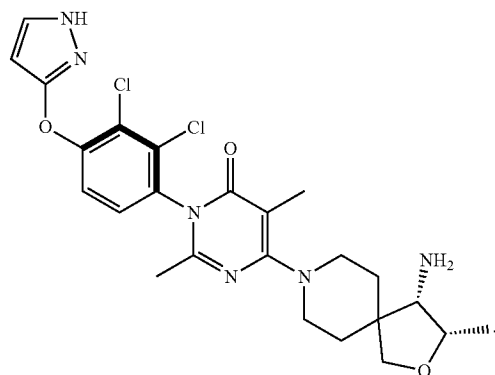
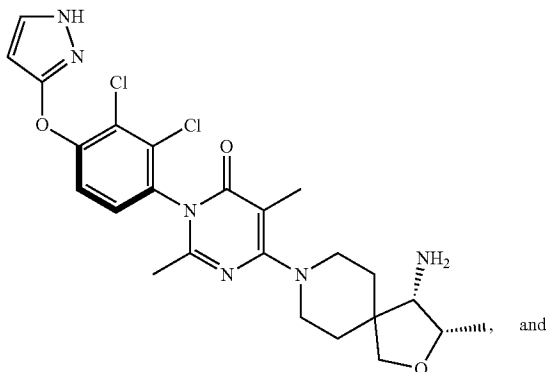
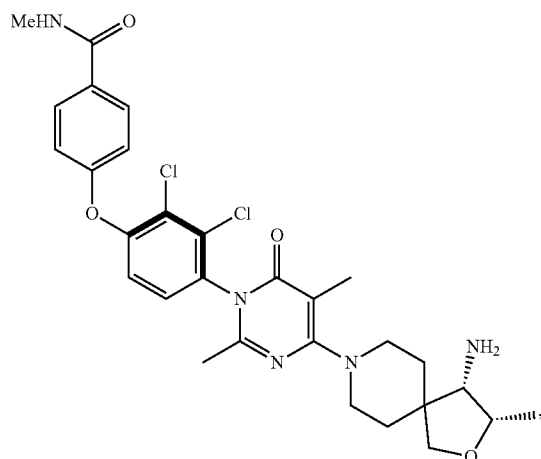
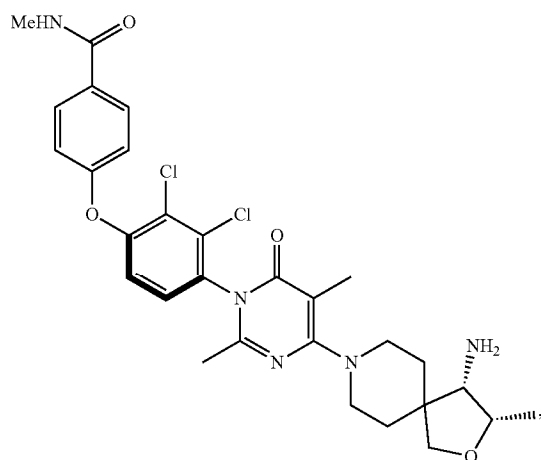
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Also provided are embodiments wherein any embodiment above may be combined with any one or more of these embodiments, provided the combination is not mutually exclusive.

5 As used herein, two embodiments are “mutually exclusive” when one is defined to be something which is different than the other. For example, an embodiment wherein two groups combine to form a cycloalkyl is mutually exclusive with an embodiment in which one group is ethyl the other group is hydrogen. Similarly, an embodiment wherein one group is CH₂ is mutually exclusive with an embodiment wherein the same group is NH.

10 Also provided is a compound selected from the Examples disclosed herein.

IV. Composition

Also provided is a pharmaceutical composition comprising a compound as disclosed herein, together with a pharmaceutically acceptable carrier.

In certain embodiments, the pharmaceutical composition is formulated for oral administration.

25 In certain embodiments, the pharmaceutical composition is formulated for parenteral administration.

In certain embodiments, the pharmaceutical composition is formulated for intravenous administration.

In certain embodiments, the pharmaceutical composition is formulated for subcutaneous administration.

30 In certain embodiments, the oral pharmaceutical composition is selected from a tablet and a capsule.

While it may be possible for the compounds of the subject invention to be administered as the raw chemical, it is also possible to present them as a pharmaceutical formulation.

35 Accordingly, provided herein are pharmaceutical formulations which comprise one or more of certain compounds disclosed herein, or one or more pharmaceutically acceptable salts, esters, prodrugs, amides, or solvates thereof, together with one or more pharmaceutically acceptable carriers thereof and optionally one or more other therapeutic ingredients. The carrier(s) must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Proper formulation is dependent upon the route of administration selected. Any of the well-known techniques, carriers, and excipients may be used as suitable and as understood in the art. The pharmaceutical compositions disclosed herein may be manufactured in any manner known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrap-
40 ping or compression processes.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous, intraarticular, and intramedullary), intraperitoneal, transmucosal, transdermal, rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Typically, these methods include the step of bringing into association a compound of the subject invention or a pharmaceutically acceptable salt, ester, amide, prodrug or solvate thereof (“active ingredient”) with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active
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ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations of the compounds disclosed herein suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

Pharmaceutical preparations which can be used orally include tablets, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. Tablets may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with binders, inert diluents, or lubricating, surface active or dispersing agents. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein. All formulations for oral administration should be in dosages suitable for such administration. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in powder form or in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or sterile pyrogen-free water, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for parenteral administration include aqueous and non-aqueous (oily) sterile injection solutions of the active compounds which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycer-

ides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection.

Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

For buccal or sublingual administration, the compositions may take the form of tablets, lozenges, pastilles, or gels formulated in conventional manner. Such compositions may comprise the active ingredient in a flavored basis such as sucrose and acacia or tragacanth.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter, polyethylene glycol, or other glycerides.

Certain compounds disclosed herein may be administered topically, that is by non-systemic administration. This includes the application of a compound disclosed herein externally to the epidermis or the buccal cavity and the instillation of such a compound into the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as gels, liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient for topical administration may comprise, for example, from 0.001% to 10% w/w (by weight) of the formulation. In certain embodiments, the active ingredient may comprise as much as 10% w/w. In other embodiments, it may comprise less than 5% w/w. In certain embodiments, the active ingredient may comprise from 2% w/w to 5% w/w. In other embodiments, it may comprise from 0.1% to 50% w/w of the formulation.

For administration by inhalation, compounds may be conveniently delivered from an insufflator, nebulizer pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Alternatively, for administration by inhalation or insufflation, the compounds according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

Preferred unit dosage formulations are those containing an effective dose, as herein below recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations described above may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

Compounds may be administered orally or via injection at a dose of from 0.1 to 500 mg/kg per day. The dose range for adult humans is generally from 5 mg to 2 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of one or more compounds which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

V. Methods

The present invention also relates to a method of inhibiting at least one PTPN11 function comprising the step of contacting PTPN11 with a compound as described herein. The cell phenotype, cell proliferation, activity of PTPN11, change in biochemical output produced by active PTPN11, expression of PTPN11, or binding of PTPN11 with a natural binding partner may be monitored. Such methods may be modes of treatment of disease, biological assays, cellular assays, biochemical assays, or the like.

Also provided herein is a method of treatment of a PTPN11-mediated disease comprising the administration of a therapeutically effective amount of a compound as disclosed herein, or a salt or tautomer thereof, to a patient in need thereof.

In certain embodiments, the disease is cancer.

In certain embodiments, the cancer is selected from breast cancer, colon cancer, leukemia, or melanoma.

Also provided herein is a method of treatment of a PTP-mediated disease comprising the administration of a therapeutically effective amount of a compound as disclosed herein, or a salt or tautomer thereof, to a patient in need thereof.

In certain embodiments, the disease is selected from Noonan Syndrome and Leopard Syndrome.

In certain embodiments, the disease is cancer.

In certain embodiments, the cancer is selected from breast cancer, colon cancer, leukemia, or melanoma.

Also provided herein is a compound as disclosed herein for use as a medicament.

Also provided herein is a compound as disclosed herein for use as a medicament for the treatment of a PTPN11-mediated disease.

Also provided herein is a compound as disclosed herein for use as a medicament for the treatment of a PTP-mediated disease.

Also provided is the use of a compound as disclosed herein as a medicament.

Also provided is the use of a compound as disclosed herein as a medicament for the treatment of a PTPN11-mediated disease.

Also provided is a compound as disclosed herein for use in the manufacture of a medicament for the treatment of a PTPN11-mediated disease.

Also provided is the use of a compound as disclosed herein for the treatment of a PTPN11-mediated disease.

Also provided is the use of a compound as disclosed herein for the treatment of a PTP-mediated disease.

Also provided herein is a method of inhibition of PTPN11 comprising contacting PTPN11 with a compound as disclosed herein, or a salt or tautomer thereof.

Also provided herein is a method of inhibition of PTP comprising contacting PTP with a compound as disclosed herein, or a salt or tautomer thereof.

Also provided herein is a method for achieving an effect in a patient comprising the administration of a therapeutically effective amount of a compound as disclosed herein, or a salt or tautomer thereof, to a patient, wherein the effect is cognition enhancement.

In certain embodiments, the PTPN11-mediated disease is selected from Noonan Syndrome and Leopard Syndrome.

In certain embodiments, the PTPN11-mediated disease is cancer.

In certain embodiments, the PTPN11-mediated disease is selected from breast cancer, colon cancer, leukemia, or melanoma.

Also provided is a method of modulation of a PTPN11-mediated function in a subject comprising the administration of a therapeutically effective amount of a compound as disclosed herein.

Administration and Combination Therapy

The compounds can be administered in various modes, e.g. orally, topically, or by injection. The precise amount of compound administered to a patient will be the responsibility of the attendant physician. The specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diets, time of administration, route of administration, rate of excretion, drug combination, the precise disorder being treated, and the severity of the indication or condition being treated. Also, the route of administration may vary depending on the condition and its severity.

In certain instances, it may be appropriate to administer at least one of the compounds described herein (or a pharmaceutically acceptable salt, ester, tautomer, or prodrug thereof) in combination with another therapeutic agent. By way of example only, if one of the side effects experienced by a patient upon receiving one of the compounds herein is hypertension, then it may be appropriate to administer an anti-hypertensive agent in combination with the initial therapeutic agent. Or, by way of example only, the therapeutic effectiveness of one of the compounds described herein may be enhanced by administration of an adjuvant (i.e., by itself the adjuvant may only have minimal therapeutic benefit, but in combination with another therapeutic agent, the overall therapeutic benefit to the patient is enhanced). Or, by way of example only, the benefit of experienced by a patient may be increased by administering one of the compounds described herein with another therapeutic agent (which also includes a therapeutic regimen) that also has therapeutic benefit. By way of example only, in a treatment for diabetes involving administration of one of the compounds described herein, increased therapeutic benefit may result by also providing the patient with another therapeutic agent for diabetes. In any case, regardless of the disease, disorder or condition being treated, the overall benefit experienced by the patient may simply be additive of the two therapeutic agents or the patient may experience a synergistic benefit.

Specific, non-limiting examples of possible combination therapies include use of certain compounds of the invention

with anti-cancer (chemotherapeutic) drugs. Classes of anti-cancer drugs include, but are not limited to: alkylating agents, anti-metabolites, antimetotics, checkpoint inhibitors, plant alkaloids and terpenoids, topoisomerase inhibitors, cytotoxic antibiotics, aromatase inhibitors, angiogenesis inhibitors, anti-steroids and anti-androgens, mTOR inhibitors, tyrosine kinase inhibitors, and others.

For use in cancer and neoplastic diseases a PTPN11 (SHP2) inhibitor may be optimally used together with one or more of the following non-limiting examples of anti-cancer agents:

- (1) alkylating agents, including but not limited to carmustine, chlorambucil (LEUKERAN), cisplatin (PLATIN), carboplatin (PARAPLATIN), oxaliplatin (ELOXATIN), streptozocin (ZANOSAR), busulfan (MYLERAN), dacarbazine, ifosfamide, lomustine (CCNU), melphalan (ALKERAN), procarbazine (MATULAN), temozolomide (TEMODAR), thiotepe, and cyclophosphamide (ENDOXAN);
- (2) anti-metabolites, including but not limited to cladribine (LEUSTATIN), mercaptopurine (PURINETHOL), thioguanine, pentostatin (NIPENT), cytosine arabinoside (cytarabine, ARA-C), gemcitabine (GEMZAR), fluorouracil (5-FU, CARAC), capecitabine (XELODA), leucovorin (FUSILEV), methotrexate (RHEUMATREX), raltitrexed;
- (3) antimetotics, which are often plant alkaloids and terpenoids, or derivatives thereof, including but not limited to taxanes such as docetaxel (TAXITERE) and paclitaxel (ABRAXANE, TAXOL); vinca alkaloids such as vincristine (ONCOVIN), vinblastine, vindesine, and vinorelbine (NAVELBINE);
- (4) checkpoint inhibitors, such as anti-PD-1 or PD-L1 antibodies pembrolizumab (KEYTRUDA), nivolumab (OPDIVO), MEDI4736, and MPDL3280A; anti-CTLA-4 antibody ipilimumab (YERVOY); and those that target LAG3 (lymphocyte activation gene 3 protein), MR (killer cell immunoglobulin-like receptor), 4-1BB (tumour necrosis factor receptor superfamily member 9), TIM3 (T-cell immunoglobulin and mucin domain containing-3) and OX40 (tumour necrosis factor receptor superfamily member 4);
- (5) topoisomerase inhibitors, including but not limited to camptothecin (CTP), irinotecan (CAMPTOSAR), topotecan (HYCAMTIN), teniposide (VUMON), and etoposide (EPOSIN);
- (6) cytotoxic antibiotics, including but not limited to actinomycin D (dactinomycin, COSMEGEN), bleomycin (BLENOXANE) doxorubicin (ADRIAMYCIN), daunorubicin (CERUBIDINE), epirubicin (ELLENCE), fludarabine (FLUDARA), idarubicin, mitomycin (MITOSOL), mitoxantrone (NOVANTRONE), plicamycin;
- (7) aromatase inhibitors, including but not limited to aminoglutethimide, anastrozole (ARIMIDEX), letrozole (FEMARA), vorozole (RIVIZOR), exemestane (AROMASIN);
- (8) angiogenesis inhibitors, including but not limited to genistein, sunitinib (SUTENT) and bevacizumab (AVASTIN);
- (9) anti-steroids and anti-androgens such as aminoglutethimide (CYTADREN), bicalutamide (CASODEX), cyproterone, flutamide (EULEXIN), nilutamide (NILANDRON);

- (10) tyrosine kinase inhibitors, including but not limited to imatinib (GLEEVEC), erlotinib (TARCEVA), lapatinib (TYKERB), sorafenib (NEXAVAR), and axitinib (INLYTA);
- (11) mTOR inhibitors such as everolimus, temsirolimus (TORISEL), and sirolimus; (12) monoclonal antibodies such as trastuzumab (HERCEPTIN) and rituximab (RITUXAN);
- (13) other agents, such as amsacrine; Bacillus Calmette-Guérin (B-C-G) vaccine; buserelin (ETILAMIDE); chloroquine (ARALEN); clodronate, pamidronate, and other bisphosphonates; colchicine; demethoxyviridin; dichloroacetate; estramustine; filgrastim (NEUPOGEN); fludrocortisone (FLORINEF); goserelin (ZOLADEX); interferon; leucovorin; leuprolide (LUPRON); levamisole; lonidamine; mesna; metformin; mitotane (o,p'-DDD, LYSODREN); nocodazole; octreotide (SANDOSTATIN); perfosine; porfimer (particularly in combination with photo- and radiotherapy); suramin; tamoxifen; titanocene dichloride; tretinoin; anabolic steroids such as fluoxymesterone (HALOTESTIN); estrogens such as estradiol, diethylstilbestrol (DES), and dienestrol; progestins such as medroxyprogesterone acetate (MPA) and megestrol; and testosterone.

In any case, the multiple therapeutic agents (at least one of which is a compound disclosed herein) may be administered in any order or even simultaneously. If simultaneously, the multiple therapeutic agents may be provided in a single, unified form, or in multiple forms (by way of example only, either as a single pill or as two separate pills). One of the therapeutic agents may be given in multiple doses, or both may be given as multiple doses. If not simultaneous, the timing between the multiple doses may be any duration of time ranging from a few minutes to four weeks.

Thus, in another aspect, certain embodiments provide methods for treating PTPN11-mediated disorders in a human or animal subject in need of such treatment comprising administering to said subject an amount of a compound disclosed herein effective to reduce or prevent said disorder in the subject, in combination with at least one additional agent for the treatment of said disorder that is known in the art. In a related aspect, certain embodiments provide therapeutic compositions comprising at least one compound disclosed herein in combination with one or more additional agents for the treatment of PTPN11-mediated disorders.

In some embodiments, methods described herein are used to treat a disease condition comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I or pharmaceutically acceptable salt thereof, wherein the condition is cancer which has developed resistance to chemotherapeutic drugs and/or ionizing radiation.

In some embodiments, methods described herein are used to treat a disease condition comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I or pharmaceutically acceptable salt thereof, wherein the condition is cancer which has developed resistance to chemotherapeutic drugs and/or ionizing radiation.

The compounds, compositions, and methods disclosed herein are useful for the treatment of disease. In certain embodiments, the disease is one of dysregulated cellular proliferation, including cancer. The cancer may be hormone-dependent or hormone-resistant, such as in the case of breast cancers. In certain embodiments, the cancer is a solid tumor.

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In other embodiments, the cancer is a lymphoma or leukemia. In certain embodiments, the cancer is and a drug resistant phenotype of a cancer disclosed herein or known in the art. Tumor invasion, tumor growth, tumor metastasis, and angiogenesis may also be treated using the compositions and methods disclosed herein. Precancerous neoplasias are also treated using the compositions and methods disclosed herein.

Cancers to be treated by the methods disclosed herein include colon cancer, breast cancer, ovarian cancer, lung cancer and prostate cancer; cancers of the oral cavity and pharynx (lip, tongue, mouth, larynx, pharynx), esophagus, stomach, small intestine, large intestine, colon, rectum, liver and biliary passages; pancreas, bone, connective tissue, skin, cervix, uterus, corpus endometrium, testis, bladder, kidney and other urinary tissues, including renal cell carcinoma (RCC); cancers of the eye, brain, spinal cord, and other components of the central and peripheral nervous systems, as well as associated structures such as the meninges; and thyroid and other endocrine glands. The term "cancer" also encompasses cancers that do not necessarily form solid tumors, including Hodgkin's disease, non-Hodgkin's lymphomas, multiple myeloma and hematopoietic malignancies including leukemias (Chronic Lymphocytic Leukemia (CLL), Acute Lymphocytic Leukemia (ALL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML)) and lymphomas including lymphocytic, granulocytic and monocytic. Additional types of cancers which may be treated using the compounds and methods of the invention include, but are not limited to, adenocarcinoma, angiosarcoma, astrocytoma, acoustic neuroma, anaplastic astrocytoma, basal cell carcinoma, blastoglioma, chondrosarcoma, choriocarcinoma, chordoma, cranio-pharyngioma, cutaneous melanoma, cystadenocarcinoma, endotheliosarcoma, embryonal carcinoma, ependymoma, Ewing's tumor, epithelial carcinoma, fibrosarcoma, gastric cancer, genitourinary tract cancers, glioblastoma multiforme, head and neck cancer, hemangioblastoma, hepatocellular carcinoma, hepatoma, Kaposi's sarcoma, large cell carcinoma, leiomyosarcoma, leukemias, liposarcoma, lymphatic system cancer, lymphomas, lymphangiosarcoma, lymphoendotheliosarcoma, medullary thyroid carcinoma, medulloblastoma, meningioma mesothelioma, myelomas, myxosarcoma neuroblastoma, neurofibrosarcoma, oligodendroglioma, osteogenic sarcoma, epithelial ovarian cancer, papillary carcinoma, papillary adenocarci-

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nomas, paraganglioma, parathyroid tumours, pheochromocytoma, pinealoma, plasmacytomas, retinoblastoma, rhabdomyosarcoma, sebaceous gland carcinoma, seminoma, skin cancers, melanoma, small cell lung carcinoma, non-small cell lung carcinoma, squamous cell carcinoma, sweat gland carcinoma, synovioma, thyroid cancer, uveal melanoma, and Wilm's tumor.

In certain embodiments, the compositions and methods disclosed herein are useful for preventing or reducing tumor invasion and tumor metastasis.

Besides being useful for human treatment, certain compounds and formulations disclosed herein may also be useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

VI. Methods of Preparation

The chirality of the molecule is described by specifying its configuration using the Cahn-Ingold-Prelog convention, using the descriptors R and S for central chirality and R_a and S_a for axial chirality. PAC, 1996, 68, 2193, Basic terminology of stereochemistry (IUPAC Recommendations 1996), doi:10.1351/pac199668122193; Preferred IUPAC Names Chapter 9, September, 2004. Cahn, C. K. Ingold and V. Prelog, *Angew. Chem. Internat. Ed. Eng.* 5, 385-415, 511 (1966); V. Prelog and G. Helmchen, *Angew. Chem. Internat. Ed. Eng.* 21, 567-583 (1982).

SYNTHESIS SCHEMES

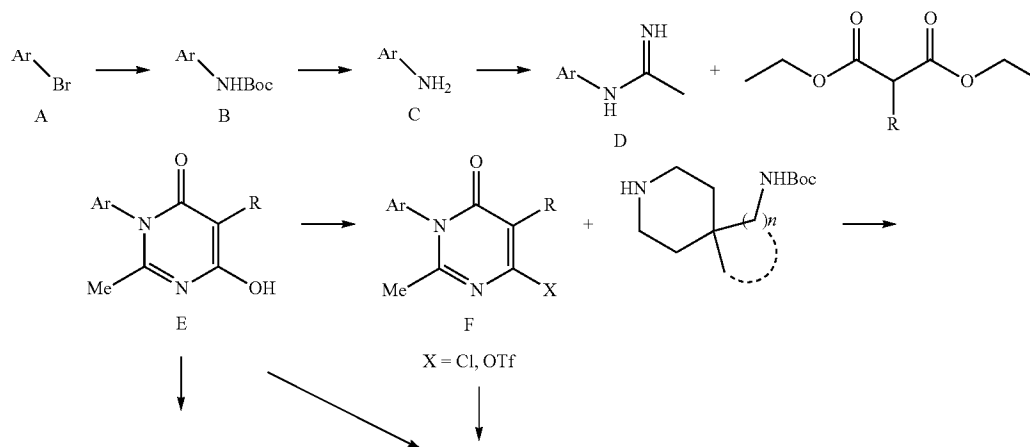
The following schemes can be used to practice the present invention.

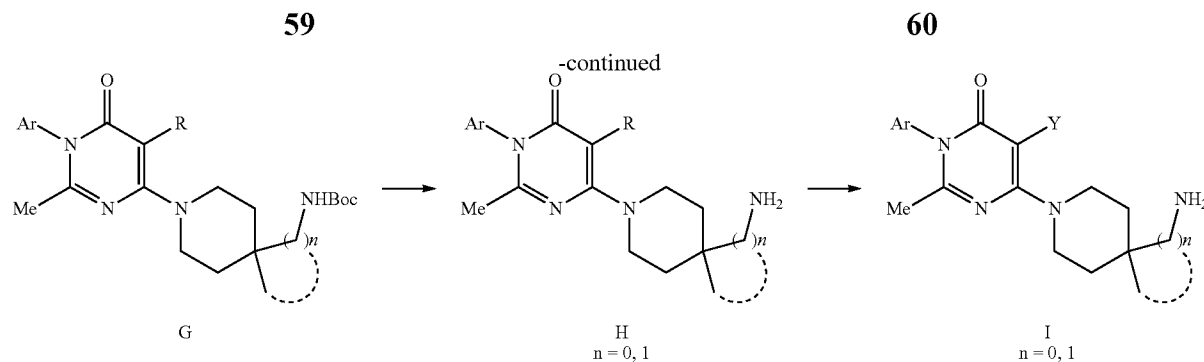
General Synthetic Methods for Preparing Compounds

The following scheme can be used as general guidance to prepare compounds of the present invention.

List of Abbreviations: Pd₂dba₃-Tris(dibenzylideneacetone)dipalladium; Xantphos—4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene; HCl=hydrogen chloride; TFA=trifluoroacetic acid; POCl₃-Phosphorus (V) oxychloride; Tf₂O—Trifluoromethanesulfonic anhydride; DIEA—Diisopropyl ethylamine; DCM=dichloromethane; BOP—(benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate); DBU—1,8-Diazabicyclo[5.4.0]undec-7-ene; NBS—N-Bromoosuccinimide; and NCS—N-Chlorosuccinimide.

Scheme I





Aryl halide A can be transformed into the corresponding protected amine B using a "Pd" catalyst and ligand such as Pd_2dba_3 and Xantphos. Amine B can be deprotected to afford C and the corresponding acetimidamide D can be formed by treatment of amine C with acetonitrile and an acid such as HCl. Aryl substituted-6-hydroxy-2-methylpyrimidin-4(3H)-one E is formed by reacting D with the desired malonate to afford E. E can be transformed to the corresponding halide by treatment with reagent e.g. POCl_3 or triflate by TiF_4 and DIEA in DCM. Subsequent $\text{S}_\text{N}\text{Ar}$ reaction can provide protected amine G that can be transformed to the unprotected amine H. F can directly transformed to H in an $\text{S}_\text{N}\text{Ar}$ reaction. Direct conversion of E to H can be performed by applying reagents such as BOP and DBU. H can be further functionalized to the corresponding bromide, chloride or CF_3 derivative (I) by applying reagent such as NBS, NCS or bis(((trifluoromethyl)sulfinyl)oxy) zinc in the presence of tert-butyl hydroperoxide.

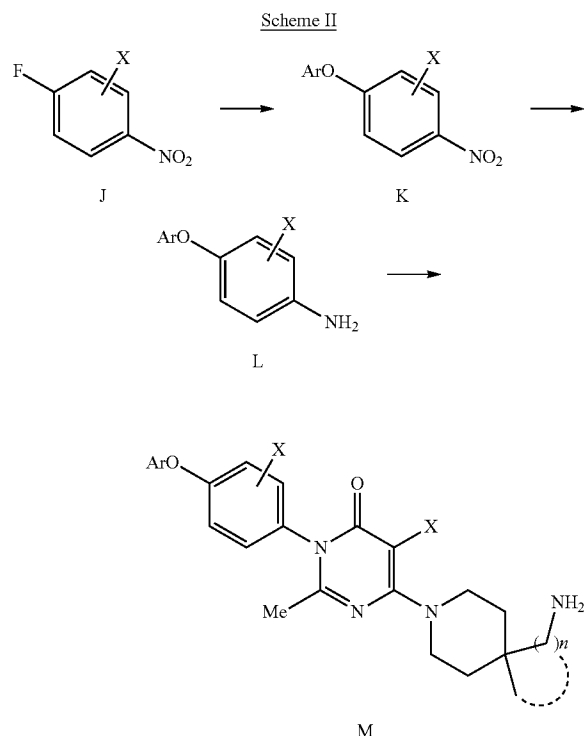
Fluoronitrobenzene derivative J can be transferred to corresponding ether K using an alcohol or phenol and a base such as sodium hydride under $\text{S}_\text{N}\text{Ar}$ reaction conditions. The nitro derivative can be reduced to the amine L using a reducing agent such as $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$. Compound L subsequently can be transformed to the desired pyrimidinone derivative M as described in Scheme I.

It will be appreciated that other synthetic routes may be available for practice of the present invention.

The invention is further illustrated by the following examples, which may be synthesized and isolated as free bases or as salts, for example TFA or HCl salts.

VII. Examples

List of Abbreviations

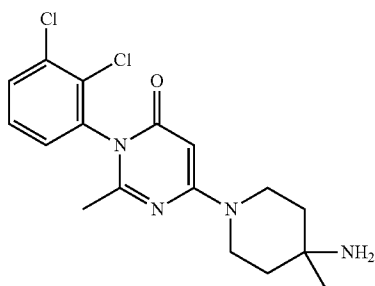


mg—milligram; mL—milliliter; μL —microliter; M=molar; mmol—millimol; h=hour; min.=minute; Rt —Room Temperature; N_2 =nitrogen; HCl=hydrogen chloride; H_2O =water; MS=mass spectrometry; ES^+ =electrospray positive ionization; $^1\text{H-NMR}$ =proton nuclear magnetic resonance; MHz=megahertz; DMSO-d_6 =dimethyl sulfoxide deuterated-6; H=hydrogen; rt =room temperature; $^\circ\text{C}$ =degrees Celsius; Br_2 =bromine; NaOH=sodium hydroxide; NaHSO_3 =sodium bisulfate; K_2CO_3 =potassium carbonate; NMP=N-Methyl-2-pyrrolidone; BOP=(benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate); DBU=1,8-Diazabicyclo[5.4.0]undec-7-ene; DIEA=Diisopropyl ethylamine; MW=microwave; KF=potassium fluoride; Pd(dppf)Cl_2 =[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride; Pd_2dba_3 -Tris(dibenzylideneacetone)dipalladium; POCl_3 -Phosphorus (V) oxychloride; PE=petroleum ether; EA=ethyl acetate; CDCl_3 =deuterated chloroform; MeOH=methanol; D_2O =deuterated water; HPLC=high pressure liquid chromatography; DMSO=dimethyl sulfoxide; MeCN (or ACN)=acetonitrile; NIS=N-iodosuccinimide; DMF=dimethylformamide; K_3PO_4 =potassium phosphate, tribasic; Et_2O =Diethyl ether; EtOAc=Ethyl acetate; EtOH=Ethanol; NaOMe=Sodium methoxide; NaOEt=Sodium ethoxide; NCS=N-Chlorosuccinimide; TBDMS=TBS=tert-butyldimethylsilyl; TFA=trifluoroacetic acid; DCM=dichloromethane; Xantphos=4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene.

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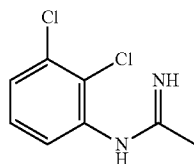
Example 1

6-(4-Amino-4-methylpiperidin-1-yl)-3-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one TFA salt



Step 1

N-(2,3-Dichlorophenyl)acetimidamide



Method A

To a mixture of 2,3-dichloroaniline (1.6 g, 10 mmol) and MeCN (615 mg, 15.0 mmol) in 1,2-dichloroethane (10 mL) was added AlCl_3 (1.46 g, 1.10 mmol) at 0° C. The reaction mixture was stirred for 10 min then it was heated to 100° C. for 18 h in a sealed tube. The reaction was cooled to rt, ice-water (30 mL) was added, and the aqueous phase was extracted with DCM (50 mL $\times$ 2). Subsequently, 2 M NaOH was added to adjust the pH to 10 and the aqueous phase was extracted with DCM (50 mL $\times$ 3). The combined organic layers were dried over MgSO_4 and concentrated under reduced pressure to afford the title compound as a beige oil (950 mg, 47%). MS (ES+) $\text{C}_8\text{H}_8\text{Cl}_2\text{N}_2$ requires: 202, found: 203[M+H]⁺. The product was used in the next step without further purification.

Method B

In a high pressure reaction vessel MeCN (123 mL) was bubbled with HCl gas for 15 seconds at 0° C. to give a saturated solution. 2,3-Dichloroaniline (7.30 mL, 61.7 mmol) was added and a white solid precipitate formed. The vessel was sealed and the mixture was heated at 120° C. for 16 h. The reaction mixture was cooled to rt and the white precipitate was collected by vacuum filtration. The solid was rinsed with Et_2O (20 mL $\times$ 3) and dried under vacuum to afford the title compound (14.5 g, 98% yield) as a white solid. MS (ES+) $\text{C}_8\text{H}_8\text{Cl}_2\text{N}_2$ requires: 202, found: 203 [M+H]⁺.

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Step 2

3-(2,3-Dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one

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Method A

To a solution of N-(2,3-dichlorophenyl)acetimidamide (950 mg, 4.68 mmol) in 2-methoxyethan-1-ol (10 mL) was added diethyl malonate (2.99 g, 18.7 mmol) and NaOMe in MeOH (4 M, 4.7 mL, 18 mmol). The mixture was heated at 120° C. for 16 h. The reaction mixture was cooled to rt, it was poured onto water (50 mL) and it was extracted with Et_2O (50 mL). The aqueous layer was acidified to pH-2 with HCl (6 M, aq.) and the solid precipitate was collected by filtration to afford the title compound (1 g, 77%) as a white solid. MS (ES+) $\text{C}_{11}\text{H}_8\text{Cl}_2\text{N}_2\text{O}_2$ requires: 270, found: 271 [M+H]⁺.

Method B

To a suspension of N-(2,3-dichlorophenyl)acetimidamide (30 g, 125 mmol) in EtOH (125 mL) were added diethyl malonate (38.2 mL, 250 mmol) and NaOEt (20% in EtOH) (140 mL, 376 mmol) and the resulting mixture was stirred in a sealed tube at 120° C. for 18 h. The reaction mixture was allowed to cool to room temperature and the volatiles were removed under reduced pressure. Water (30 mL) was added to the residue, the mixture was cooled to 0° C. and the mixture was acidified to pH-2 with HCl (6 M, aq.). The mixture was allowed to warm to rt and stirred for 1 h. The solid was collected by vacuum filtration, it was rinsed with Et_2O (20 mL $\times$ 2) and dried under reduced pressure to afford the title compound (26.3 g, 77%) as a tan solid (mixture of enantiomers). MS (ES+) $\text{C}_{11}\text{H}_8\text{Cl}_2\text{N}_2\text{O}_2$ requires: 270, found: 271 [M+H]⁺.

The compound of Step 2 can exist as a mixture of 3-(S_a)-(2,3-Dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one and 3-(R_a)-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one

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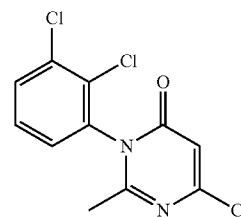
Step 3

6-Chloro-3-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one

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A mixture of 1-(2,3-dichlorophenyl)-2-methylpyrimidine-4,6(1H, 5H)-dione (250 mg, 0.92 mmol) in POCl_3 (5 mL) was heated at 100° C. for 4 hrs. The reaction mixture

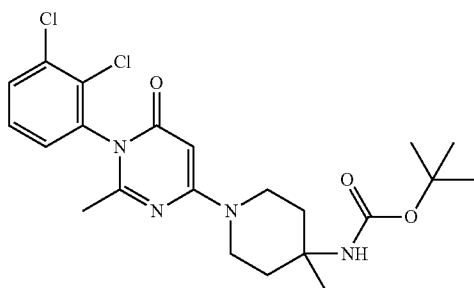
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was cooled to rt, it was concentrated under reduced pressure and it was slowly added to ice-water. The mixture was extracted with EtOAc (15 mL×3). The organic layer was dried and concentrated under reduced pressure. The residue was purified by flash column chromatography (EtOAc in PE, 20 to 50%) to afford the title compound as a beige solid (100 mg, 37.5%) (mixture of enantiomers). MS (ES+) $C_{11}H_7Cl_3N_2O$ requires: 288, found: 289[M+H]⁺.

The compound of Step 3 can exist as a mixture of 6-Chloro-3-(S_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one and 6-chloro-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one.

Step 4

Tert-butyl(1-(1-(2,3-dichlorophenyl)-2-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-4-methylpiperidin-4-yl)carbamate



A mixture of 6-chloro-3-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (100 mg, 0.35 mmol), tert-butyl (4-methylpiperidin-4-yl)carbamate (89 mg, 0.42 mmol) and DIEA (134 mg, 1.04 mmol) in DMF (3 mL) was stirred at 100° C. for 3 h. The reaction mixture was diluted with water, it was extracted with EtOAc (15 mL×3). The organic layer was washed with brine, dried over MgSO₄ and concentrated under reduced pressure to afford the title compound. MS (ES+) $C_{22}H_{28}Cl_2N_4O_3$ requires: 466, found: 467[M+H]⁺. The product was used in the next step without further purification.

The compound of Step 4 can exist as a mixture of Tert-butyl (1-(1-(S_a)-(2,3-dichlorophenyl)-2-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-4-methylpiperidin-4-yl)carbamate and tert-butyl (1-(1-(R_a)-(2,3-dichlorophenyl)-2-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-4-methylpiperidin-4-yl)carbamate.

Step 5

6-(4-amino-4-methylpiperidin-1-yl)-3-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one TFA salt

To a solution of tert-butyl (1-(1-(2,3-dichlorophenyl)-2-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-4-methylpiperidin-4-yl)carbamate (0.35 mmol, crude) in DCM (3 mL) was added TFA (1 mL). The resulting solution was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was purified by prep-HPLC (Mobile phase: A=0.01% TFA/H₂O, B=MeCN; Gradient: B=60%-95% in 18 min; Column: Venusil CBP C18 (L) C18, 10 μm, 21.2 mm×250 mm, Cat. NO.: VX902520-L) to afford the title compound as a white solid (110 mg, 86%); MS (ES+) $C_{17}H_{20}Cl_2N_4O$ requires: 366, found: 367 [M+H]⁺; ¹H NMR (500 MHz, DMSO) δ 8.03 (s, 3H), 7.80 (dd, J=7.8, 1.7 Hz,

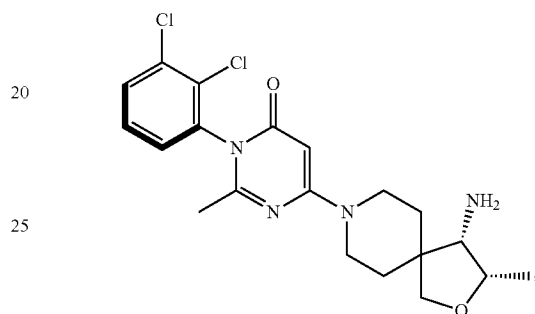
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1H), 7.61-7.46 (m, 2H), 5.47 (s, 1H), 4.08-3.82 (m, 2H), 3.34-3.29 (m, 2H), 2.00 (s, 3H), 1.71 (t, J=5.5 Hz, 4H), 1.37 (s, 3H).

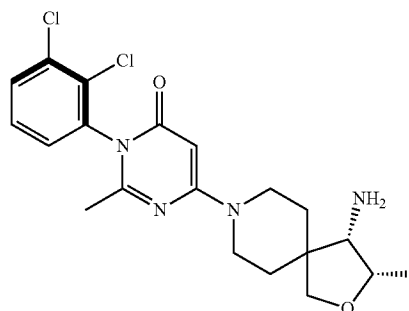
The compound of Example 1 can exist as a mixture of 6-(4-amino-4-methylpiperidin-1-yl)-3-(S_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one TFA salt and 6-(4-amino-4-methylpiperidin-1-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one TFA salt.

Example 2a and 2b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 2a)



and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 2b)



To a suspension of 3-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one (Example 1, Step 2, 3.50 g, 12.9 mmol) and (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (3.45 g, 14.2 mmol) in MeCN (43 mL) were added DBU (6.23 mL, 41.3 mmol) and BOP (6.85 g, 15.5 mmol) and the resulting mixture was stirred at rt for 24 h. The mixture was concentrated under reduced pressure. The residue was purified via silica gel chromatography (2-15% MeOH with 2% NH₄OH in DCM) to afford the title compounds as a mixture of diastereomers (4.2 g, 77% yield) as an off-white solid. The mixture of diastereomers were separated by Chiral SFC (Mobile phase: CO₂/methanol (0.25% isopropylamine)=60/40; Flow rate: 80 g/min; 5 min; Column temperature: 25° C.; Back pressure: 100 bar; Column: ES Industries ChromegaChiral CCS, 2.0×25.0 cm) to afford the title compounds, Example 2a (19.7 g, 48%) as a white solid and Example 2b (11.6 g, 28%) as a white solid.

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Example 2a 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one: MS (ES⁺) C₂₀H₂₄Cl₂N₄O₂ requires: 422, found: 423 [M+H]⁺; ¹H NMR (600 MHz, CDCl₃) δ 7.57 (dd, J=8.1, 1.5 Hz, 1H), 7.35 (t, J=8.0 Hz, 1H), 7.21 (dd, J=7.9, 1.5 Hz, 1H), 5.45 (s, 1H), 4.21-4.16 (m, 1H), 3.86 (br s, 2H), 3.79 (d, J=8.7 Hz, 1H), 3.68 (d, J=8.7 Hz, 1H), 3.40 (ddd, J=13.1, 9.1, 3.8 Hz, 1H), 3.31 (ddd, J=13.3, 9.4, 3.2 Hz, 1H), 2.98 (d, J=4.5 Hz, 1H), 2.06 (s, 3H), 1.84 (ddd, J=13.4, 9.4, 3.9 Hz, 1H), 1.75-1.60 (m, 3H), 1.23 (d, J=6.4 Hz, 3H).

Example 2b 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-

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methylpyrimidin-4(3H)-one: MS (ES⁺) C₂₀H₂₄Cl₂N₄O₂ requires: 422, found: 423 [M+H]⁺; ¹H NMR (600 MHz, CDCl₃) δ 7.57 (dd, J=8.2, 1.5 Hz, 1H), 7.36 (t, J=8.0 Hz, 1H), 7.21 (dd, J=7.9, 1.5 Hz, 1H), 5.46 (s, 1H), 4.22-4.14 (m, 1H), 3.95-3.82 (m, 2H), 3.80 (d, J=8.7 Hz, 1H), 3.68 (d, J=8.7 Hz, 1H), 3.45-3.36 (m, 1H), 3.29 (ddd, J=13.3, 9.5, 3.3 Hz, 1H), 2.99 (d, J=4.6 Hz, 1H), 2.07 (s, 3H), 1.84 (ddd, J=13.4, 9.4, 3.9 Hz, 1H), 1.77-1.70 (m, 1H), 1.70-1.61 (m, 2H), 1.24 (d, J=6.4 Hz, 3H).

The following Examples were synthesized with synthetic methods that were similar to that used for Example 1 and 2, and can generally be made by methods disclosed herein. The Examples may be made as free bases or as TFA salts.

TABLE 1

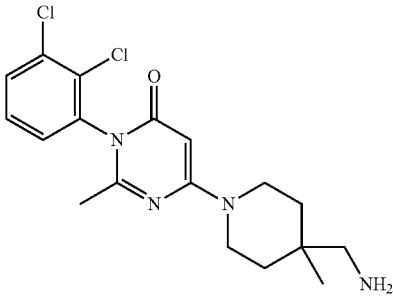
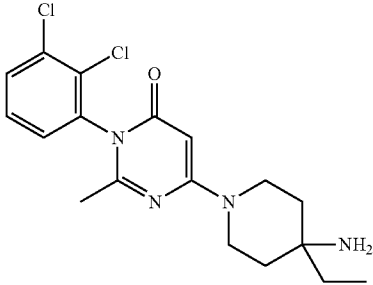
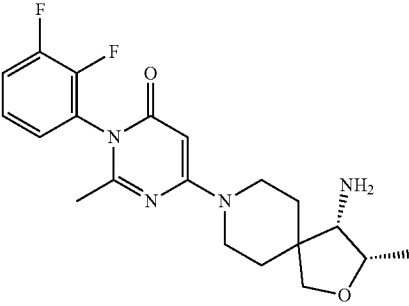
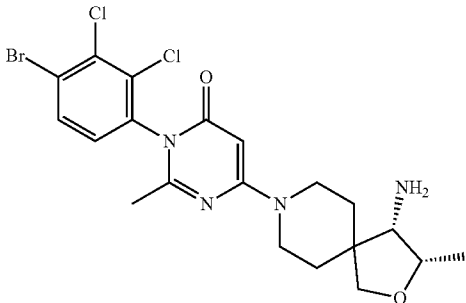
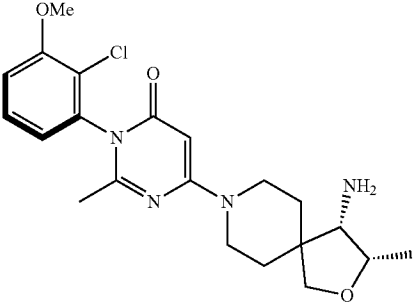
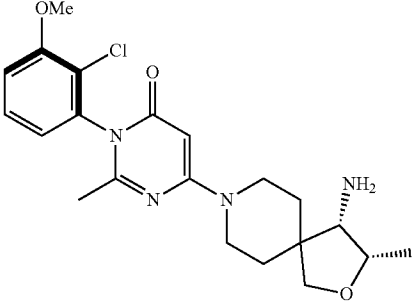
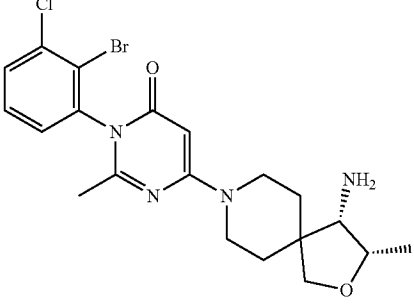
Examples 3-8			
Ex #	Structure and IUPAC Name	Calc. Mass	Mass [M + H] ⁺
3	 6-[4-(aminomethyl)-4-methylpiperidin-1-yl]-3-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one	380	381
4	 6-(4-amino-4-ethylpiperidin-1-yl)-3-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one	380	381
5	 6-[(3S,4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(2,3-difluorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one	390	391

TABLE 1-continued

Examples 3-8				
Ex #	Structure and IUPAC Name	Calc. Mass	Mass [M + H] ⁺	
6	 6-((3S,4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(4-bromo-2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one	500/502	501/503	
7a	 6-((3S,4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-methoxyphenyl)-2-methylpyrimidin-4(3H)-one	418	419	
7b	 6-((3S,4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2-chloro-3-methoxyphenyl)-2-methylpyrimidin-4(3H)-one	418	419	
8	 6-((3S,4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(2-bromo-3-chlorophenyl)-2-methylpyrimidin-4(3H)-one	466	467	

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The compound of Example 3 can exist as a mixture of 6-[4-(aminomethyl)-4-methylpiperidin-1-yl]-3-(S_a)-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one and 6-[4-(aminomethyl)-4-methylpiperidin-1-yl]-3-(R_a)-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one.

The compound of Example 4 can exist as a mixture of 6-(4-amino-4-ethylpiperidin-1-yl)-3-(S_a)-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one and 6-(4-amino-4-ethylpiperidin-1-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one.

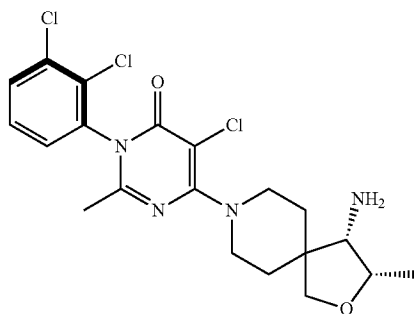
The compound of Example 5 can exist as a mixture of 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(S_a)-(2,3-difluorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one and 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(R_a)-(2,3-difluorophenyl)-2-methyl-3,4-dihydropyrimidin-4-one.

The compound of Example 6 can exist as a mixture of 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(S_a)-(4-bromo-2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one and 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(R_a)-(4-bromo-2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one.

The compound of Example 8 can exist as a mixture of 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(S_a)-(2-bromo-3-chlorophenyl)-2-methylpyrimidin-4(3H)-one and 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(R_a)-(2-bromo-3-chlorophenyl)-2-methylpyrimidin-4(3H)-one.

Example 9

6-[(3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-5-chloro-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one

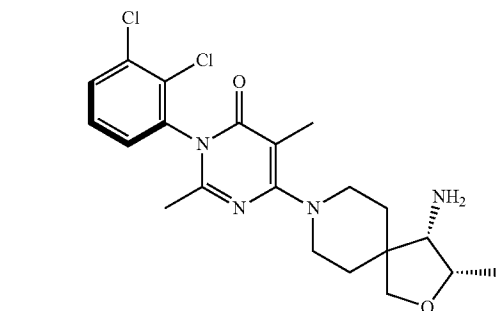


To a solution of 6-(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 2b; 2.5 g, 5.9 mmol) in DCM (30 mL) was added NCS (946 mg, 7.09 mmol) and the resulting mixture was stirred at rt for 10 min. The mixture was concentrated under reduced pressure. The residue was purified via silica gel chromatography (2-15% MeOH with 2% NH_4OH in DCM) to afford the title compound (2.1 g, 78% yield) as a white solid. MS (ES^+) $\text{C}_{20}\text{H}_{23}\text{Cl}_3\text{N}_4\text{O}_2$ requires: 456, found: 457 [$\text{M}+\text{H}$] $^+$; ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 7.83 (dd, $J=8.1, 1.6$ Hz, 1H), 7.62 (dd, $J=8.0, 1.6$ Hz, 1H), 7.57 (t, $J=8.0$ Hz, 1H), 4.09-4.01 (m, 1H), 3.96-3.87 (m, 2H), 3.67 (d, $J=8.4$ Hz, 1H), 3.53-3.46 (m, 2H), 3.40 (ddd, $J=12.9, 9.3, 3.1$ Hz, 1H), 2.91 (d, $J=5.1$ Hz, 1H), 2.00 (s, 3H), 1.81 (ddd, $J=13.2, 9.3, 3.6$ Hz, 1H), 1.71-1.65 (m, 1H), 1.59-1.54 (m, 1H), 1.54-1.48 (m, 1H), 1.08 (d, $J=6.4$ Hz, 3H).

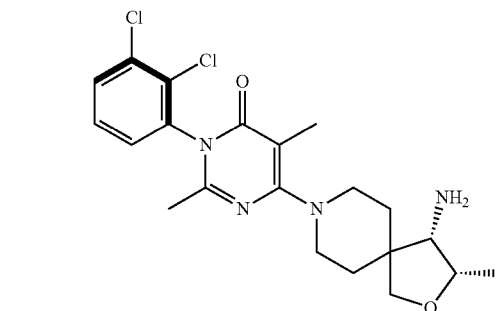
70

Example 10a and 10b

6-[(3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(S_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10a)



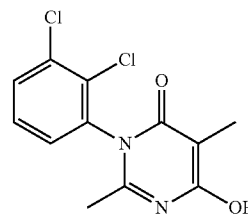
and 6-[(3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl]-3-(R_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10b)



Method A

Step 1

3-(2,3-Dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one



To a suspension of N-(2,3-dichlorophenyl)acetimidamide hydrochloride (Example 1, step 1) (44 g, 184 mmol) in EtOH (184 mL) were added diethyl 2-methylmalonate (62.6 mL, 367 mmol) and EtONa (206 mL, 551 mmol) and the resulting mixture was stirred in a sealed tube at 120° C. for 18 h. The reaction mixture was allowed to cool to room temperature and the volatiles were removed under reduced pressure. Water (200 mL) was added, the mixture was cooled to 0° C., and HCl (6 M) was added to adjust the pH to 2. The reaction was allowed to warm to rt and was stirred

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for 1 h. The reaction mixture was filtered through glass fiber paper, the solid was rinsed with Et₂O, collected, and dried under reduced pressure to afford the title compound (44.6 g, 156 mmol, 85% yield) as a pale yellow solid. MS (ES⁺) C₁₂H₁₀Cl₂N₂O₂ requires: 284, found: 285 [M+H]⁺.

The compound of Step 1 can exist as a mixture of 3-(S_a)-(2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one and 3-(R_a)-(2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one.

Step 2

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10a) and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10b)

To a suspension of 3-(2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one (30 g, 105 mmol) and (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (28.1 g, 116 mmol) in acetonitrile (351 mL) were added DBU (50.8 mL, 337 mmol) and BOP (51.2 g, 116 mmol) and the resulting mixture was stirred at rt for 24 h. (3S, 4S)-3-Methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (12.8 g, 52.5 mmol) and DBU (15.8 mL, 105 mmol) were added and the mixture was stirred at rt for an additional 24 h. The reaction mixture was concentrated under reduced pressure. The residue was partitioned between DCM (200 mL) and water (200 mL), the layers were separated and the aqueous layer was extracted with DCM (200 mL×3). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified via silica gel chromatography (2-15% MeOH with 2% NH₄OH in DCM) to afford the title compounds as a mixture of diastereomers as an off-white solid. The mixture of diastereomers were separated by Chiral SFC (Mobile phase: CO₂/methanol (0.25% isopropylamine)=60/40; Flow rate: 80 g/min; 5 min; Column temperature: 25° C.; Back pressure: 100 bar; Column: Chiral Technologies Chiralpak AD-H, 2.1×25.0 cm) to afford the title compounds Example 10a (19.51 g, 42%, 98% ee) as a white solid (a single diastereomer) and Example 10b (19.25 g, 42%, >99% ee) as a white solid (a single diastereomer).

Example 10a 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES⁺) C₂₁H₂₆Cl₂N₄O₂ requires: 436, found: 437 [M+H]⁺; ¹H NMR (600 MHz, DMSO-d₆) δ 7.83-7.77 (m, 1H), 7.57-7.50 (m, 2H), 4.09-4.02 (m, 1H), 3.66 (d, J=8.4 Hz, 1H), 3.58-3.51 (m, 2H), 3.49 (d, J=8.4 Hz, 1H), 3.21 (ddd, J=12.9, 9.3, 3.2 Hz, 1H), 3.13 (ddd, J=12.9, 9.4, 3.0 Hz, 1H), 2.89 (d, J=5.1 Hz, 1H), 1.97 (s, 3H), 1.89 (s, 3H), 1.78 (ddd, J=13.3, 9.5, 3.6 Hz, 1H), 1.66 (ddd, J=13.1, 9.1, 3.7 Hz, 1H), 1.57-1.52 (m, 1H), 1.52-1.47 (m, 1H), 1.08 (d, J=6.4 Hz, 3H).

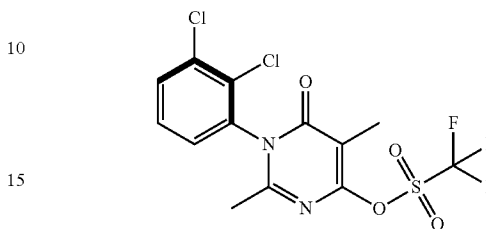
Example 10b 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES⁺) C₂₁H₂₆Cl₂N₄O₂ requires: 436, found: 437 [M+H]⁺; ¹H NMR (600 MHz, DMSO-d₆) δ 7.83-7.77 (t, J=4.8 Hz, 1H), 7.57-7.50 (d, J=4.8 Hz, 2H), 4.09-4.02 (m, 1H), 3.65 (d, J=8.4 Hz, 1H), 3.58-3.51 (m, 2H), 3.49 (d, J=8.4 Hz, 1H), 3.22 (ddd, J=13, 9.5, 2.5 Hz, 1H), 3.12 (ddd, J=13.0, 9.5, 2.5 Hz, 1H), 2.89 (d, J=5.1 Hz, 1H), 1.97 (s, 3H), 1.89 (s, 3H), 1.78 (ddd, J=13.2, 9.5, 3.7 Hz, 1H), 1.66 (ddd, J=13.2, 9.5, 3.7 Hz, 1H), 1.58-1.46 (m, 2H), 1.28 (bs, 2H), 1.08 (d, J=6.4 Hz, 3H).

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Method B

Step 1

5 1-(R_a)-(2,3-Dichlorophenyl)-2,5-dimethyl-6-oxo-1,6-dihydropyrimidin-4-yl trifluoromethanesulfonate



To a mixture of 3-(S_a)-(2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one and 3-(R_a)-(2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one (Example 10, Method A, Step 1) (100 g, 351 mmol) and DIPEA (183 mL, 1.05 mol) in DCM (1.00 L) was added Tf₂O (116 mL, 701 mmol, 116 mL) drop-wise at -25° C. The resulting mixture was stirred at -25° C.~20° C. for 1 h and at 20° C. for 18 h. Two batches of the reaction mixture were combined. To the reaction mixture was added water (1 L), the resulting mixture was extracted with CH₂Cl₂ (1 L×2). The combined organic layers were washed with 1 M HCl (1 L, aq), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc in PE, 20%) to afford a mixture of diastereomers (144 g, 336 mmol, 47.8% yield) as a brown solid. The mixture of diastereomers was separated by SFC (column: DAICEL CHIRALPAK AD (250 mm×50 mm, 10 μm); mobile phase: [Neu-IPA]; B %: 15%-15%, 2.9 min, 1,100 min) to afford the title compound (56.4 g, 18%, ee=99.5%) as a brown solid. MS (ES⁺) C₁₃H₉Cl₂F₃N₂O₄S requires: 416, found: 417 [M+H]⁺.

Step 2

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichlorophenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 10b)

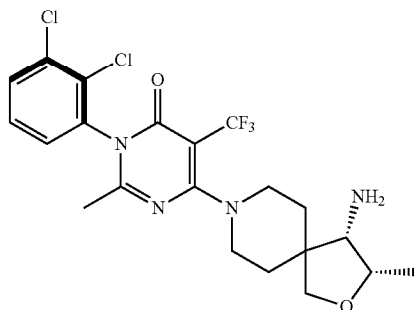
To a solution of (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine (23.3 g, 95.6 mmol, 1.05 eq, 2HCl) in DMF (50.0 mL) was added DIPEA (47.1 g, 364 mmol, 63.5 mL) and a solution of 1-(R_a)-(2,3-dichlorophenyl)-2,5-dimethyl-6-oxo-1,6-dihydropyrimidin-4-yl trifluoromethanesulfonate (38.0 g, 91.1 mmol, 1.00 eq) in DMF (200 mL) at 0° C. The resulting mixture was stirred at 15° C. for 18 h and (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine (1.11 g, 4.55 mmol, 2HCl) was added. The resulting mixture was stirred at 25° C. for 15 h. The reaction mixture was concentrated under reduced pressure. K₂CO₃ (15%, 500 mL, aq.) was added and the aqueous phase was extracted with DCM (200 mL×3). The combined organic layers were washed with brine (400 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, MeOH in DCM, 5 to 20%) followed by reversed-phase column chromatography (0.1% NH₃·H₂O, MeCN: H₂O (65:35)). The residue was dissolved in hot MeOH (250 mL), to the resulting solution was added activated carbon (2.70 g) and the mixture was stirred at 60° C. for 15 min. The mixture was filtered and

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concentrated under reduced pressure to afford the title compound Example 10b (26.5 g, 66.5%) as off-white solid.

Example 11

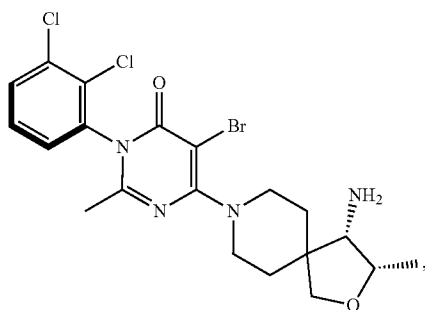
6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(*R_a*)-(2,3-dichlorophenyl)-2-methyl-5-(trifluoromethyl)pyrimidin-4(3H)-one TFA salt



A suspension of 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-chloro-3-(*R_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 9) (35 mg, 0.083 mmol), TFA (9.6 μ L, 0.12 mmol) and bis(((trifluoromethyl)sulfinyl)oxy)zinc (55 mg, 0.16 mmol) in DCE (591 μ L) was sonicated to give a uniform suspension then cooled to $^{\circ}$ C. and tert-butyl hydroperoxide (34 μ L, 0.25 mmol) was added dropwise. The resulting mixture was warmed to rt and stirred for 2 h. Water (1 mL) was added, the layers were separated and the aqueous layer was extracted with DCM (1 mL $\times$ 3). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by mass-triggered preparative HPLC (Mobile phase: A=0.1% TFA/H₂O, B=0.1% TFA/MeCN; Gradient: B=20-50%; 20 min; Column:)(Bridge C18, 5 μ m, 19 mm $\times$ 150 mm) to afford the title compound (15 mg, 30% yield) as a white solid (single diastereomer): MS (ES) C₂₁H₂₃Cl₂F₃N₄O₂ requires: 490, found: 491 [M+H]⁺; ¹H NMR (600 MHz, DMSO-d₆) δ 7.90 (s, 3H), 7.84 (dd, J=7.9, 1.8 Hz, 1H), 7.63-7.56 (m, 2H), 4.24-4.17 (m, 1H), 3.96 (d, J=14.0 Hz, 1H), 3.91-3.83 (m, 2H), 3.72 (d, J=9.0 Hz, 1H), 3.50-3.44 (m, overlap H₂O, 1H), 3.32-3.16 (m, overlap H₂O, 2H), 2.03 (s, 3H), 1.78 (d, J=13.8 Hz, 3H), 1.61 (d, J=13.2 Hz, 1H), 1.21 (d, J=6.5 Hz, 3H).

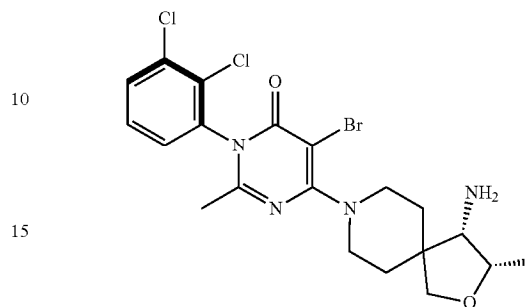
Example 12a and 12b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*S_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 12a)



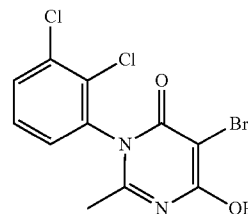
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and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*R_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 12b):



Step 1

5-Bromo-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one



To a solution of 3-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one (Example 1, step 2) (540 mg, 2 mmol) in DCM (8 mL) was added a solution of NBS (356 mg, 2 mmol) in DCM (2 mL). The reaction mixture was stirred at rt for 2 h. The reaction mixture was filtered to afford the title compound as a white solid (680 mg, 97.1%). MS (ES⁺) C₁₁H₇BrCl₂N₂O₂ requires: 350, found: 351 [M+H]⁺.

The compound of Step 1 can exist as a mixture of 5-bromo-(*S_a*)-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one and 5-bromo-(*R_a*)-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one.

Step 2

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*R_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 12a) and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*S_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one (Example 12b)

5-Bromo-3-(2,3-dichlorophenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one (80 mg, 0.23 mmol), (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine (67 mg, 0.27 mmol), BOP (121 mg, 0.27 mmol) and DBU (174 mg, 1.14 mmol) in MeCN (2 mL) was stirred at rt for 2 h. The mixture was concentrated and purified by prep-HPLC (NH₄HCO₃) to afford a solid. The mixture of diastereomers were separated by Chiral SFC (Mobile phase: CO₂/methanol (0.2% MEA)=30/70; Flow rate: 80 g/min; 5 min; Column temperature: 35 $^{\circ}$ C.; Back pressure: 100 bar; Column: AD

75

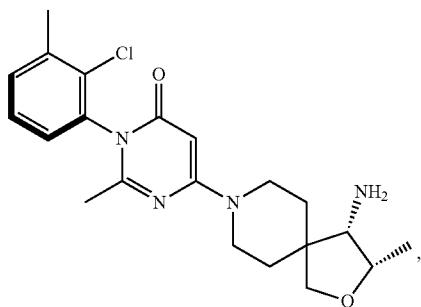
20*250 mm, 10 um (Daicel) to afford Example 12a as white solid (18 mg, 16%) and Example 12b as a white solid (15 mg, 13%).

Example 12a 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*S_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one: MS (ES+) $C_{20}H_{23}BrCl_2N_4O_2$ requires: 500, 502, found: 501, 503 [M+H]⁺; ¹H NMR (500 MHz, DMSO) δ 7.83 (dd, J=8.1, 1.5 Hz, 1H), 7.62 (dd, J=8.0, 1.5 Hz, 1H), 7.56 (t, J=8.0 Hz, 1H), 4.09-4.02 (m, 1H), 3.93-3.82 (m, 2H), 3.66 (d, J=8.5 Hz, 1H), 3.50-3.36 (m, 3H), 2.91 (d, J=5.1 Hz, 1H), 2.00 (s, 3H), 1.86-1.78 (m, 1H), 1.68-1.59 (m, 1H), 1.61-1.47 (m, 2H), 1.08 (d, J=6.4 Hz, 3H).

Example 12b 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-bromo-3-(*R_a*)-(2,3-dichlorophenyl)-2-methylpyrimidin-4(3H)-one: MS (ES+) $C_{20}H_{23}BrCl_2N_4O_2$ requires: 500, 502, found: 501, 503 [M+H]⁺; ¹H NMR (500 MHz, DMSO) δ 7.83 (dd, J=8.0, 1.6 Hz, 1H), 7.62 (dd, J=7.9, 1.5 Hz, 1H), 7.56 (t, J=8.0 Hz, 1H), 4.10-4.00 (m, 1H), 3.94-3.82 (m, 2H), 3.66 (d, J=8.4 Hz, 1H), 3.53-3.36 (m, 3H), 2.91 (d, J=5.0 Hz, 1H), 2.00 (s, 3H), 1.81 (ddd, J=13.0, 9.3, 3.4 Hz, 1H), 1.69 (ddd, J=12.9, 9.1, 3.6 Hz, 1H), 1.61-1.46 (m, 2H), 1.08 (d, J=6.4 Hz, 3H).

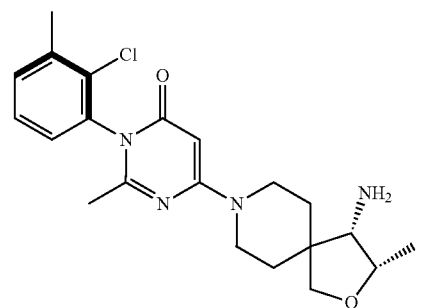
Example 13a and 13b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(*S_a*)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 13a)



and

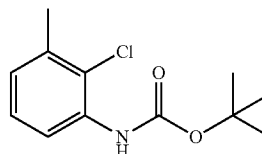
6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(*S_a*)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 13b)



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Step 1

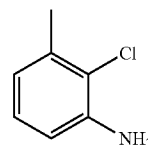
Tert-butyl 2-chloro-3-methylphenylcarbamate



A mixture of 1-bromo-2-chloro-3-methylbenzene (4.0 g, 19.6 mmol), Pd₂dba₃ (1014 mg, 0.98 mmol), Xantphos (566 mg, 0.98 mmol), tert-butyl carbamate (3.41 g, 29.4 mmol) and Cs₂CO₃ (9.58 g, 29.4 mmol) in dioxane (120 mL) was stirred at 110° C. for 2 h and then cooled to rt. Water (150 mL) was added and the mixture was extracted by EtOAc (150 mL×2). The organic layer was dried and concentrated. The residue was purified by flash column chromatography (PE, 100%) to afford the title compound (4.2 g, 89%). MS (ES+) $C_{12}H_{16}ClNO_2$ requires: 241, found: 187 [M-55+H]⁺.

Step 2

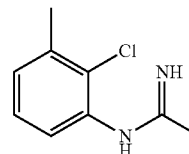
2-Chloro-3-methylaniline



A mixture of tert-butyl 2-chloro-3-methylphenylcarbamate (4.2 g, 17.4 mmol) in 2 M HCl (40 mL in MeOH) was stirred at rt for 1 h. The mixture was concentrated, washed with Et₂O (40 mL), and dried under vacuum to afford the title compound as a yellow solid (2.3 g, 93%). MS (ES+) C_7H_8ClN requires: 141, found: 142 [M+H]⁺.

Step 3

N-(2-Chloro-3-methylphenyl)acetimidamide

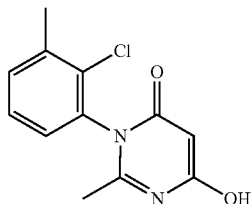


A mixture of 2-chloro-3-methylaniline (1.4 g, 9.93 mmol) in HCl (1N in CH₃CN, 15 mL) was heated at 120° C. for 4 h. The reaction mixture was cooled to rt, it was filtered and the filter cake was washed by Et₂O (15 mL). The filter cake was dried under vacuum to afford the title compound (1.2 g, 66%). MS (ES+) $C_9H_{11}ClN_2$ requires: 182, found: 183 [M+H]⁺.

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Step 4

3-(2-Chloro-3-methylphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one



To a solution of N-(2-chloro-3-methylphenyl)acetimidamide (1.2 g, 6.6 mmol) in 2-methoxyethanol (12 mL) was added diethyl malonate (4.22 g, 26.36 mmol) and $\text{CH}_3\text{ONa}/\text{CH}_3\text{OH}$ (4 M, 6.6 mL, 26 mmol). The mixture was heated at 120° C. for 16 h. The mixture was cooled to rt, it was poured into water (50 mL) and the aqueous phase was washed with Et_2O (50 mL). The aqueous layer was acidified to pH-2 with 6N HCl and it was extracted with EtOAc (50 mL $\times$ 3). The organic layer was dried and concentrated. The residue was washed with EtOAc to afford the title compound (300 mg, 18%) as a white solid. MS (ES+) $\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O}_2$ requires: 250, found: 251[M+H]⁺.

The compound of Step 4 can exist as a mixture of 3-(S_a) (2-chloro-3-methylphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one and 3-(R_a) (2-chloro-3-methylphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one.

Step 5

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 13a) and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 13b)

To a suspension of 3-(2-chloro-3-methylphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one (150 mg, 0.6 mmol) and (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (173 mg, 0.72 mmol) in MeCN (3 mL) were added DBU (183 mg, 1.2 mmol) and BOP (318 mg, 0.72 mmol) and the resulting mixture was stirred at rt for 1 h. The mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (A:water(0.05% TFA), B:ACN), 18-28% B in 10 min, stop at 15 min. Column: Sunfire prep C18 10 μm , OBD 19 $\times$ 250 mm) to afford the title compound TFA salt Example 13a, (40 mg, 25%) and the title compound TFA salt Example 13b (60 mg, 38%).

Example 13a 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) $\text{C}_{23}\text{H}_{28}\text{ClF}_3\text{N}_4\text{O}_4$ requires: 402, found: 403 [M+H]⁺; ¹H NMR (500 MHz, DMSO) δ 7.92 (s, 3H), 7.49-7.48 (m, 1H), 7.41-7.38 (m, 1H), 7.29-7.28 (m, 1H), 5.43 (s, 1H), 4.21-4.19 (m, 2H), 3.87-3.85 (m, 1H), 3.70-3.68 (m, 1H), 3.41-3.39 (m, 2H), 3.05-3.02 (m, 2H), 2.41 (s, 3H), 1.96 (s, 3H), 1.69-1.68 (m, 3H), 1.55-1.53 (m, 1H), 1.21-1.20 (d, J=7 Hz, 3H).

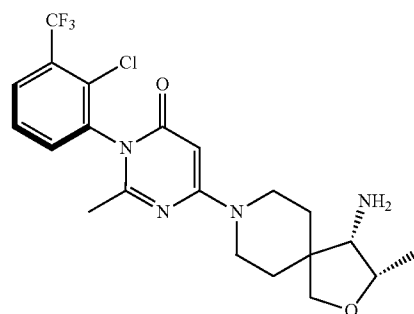
Example 13b 6-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2-chloro-3-methylphenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) $\text{C}_{23}\text{H}_{28}\text{ClF}_3\text{N}_4\text{O}_4$ requires: 402, found: 403 [M+H]⁺; ¹H NMR (500 MHz, DMSO) δ 8.01(s, 3H), 7.49-7.47 (m, 1H),

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7.41-7.38 (m, 1H), 7.29-7.28 (m, 1H), 5.43 (s, 1H), 4.22-4.19 (m, 2H), 3.88-3.86 (m, 2H), 3.69-3.68 (m, 1H), 3.42-3.40 (m, 1H), 3.06-3.00 (m, 2H), 2.41 (s, 3H), 1.96 (s, 3H), 1.71-1.68 (m, 3H), 1.54-1.51 (m, 1H), 1.22-1.21 (d, J=6 Hz, 3H).

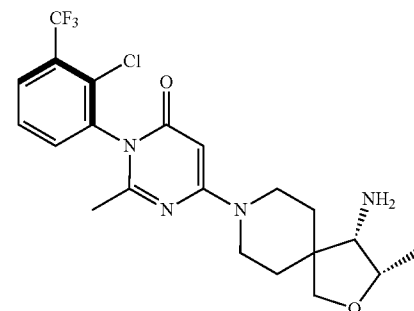
Example 14a and 14b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-(trifluoromethyl)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 14a)



and

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-(trifluoromethyl)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 14b):



Examples 14a and 14b were synthesized with synthetic methods that were similar to that used for Examples 13a and 13b, and can generally be made by methods disclosed herein. The Examples may be made as free bases or as TFA salts.

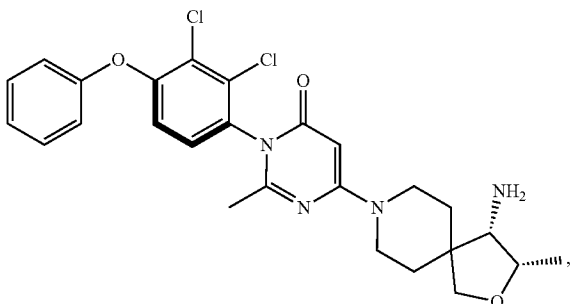
Example 14a 6-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2-chloro-3-(trifluoromethyl)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) $\text{C}_{21}\text{H}_{24}\text{ClF}_3\text{N}_4\text{O}_2$ requires: 456, found: 457 [M+H]⁺.

Example 14b 6-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2-chloro-3-(trifluoromethyl)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) $\text{C}_{21}\text{H}_{24}\text{ClF}_3\text{N}_4\text{O}_2$ requires: 456, found: 457 [M+H]⁺.

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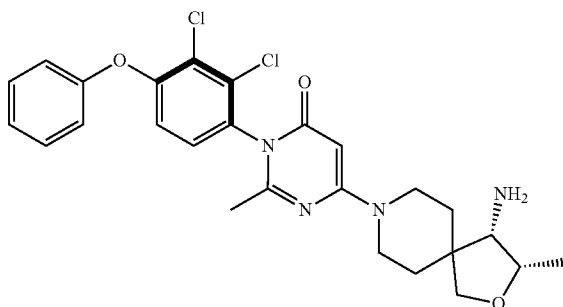
Example 15a and 15b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one (Example 15a)



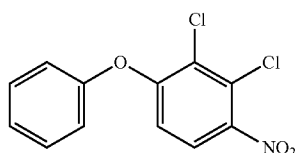
and

6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one (Example 15b)



Step 1

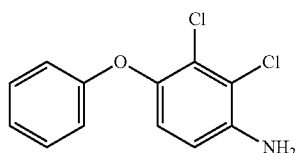
2,3-Dichloro-1-nitro-4-phenoxybenzene



To a solution of phenol (1.1 g, 12 mmol) in dry N,N-dimethylformamide (15 mL) was added NaH (520 mg, 13 mmol, 60%) by small portions at 0° and the resulting mixture was stirred at 0° C. for 10 min. 2,3-Dichloro-1-fluoro-4-nitrobenzene (2.1 g, 10 mmol) was added and the mixture was warmed to rt and stirred overnight. The mixture was purified by silica gel chromatography (EtOAc in PE, 0 to 15%) to afford the title compound (2.0 g, 67%) as a yellow solid. MS (ES+) C₁₂H₇Cl₂NO₃ requires 282; found: 283.

Step 2

2,3-Dichloro-4-phenoxyaniline

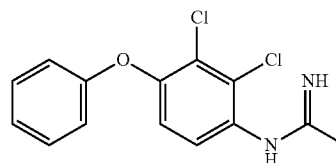


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2,3-Dichloro-1-nitro-4-phenoxybenzene (1.8 g, 6.4 mmol) and SnCl₂·2H₂O (14.3 g, 64 mmol) in EtOH (20 mL) was stirred at 85° C. for 5 h. The resulting mixture was adjusted to pH>9 with aq. sat. NaHCO₃, the solid was filtered and washed with EtOAc (50 mL×3). The organic phase was dried, concentrated under reduced pressure and purified by silica gel chromatography (EtOAc in PE, 0 to 40%) to afford the title compound (1.2 g, 73%) as a tan solid. MS (ES+) C₁₂H₉Cl₂NO requires 252; found: 253.

Step 3

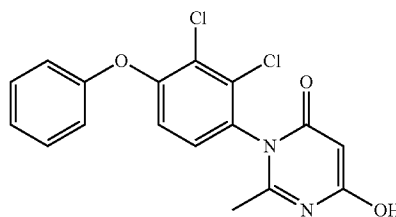
N-(2,3-Dichloro-4-phenoxyphenyl)acetimidamide



2,3-Dichloro-4-phenoxyaniline (1.0 g, 3.9 mmol) in HCl/MeCN (1 M, 20 mL) was heated in a sealed tube at 120° C. overnight. The mixture was cooled to rt, the solid was filtered and washed with MeCN (5 mL) to afford the title compound (0.9 g, 70%) as a white solid. MS (ES+) C₁₄H₁₂Cl₂N₂ requires 293; found: 294.

Step 4

3-(2,3-dichloro-4-phenoxyphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one



The reaction was performed as described for Example 1, Step 2, Method B to provide the title compound (0.5 g, 54%) as a tan solid. MS (ES+) C₁₇H₁₂Cl₂N₂O₃ requires 361; found 362.

The compound of Step 4 can exist as a mixture of 3-(S_a)-(2,3-dichloro-4-phenoxyphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one and 3-(R_a)-(2,3-dichloro-4-phenoxyphenyl)-6-hydroxy-2-methylpyrimidin-4(3H)-one.

Step 5

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one (Example 15a) and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one (Example 15b)

The reaction was performed as described for Example 2, Step 1. The mixture of diastereomers were separated by Chiral SFC chiral-HPLC (co-solvent: MeOH (0.2% methanol-ammonia), Column: AD-H, 4.6×100 mm) to provide title compounds Example 15a (25 mg, 18%) and 15b (27 mg, 19%).

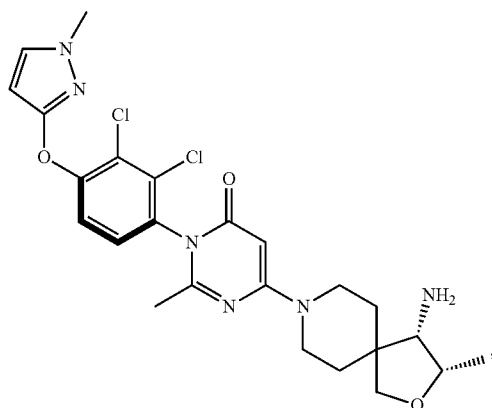
81

Example 15a 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one: MS (ES+) C₂₆H₂₈Cl₂N₄O₃ requires: 514, found: 515 [M+H]⁺; ¹H NMR (400 MHz, MeOD) δ 7.44 (m, 2H), 7.37 (dd, J=9, 2 Hz, 1H), 7.24 (t, J=7.5 Hz, 1H), 7.12 (dd, J=9, 1 Hz, 1H), 7.07 (dd, J=9, 2 Hz, 1H), 5.51 (s, 1H), 4.25 (m, 1H), 4.07 (m, 2H), 3.88 (d, J=9 Hz, 1H), 3.74 (m, 1H), 3.73 (d, J=9 Hz, 1H), 3.42 (m, 1H), 3.03 (d, J=5 Hz, 1H), 2.13 (s, 3H), 1.83-1.67 (m, 4H), 1.23 (d, J=6 Hz, 3H).

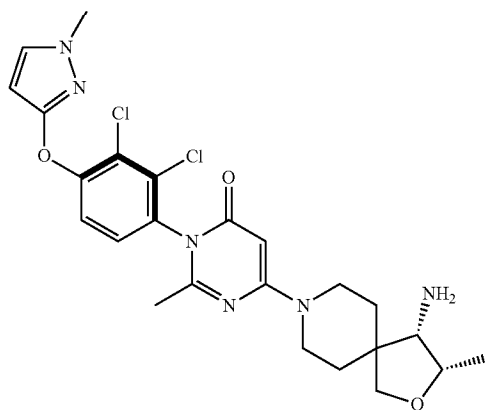
Example 15b 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-phenoxyphenyl)-2-methylpyrimidin-4(3H)-one: MS (ES+) C₂₆H₂₈Cl₂N₄O₃ requires 514; found: 515 [M+H]⁺; ¹H NMR (500 MHz, MeOD) δ 7.51-7.41 (m, 2H), 7.36 (dd, J=8.8, 2.7 Hz, 1H), 7.24 (t, J=7.4 Hz, 1H), 7.12 (dd, J=8.6, 0.8 Hz, 2H), 7.07 (dd, J=8.8, 1.8 Hz, 1H), 5.51 (d, J=7.5 Hz, 1H), 4.30-4.21 (m, 1H), 4.07 (d, J=4.8 Hz, 1H), 4.07 (m, 1H), 3.89 (d, J=8.7 Hz, 1H), 3.74 (d, J=8.7 Hz, 1H), 3.42 (t, J=10.0 Hz, 1H), 3.03 (d, J=5.0 Hz, 1H), 2.14 (d, J=3.9 Hz, 3H), 1.89-1.61 (m, 4H), 1.23 (dd, J=11.7, 6.4 Hz, 3H).

Example 16a and 16b

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-(1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 16a)



and
6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-(1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt (Example 16b)



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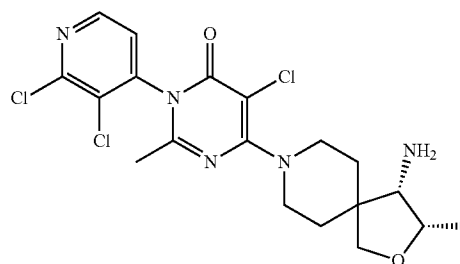
Examples 16a and 16b were synthesized with synthetic methods that were similar to that used for Examples 15a and 15b, and can generally be made by methods disclosed herein. The Examples may be made as free bases or as TFA salts.

Example 16a 6-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) C₂₄H₂₈Cl₂N₆O₃ requires: 518, found: 519 [M+H]⁺.

Example 16b 6-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2-methylpyrimidin-4(3H)-one TFA salt: MS (ES+) C₂₄H₂₈Cl₂N₆O₃ requires: 518, found: 519 [M+H]⁺.

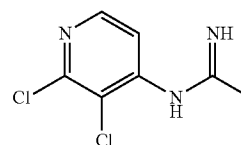
Example 17

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-chloro-3-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one



Step 1

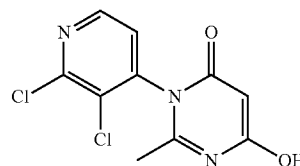
N-(2,3-Dichloropyridin-4-yl)acetimidamide



To a solution of 2,3-dichloro-4-iodopyridine (1 g mg, 3.6 mmol) in DMF (5 ml) were added acetimidamide hydrochloride (345 mg, 3.65 mmol), Cs₂CO₃ (2.4 g, 7.4 mmol) and Cu(I)I (70 mg, 0.36 mmol) and the resulting mixture was stirred at 90° C. for 12 h. The reaction mixture was diluted with MeCN (20 ml), filtered through Celite, and the filtrate was concentrated under reduced pressure to provide the title compound as a gray solid (0.83 g, 100%).

Step 2

3-(2,3-Dichloropyridin-4-yl)-6-hydroxy-2-methylpyrimidin-4(3H)-one



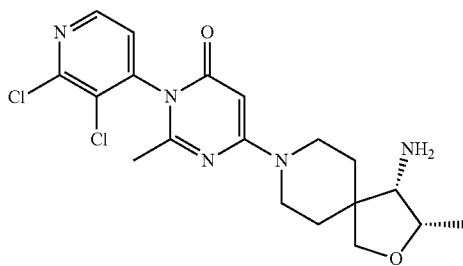
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To a suspension of N-(2,3-dichloropyridin-4-yl)acetimidamide(1) (500 mg, 2.45 mmol) in THF (5 mL) was added bis(2,4,6-trichlorophenyl) malonate (1.2 g, 2.6 mmol) and the resulting mixture was stirred at 90° C. for 2 h. The volatiles were removed under reduced pressure. The reaction mixture was diluted with EtOAc (20 mL) and washed with water (5 mL). The layers were separated, and the organic layer was washed with brine (5 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified via silica gel chromatography (0-20% MeOH in DCM to provide the title compound (280 mg, 42% yield) as a light orange solid. MS (ES+) C₁₀H₇Cl₂N₃O₂ requires: 271, found: 272 [M+H]⁺.

The compound of Step 2 can exist as a mixture of 3-(R_a)-(2,3-Dichloropyridin-4-yl)-6-hydroxy-2-methylpyrimidin-4(3H)-one and 3-(S_a)-(2,3-dichloropyridin-4-yl)-6-hydroxy-2-methylpyrimidin-4(3H)-one.

Step 3

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one



To 3-(2,3-dichloropyridin-4-yl)-6-hydroxy-2-methylpyrimidin-4(3H)-one (100 mg, 0.37 mmol), (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (89 mg, 0.37 mmol), BOP (244 mg, 0.551 mmol) in DMF (2 mL) was added DBU (0.11 mL, 0.73 mmol) and the resulting mixture was stirred at rt for 1 h. The residue was purified by mass-triggered preparative HPLC (Mobile phase: A=0.1% TFA/H₂O, B=0.1% TFA/MeCN; Gradient: B=10-50%; 20 min; Column: C18) to provide the title compound (92 mg, 0.22 mmol, 59% yield) as a white solid. MS (ES+) C₁₉H₂₃Cl₂N₅O₂ requires: 423, found: 424 [M+H]⁺.

The compound of Step 3 can exist as a mixture of 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one.

Step 4

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-chloro-3-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one

A vial was charged with 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one (40 mg, 0.047 mmol), NCS (15 mg, 0.11 mmol) and DCM (1.0 mL). The mixture was stirred at rt for 5 min. The volatiles were removed under reduced pressure. The residue was purified by mass-triggered preparative HPLC (Mobile phase: A=0.1% TFA/H₂O, B=0.1% TFA/MeCN; Gradient: B=10-40%; 20 min; Col-

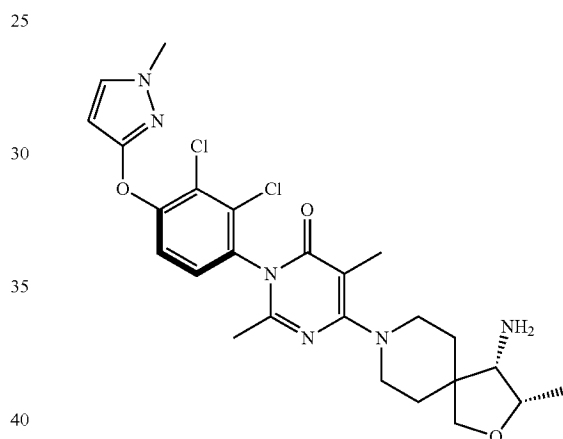
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umn: C18) to provide the title compound (12 mg, 0.026 mmol, 11% yield) as a white powder. MS (ES+) C₁₉H₂₂Cl₃N₅O₂ requires: 457, found: 458 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ 8.64 (d, J=5.0 Hz, 1H); 7.94 (m, 2H); 7.82 (m, 1H); 4.16-4.26 (m, 2H); 3.86 (m, 1H); 3.71 (d, J=10 Hz, 1H); 3.17-3.26 (m, 3H); 2.81 (d, J=10.68 Hz, 1H), 2.06 (s, 3H), 1.73-1.81 (m, 3H), 1.62 (m, 1H), 1.22 (d, J=5.0 Hz, 3H).

The compound of Example 17 can exist as a mixture of 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-chloro-3-(S_a)-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one and 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-5-chloro-3-(R_a)-(2,3-dichloropyridin-4-yl)-2-methylpyrimidin-4(3H)-one.

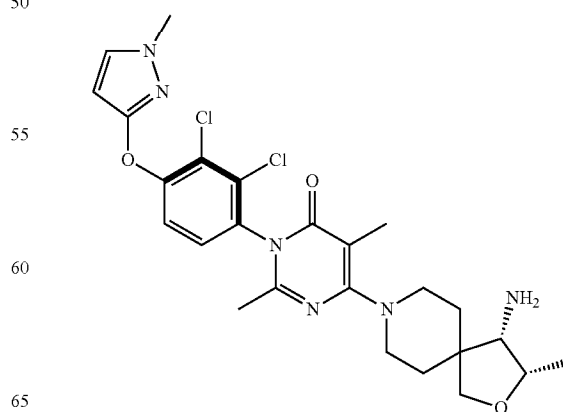
Example 18a and 18b

20 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-(1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 18a)



and

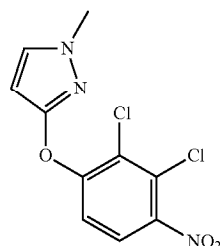
25 6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-(1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 18b):



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Step 1

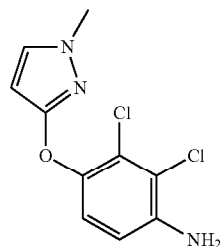
3-(2,3-Dichloro-4-nitrophenoxy)-1-methyl-1H-pyrazole



A mixture of 2,3-dichloro-1-fluoro-4-nitrobenzene (1.3 g, 6.2 mmol), 1-methyl-1,2-dihydro-3H-pyrazol-3-one (729 mg, 7.4 mmol), K_2CO_3 (1.7 g, 12.4 mmol) in MeCN (30 mL) was stirred at rt for 3 h. The reaction mixture was poured into water (50 ml) and extracted with EtOAc (100 mL $\times$ 3). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated and the residue was purified by silica gel chromatography (EtOAc in PE, 10%) to provide the title compound (1.5 g, 84%) as a white solid. MS (ES $^+$) $C_{10}H_7Cl_2N_3O_3$ requires: 287; found: 288 [M+H] $^+$.

Step 2

2,3-Dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)aniline

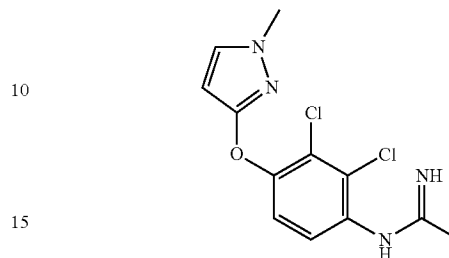


To a solution of 3-(2,3-dichloro-4-nitrophenoxy)-1-methyl-1H-pyrazole (1.5 g, 5.2 mmol) in AcOH (10 mL) was added Fe (1.4 g, 26 mmol). The resulting mixture was stirred at 60° C. for 2 h. The reaction mixture was poured into ice water (60 ml) and extracted with EtOAc (100 mL $\times$ 3). The combined organic phases were dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel chromatography (EtOAc in PE, 50%) to provide the title compound (1.2 g, 90%) as a yellow solid. MS (ES $^+$) $C_{10}H_9Cl_2N_3O$ requires: 257; found: 258 [M+H] $^+$.

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Step 3

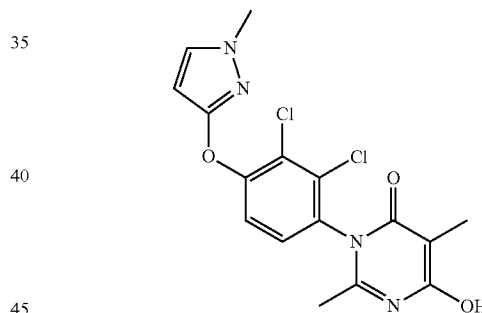
N-(2,3-Dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)acetimidamide



A mixture of 2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)aniline (1.2 g, 4.7 mmol) in HCl/CH₃CN (1 M, 10 mL) was stirred in a seal tube at 120° C. for 24 h. The mixture was concentrated and the residue was washed with Et₂O (10 mL). The precipitate was filtered to provide the title compound (1.2 g, 86%) as a white solid. MS (ES $^+$) $C_{12}H_{12}Cl_2N_4O$ requires: 298; found: 299 [M+H] $^+$.

Step 4

3-(2,3-Dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one



To a solution of diethyl 2-methylmalonate (1.4 g, 8.0 mmol) in EtOH (6 mL) was added CH₃ONa/CH₃OH (4 M, 2 mL). The mixture was stirred at rt for 10 min. N-(2,3-Dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)acetimidamide (600 mg, 2 mmol) was added and the mixture was stirred at 120° C. for 4 h. The mixture was poured into water (30 mL) and extracted with Et₂O (30 mL $\times$ 2). The pH of the aqueous layer was adjusted pH 5 by AcOH and extracted with EtOAc (80 mL $\times$ 3). The combined organic phases were dried (Na_2SO_4), filtered and concentrated. The crude product was washed with Et₂O (10 mL) to provide the title compound (320 mg, 42%) as a white solid. MS (ES $^+$) $C_{16}H_{14}Cl_2N_4O_3$ requires: 380; found: 381 [M+H] $^+$.

The compound of Step 4 can exist as a mixture of 3-(S_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one and 3-(R_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one.

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Step 5

6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 18a) and 6-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one (Example 18b)

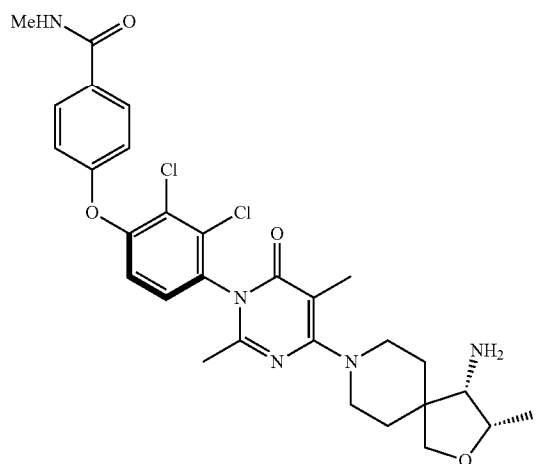
To a suspension of 3-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one (100 mg, 0.26 mmol) and (3S, 4S)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-amine dihydrochloride (126 mg, 0.52 mmol) in MeCN (8 ml) was added DBU (395 mg, 2.6 mmol) and BOP (230 mg, 0.52 mmol) and the resulting mixture was stirred at rt for 48 h. The mixture was concentrated under reduced pressure and the residue was purified by preparative HPLC (Mobile phase: A=0.05% TFA/H₂O, B=MeCN; Gradient: B=21-31%; 10 min; Column: C18) and the diastereomers were separated using a Chiralpak OZ Column (20x250 mm) with CO₂ and 0.2% methanol ammonia(45:55) as an eluent, a flow rate of 80 g/min and UV detection (214 nm) to provide Example 18a (7 mg, 5%) and Example 18b (9 mg, 7%) as a white solid.

Example 18a 6-((3 S, 4 S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(S_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES+) C₂₅H₃₀Cl₂N₆O₃ requires: 532 found: 533 [M+H]⁺; ¹H NMR (500 MHz, MeOD) δ 7.46 (d, J=2.5 Hz, 1H), 7.14-7.23 (m, 2H), 5.84 (d, J=2.5 Hz, 1H), 4.12-4.15 (m, 1H), 3.71-3.78 (m, 1H), 3.62-3.68 (m, 6H), 3.17-3.20 (m, 3H), 2.95-2.96 (m, 1H), 1.99 (s, 3H), 1.90 (s, 3H), 1.57-1.78 (m, 4H), 1.18 (s, 1H), 1.12 (d, J=6 Hz, 3H).

Example 18b 6-((3 S, 4 S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-3-(R_a)-(2,3-dichloro-4-((1-methyl-1H-pyrazol-3-yl)oxy)phenyl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES+) C₂₅H₃₀Cl₂N₆O₃ requires: 532 found: 533 [M+H]⁺; ¹H NMR (500 MHz, MeOD) δ 7.58 (d, J=2.5 Hz, 1H), 7.26-7.36 (m, 2H), 5.96 (d, J=3 Hz, 1H), 4.24-4.26 (m, 1H), 3.83-3.89 (m, 1H), 3.74-3.80 (m, 6H), 3.18-3.29 (m, 3H), 3.05-3.06 (m, 1H), 2.69 (s, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 1.66-1.88 (m, 4H), 1.23 (d, J=7 Hz, 3H).

Example 19a and 19b

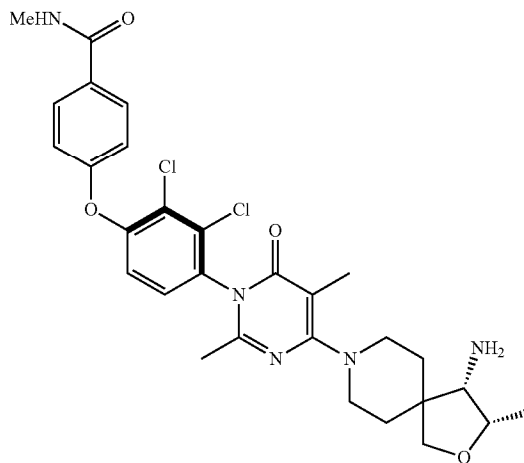
4-(4-(4-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethyl-6-oxopyrimidin-1(6H)-yl)-(S_a)-(2,3-dichlorophenoxy)-N-methylbenzamide (Example 19a)



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and

4-(4-(4-((3S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethyl-6-oxopyrimidin-1(6H)-yl)-(R_a)-(2,3-dichlorophenoxy)-N-methylbenzamide (Example 19b)



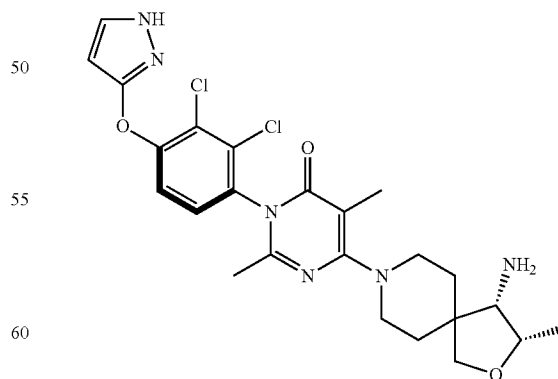
Examples 19a and 19b were synthesized with synthetic methods that were similar to that used for Examples 15a and 15b, and can generally be made by methods disclosed herein. The Examples may be made as free bases or as TFA salts.

Example 19a 4-(4-(4-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethyl-6-oxopyrimidin-1 (6H)-yl)-(S_a)-(2,3-dichlorophenoxy)-N-methylbenzamide: MS (ES+) C₂₉H₃₃Cl₂N₅O₄ requires: 585, found: 586 [M+H]⁺.

Example 19b 4-(4-(4-((3 S, 4S)-4-Amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethyl-6-oxopyrimidin-1 (6H)-yl)-(R_a)-(2,3-dichlorophenoxy)-N-methylbenzamide: MS (ES+) C₂₉H₃₃Cl₂N₅O₄ requires: 585, found: 586 [M+H]⁺.

Example 20a and 20b

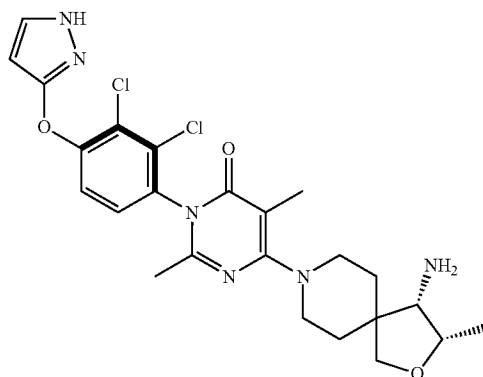
3-(4-((1H-Pyrazol-3-yl)oxy)-(S_a)-2,3-dichlorophenyl)-6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one (Example 20a)



and

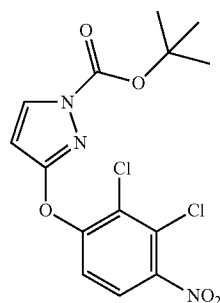
3-(4-((1H-pyrazol-3-yl)oxy)-(R_a)-2,3-dichlorophenyl)-6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one (Example 20b)

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Step 1

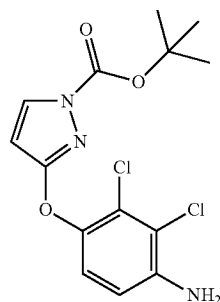
tert-Butyl 3-(2,3-dichloro-4-nitrophenoxy)-1H-pyrazole-1-carboxylate



A mixture of tert-butyl 3-oxo-2,3-dihydro-1H-pyrazole-1-carboxylate (687 mg, 3.73 mmol), 2,3-dichloro-1-fluoro-4-nitrobenzene (650 mg, 3.11 mmol) and K_2CO_3 (858 mg, 6.22 mmol) in DMSO (10 mL) was stirred at rt for 4 h. Water (20 mL) was added and the mixture was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 , filtered and purified by column chromatography (EtOAc in PE; 20 to 50%) to afford the title compound (800 mg, 67%) as a yellow solid. MS (ES+) $C_{14}H_{13}Cl_2N_3O_5$ requires: 373, found: 318 $[M-56+H]^+$.

Step 2

tert-Butyl 3-(4-amino-2,3-dichlorophenoxy)-1H-pyrazole-1-carboxylate

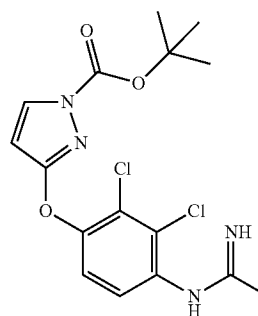


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A suspension of tert-butyl 3-(2,3-dichloro-4-nitrophenoxy)-1H-pyrazole-1-carboxylate (1.2 g, 3.2 mmol), Pt/C (200 mg) in EtOH (100 mL) was stirred under H_2 atmosphere at rt for 16 h. The mixture was filtered and the filtrate was concentrated to afford the title compound (1.1 g, 100%) as a beige solid. MS (ESI+) $C_{14}H_{15}Cl_2N_3O_3$ requires: 343, found: 288 $[M-56+H]^+$.

Step 3

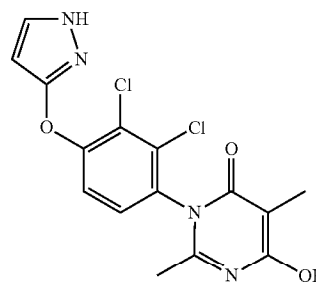
N-(4-((1H-Pyrazol-3-yl)oxy)-2,3-dichlorophenyl)acetimidamide



A mixture of tert-butyl 3-(4-amino-2,3-dichlorophenoxy)-1H-pyrazole-1-carboxylate (1.1 g, 3.2 mmol) in HO/ CH_3CN (1 M, 20 mL) was stirred in a sealed tube at 110° C. for 4 h. The mixture was concentrated to give N-(4-((1H-pyrazol-3-yl)oxy)-2,3-dichlorophenyl)acetimidamide as a crude product. MS (ESI+) $C_{11}H_{10}Cl_2N_4O$ requires: 284, found: 285 $[M+H]^+$.

Step 4

3-(4-((1H-Pyrazol-3-yl)oxy)-2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one



A mixture of N-(4-((1H-pyrazol-3-yl)oxy)-2,3-dichlorophenyl)acetimidamide (600 mg, 2.11 mmol) diethyl 2-methylmalonate (1.84 g, 10.6 mmol) in EtOH (10 mL) was added CH_3ONa/CH_3OH (4 M, 2.64 mL). The mixture was stirred at 110° C. for 5 h. The mixture was concentrated and dissolved in water (20 mL). The aqueous layer was extracted with EtOAc (20 mL) and adjusted pH to 5 with 6 N HCl. Solid was precipitated out, it was collected by filtration to give the title compound (300 mg, 38.7%) as a beige solid. MS (ESI+) $C_{15}H_{12}Cl_2N_4O_3$ requires: 366, found: 367 $[M+H]^+$.

The compound of Step 4 can exist as a mixture of 3-(4-((1H-pyrazol-3-yl)oxy)-(S_d)-2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one and 3-(4-((1H-

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pyrazol-3-yl)oxy)-(R_a)-2,3-dichlorophenyl)-6-hydroxy-2,5-dimethylpyrimidin-4(3H)-one.

Step 5

3-(4-((1H-Pyrazol-3-yl)oxy)-(S_a)-2,3-dichlorophenyl)-6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one (Example 20a) and 3-(4-((1H-pyrazol-3-yl)oxy)-(R_a)-2,3-dichlorophenyl)-6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one (Example 20b)

The experiment was performed as described for Examples 18a and 18b, step 5. The mixture was separated using a Chiralpak OZ Column (20×250 mm) with CO₂ and 0.2% methanol ammonia(50:50) as an eluent, a flow rate of 80 g/min and UV detection (214 nm) to provide Example 20a (15 mg, 11%) and Example 20b (13 mg, 11%) as a white solid.

Example 20a 3-(4-((1H-Pyrazol-3-yl)oxy)-(S_a)-2,3-dichlorophenyl)-6-((3S, 4S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES+) C₂₄H₂₈Cl₂N₆O₃ requires: 518, found: 519 [M+H]⁺; 1H NMR (500 MHz, DMSO) δ 12.57 (d, J=0.4 Hz, 1H), 7.78 (s, 1H), 7.48 (d, J=8.9 Hz, 1H), 7.18 (d, J=8.9 Hz, 1H), 6.05 (s, 1H), 4.12-3.99 (m, 1H), 3.67 (d, J=8.4 Hz, 1H), 3.60-3.52 (m, 2H), 3.50 (d, J=8.4 Hz, 1H), 3.23-3.07 (m, 2H), 2.93 (d, J=4.9 Hz, 1H), 2.00 (s, 3H), 1.89 (s, 3H), 1.81-1.74 (m, 1H), 1.70-1.63 (m, 1H), 1.59-1.47 (m, 2H), 1.23 (s, 1H), 1.09 (d, J=6.3 Hz, 3H).

Example 20b 3-(4-((1H-Pyrazol-3-yl)oxy)-(R_a)-2,3-dichlorophenyl)-6-((3 S, 4 S)-4-amino-3-methyl-2-oxa-8-azaspiro[4.5]decan-8-yl)-2,5-dimethylpyrimidin-4(3H)-one: MS (ES+) C₂₄H₂₈Cl₂N₆O₃ requires: 518, found: 519 [M+H]⁺; 1H NMR (500 MHz, DMSO) δ 12.57 (s, 1H), 7.81-7.75 (m, 1H), 7.48 (d, J=8.9 Hz, 1H), 7.18 (d, J=9.0 Hz, 1H), 6.05 (t, J=2.1 Hz, 1H), 4.10-4.01 (m, 1H), 3.67 (d, J=8.5 Hz, 1H), 3.61-3.51 (m, 2H), 3.50 (d, J=8.5 Hz, 1H), 3.23-3.18 (m, 1H), 3.15-3.06 (m, 1H), 2.93 (d, J=4.9 Hz, 1H), 2.00 (s, 3H), 1.89 (s, 3H), 1.80-1.75 (m, 1H), 1.70-1.63 (m, 1H), 1.58-1.48 (m, 2H), 1.32-1.19 (m, 2H), 1.09 (d, J=6.4 Hz, 3H).

Example 21: Biological Activity Assay

The activity of the compounds in the Examples disclosed herein as PTPN11 inhibitors is illustrated in the following assays.

PTPN11 Enzymatic Assay

Recombinant full-length wild-type and E76K mutant human PTPN11 proteins were cloned, expressed (*E. coli* system), and isolated via a two-step purification of Ni affinity followed by S75 size exclusion chromatography.

Phosphatase activity of full length wild-type PTPN11 (PTPN11-WT) or PTPN11-E76K mutant enzyme was measured using the fluorogenic 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; Molecular Probes) as the substrate. Enzyme (250 pM) was incubated with or without increasing concentrations of compounds in assay buffer (62.5 mM HEPES, 125 mM NaCl, 1 mM EDTA, 1.25 mM TECP, 0.1% BSA) for 30 min at room temperature. Reaction was initiated by addition of DiFMUP (50 μM) at room temperature in 384-well black plate with a final reaction volume of 20 μL in assay buffer. After 1 hour, DiFMUP fluorescence signal was measured (Ex:340/Em:460) using Envision plate reader. Dose-response curves were analyzed using IC₅₀ regression curve fitting (GeneData Screener).

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Curves were normalized to a high controls without inhibitor, and low controls without substrate. Results are provided below in Table 2.

TABLE 2

Biological Activity for inhibition of PTPN11-E76K mutant enzyme	
Example #	IC ₅₀ , nM
1	510
2a	7047
2b	122
3	310
4	480
5	1172
6	203
7a	>10000
7b	153
8	360
9	45
10a	2583
10b	28
11	366
12a	3457
12b	20
13a	7500
13b	186
14a	>1000
14b	703
15a	>1000
15b	148
16a	9552
16b	92
17	618
18a	511
18b	3
19b	12
20b	5

ERK Phosphorylation (Phospho-ERK) Target Engagement Assay

KYSE-520 cells (10 k cells/well) are plated onto 384-well plate in 20 μL of medium (RPMI-1640, without phenol red, containing 10% FBS) and incubated at 37° C., 5% CO₂ 16 h. DMSO (control) or increasing concentrations of compounds are diluted in medium, added to the 384-well plate (10 μL/well, final DMSO concentration of 1%), and cells are then incubated with compounds for 2 hr. Phospho-ERK levels are measured using a TR-FRET based phospho-ERK1/2 HTRF kit (CisBio, 64ERKPEH) following manufacturer's recommendations, and fluorescence signal was measured at 665 nm and 620 nm using Synergy Neo plate reader. Dose-response curves were analyzed using IC₅₀ regression curve fitting (GeneData Screener). Curves were normalized to a high controls without inhibitor, and low controls with 1 μM of selumetinib. Some compounds of this invention showed IC₅₀<1 μM.

Colony Formation Assay

KYSE-520 cells (2000 cells/well) are plated in 6-well plate containing 2 mL of medium (RPMI-1640, containing 10% FBS), in presence of DMSO (control; 1% final concentration) or increasing compound concentration. After 14 days of culture at 37° C. in a humidified 5% CO₂ incubator, colonies are fixed and stained with 0.1% crystal violet and 15% ethanol solution. Plates are imaged and colony area quantified and normalized to DMSO with ImageJ, Colony Area plugin. (Guzman, Camilo, PloS one 2014). Some compounds of this invention displayed IC₅₀<1 μM.

In Vivo Studies

The in vivo anti-tumor efficacy of Example 2b was evaluated in NSG (NOD scid gamma) mice with subcutaneously implanted KYSE520 xenografts. Example 2b was administered by oral gavage once a day for 21 days at dosing levels of 300 mg/kg, 50 mg/kg, 15 mg/kg and 5 mg/kg. Example 2b exhibited a dose-dependent tumor growth inhibition in the KYSE520 xenograft model at tolerated dose, as shown in FIG. 1.

The in vivo anti-tumor efficacy of Example 10b was evaluated in NSG mice with subcutaneously implanted KYSE520 xenografts. Example 10b was administered by oral gavage once a day for 21 days at 200 mg/kg, 150 mg/kg, 100 mg/kg, 30 mg/kg, 10 mg/kg and 3 mg/kg.

Example 10b exhibited a dose-dependent tumor growth inhibition in the KYSE520 xenograft model at tolerated dose, as shown in FIG. 2.

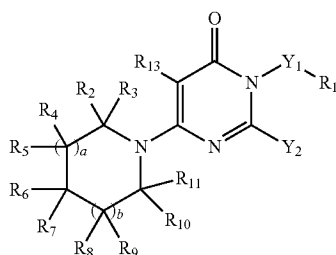
All references, patents or applications, U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein in their entireties. Where any inconsistencies arise, material literally disclosed herein controls.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

VIII. Embodiments

For further illustration, additional non-limiting embodiments of the present invention are set forth below.

Embodiment 1 is a compound represented by Formula I



or a salt, ester, or prodrug thereof, wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y₁ is a direct bond or CR₁₇R₁₈;

Y₂ is selected from the group consisting of C₁₋₄alkyl, amino, C₁₋₄alkylC(O)O—, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

R₁ is selected from the group consisting of C₆₋₁₀aryl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R₁ is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, N₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆,

S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄;

R₂, R₃, R₁₀, and R₁₁ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and C₃₋₈cycloalkyl;

R₄, R₅, R₈, and R₉ are each independently selected from the group consisting of hydrogen, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, amino, hydroxy, C₃₋₈cycloalkyl, halo, and C₁₋₄alkylamino;

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

R₇ is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino, and C₁₋₄aminoalkyl;

or R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated or unsaturated ring formed by R₆ and R₇ is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

any two groups of R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ and R₁₁ can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices elected from N, O and S; any two groups of R₂, R₄, R₆, R₈ and R₁₀ can form a direct bond, or a 1 or 2 atom carbon bridge;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, —NH—NHR₁₉, —NHR₁₉, —OR₁₉, —NHC(O)R₁₉, —NHC(O)NHR₁₉, —NHS(O)₂NHR₁₉, —NHS(O)₂R₁₉, —C(O)OR₁₉, —C(O)NR₁₉R₂₀, —C(O)NH(CH₂)_qOH, —C(O)NH(CH₂)_qR₂₁, —C(O)R₂₁, —NH₂, —OH, —S(O)₂NR₁₉R₂₀, C₃₋₈cycloalkyl, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C₁₋₄alkyl, —OH, —NH₂, —OR₂₁, halo, cyano, and oxo;

R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of amido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

R₁₇ and R₁₈ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and CF₃;

R₁₉ and R₂₀ are each independently selected from the group consisting of hydrogen, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl; and

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each R_{21} is independently selected from the group consisting of hydrogen, —OH, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and C_{3-6} cycloalkyl.

Embodiment 2 is the compound of embodiment 1, 5 wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y_1 is a direct bond or $CR_{17}R_{18}$;

Y_2 is selected from the group consisting of C_{1-4} alkyl, 10 amino, C_{1-4} alkylC(O)O—, C_{1-4} alkylamino and C_{1-4} aminoalkyl;

R_1 is selected from the group consisting of C_{6-10} aryl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring 15 vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R_1 is optionally substituted with 1 to 5 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl) amino, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, $NR_{15}C(O)R_{14}$, $NR_{15}C(O)OR_{14}$, $NR_{14}C(O)NR_{15}R_{16}$, $NR_{15}S(O)R_{14}$, $NR_{15}S(O)_2R_{14}$, $C(O)NR_{15}R_{16}$, $S(O)NR_{15}R_{16}$, $S(O)_2NR_{15}R_{16}$, $C(O)R_{14}$, $C(O)OR_{14}$, OR_{14} , SR_{14} , $S(O)R_{14}$, and $S(O)_2R_{14}$;

R_2 , R_3 , R_{10} , and R_{11} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, and C_{3-8} cycloalkyl;

R_4 , R_5 , R_8 , and R_9 are each independently selected from the group consisting of hydrogen, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, amino, hydroxy, C_{3-8} cycloalkyl, halo, and C_{1-4} alkylamino;

R_6 is selected from the group consisting of amino, C_{1-4} aminoalkyl, and C_{1-4} alkylamino;

R_7 is selected from the group consisting of hydrogen, 35 halo, and hydroxy, or is selected from the group consisting of amido, C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{3-6} cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino, and C_{1-4} aminoalkyl;

or R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated ring formed by R_6 and R_7 is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl;

any two groups of R_2 , R_3 , R_4 , R_5 , R_7 , R_8 , R_9 , R_{10} and R_{11} can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices selected from N, O and S; any two groups of R_2 , R_4 , R_6 , R_8 and R_{10} can form a direct bond, or a 1 or 2 atom carbon bridge;

R_{13} is selected from the group consisting of hydrogen, 60 halo, cyano, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} dihydroxyalkyl, —NH—NHR₁₉, —NHR₁₉, —OR₁₉, —NHC(O)R₁₉, —NHC(O)NHR₁₉, —NHS(O)₂NHR₁₉, —NHS(O)₂R₁₉, —C(O)OR₁₉, —C(O)NR₁₉R₂₀, —C(O)NH(CH₂)_qOH, —C(O)NH(CH₂)_qR₂₁, —C(O)R₂₁, —NH₂, —OH, —S(O)₂NR₁₉R₂₀, C_{3-8} cycloalkyl, aryl, heterocycl

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having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocycl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C_{1-4} alkyl, —OH, —NH₂, —OR₂₁, halo, cyano, and oxo;

R_{14} , R_{15} and R_{16} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, C_{3-8} cycloalkyl, C_{6-10} aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and C_{1-4} alkyl; R_{17} and R_{18} are each independently selected from the group consisting of hydrogen, C_{1-4} alkyl, and CF₃;

R_{19} and R_{20} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and C_{3-6} cycloalkyl; and

each R_{21} is independently selected from the group consisting of hydrogen, —OH, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and C_{3-6} cycloalkyl.

Embodiment 3 is the compound of embodiment 1 or 2, wherein subscripts a and b are each 1.

Embodiment 4 is the compound of any one of embodiments 1 to 3, wherein Y_1 is a direct bond.

Embodiment 5 is the compound of any one of embodiments 1 to 4, wherein Y_2 is C_{1-4} alkyl.

Embodiment 6 is the compound of embodiment 5, wherein Y_2 is methyl.

Embodiment 7 is the compound of any one of embodiments 1 to 6, wherein

R_{13} is selected from the group consisting of hydrogen, halo, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} dihydroxyalkyl, C_{3-8} cycloalkyl, a 3- or 6-membered heterocycl having 1-3 heteroatoms as ring vertices selected from N, O and S; wherein heterocycl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C_{1-4} alkyl, —OH, —NH₂, OR₂₁, halo, cyano and oxo.

Embodiment 8 is the compound of embodiment 7, wherein R_{13} is selected from the group consisting of hydrogen, halo, C_{1-6} alkyl, and C_{1-6} haloalkyl.

Embodiment 9 is the compound of embodiment 8, wherein R_{13} is hydrogen, Cl, Br, methyl, or CF₃.

Embodiment 10 is the compound of any one of embodiments 1 to 9, wherein

R_1 is selected from the group consisting of C_{6-10} aryl and a 5- to 9-membered heteroaryl group having 1 to 4 heteroatoms groups as ring vertices independently selected from N, C(O), O, and S; and is optionally substituted with 1 to 5 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} hydroxyalkyl, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkenyl, $NR_{15}C(O)R_{14}$, $NR_{15}C(O)OR_{14}$, $NR_{14}C(O)NR_{15}R_{16}$, $NR_{15}S(O)R_{14}$, $NR_{15}S(O)_2R_{14}$, $C(O)NR_{15}R_{16}$, $S(O)NR_{15}R_{16}$, $S(O)_2NR_{15}R_{16}$, $C(O)R_{14}$, $C(O)OR_{14}$, OR_{14} , SR_{14} , $S(O)R_{14}$, and $S(O)_2R_{14}$.

Embodiment 11 is the compound of embodiment 10, wherein

R_1 is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted

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with 1, 2 or 3 R_{12} groups independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, and OR_{14} .

Embodiment 12 is the compound of any one of embodiments 1 to 11, wherein R_2 , R_3 , R_4 , R_5 , R_8 , R_9 , R_{10} and R_{11} are hydrogen.

Embodiment 13 is the compound of any one of embodiments 1 to 12, wherein:

R_6 is selected from the group consisting of amino, C_{1-4} aminoalkyl, and C_{1-4} alkylamino; and

R_7 is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C_{1-4} alkyl, C_{1-4} hydroxyalkyl, C_{3-6} cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one or two groups selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C_{1-4} alkyl, and C_{1-4} alkoxy.

Embodiment 14 is the compound of embodiment 13, wherein

R_6 is amino or aminomethyl; and

R_7 is selected from the group consisting of hydroxy, C_{1-4} alkyl, and C_{1-4} hydroxyalkyl.

Embodiment 15 is the compound of any one of embodiments 1 to 12, wherein

R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl.

Embodiment 16 is the compound of embodiment 15, wherein

R_6 and R_7 together with the carbon atom to which they are both attached form a 4- to 6-membered saturated ring having 1 to 3 heteroatoms as ring vertices independently selected from N and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl.

Embodiment 17 is the compound of any one of embodiments 1 to 12, wherein

R_6 and R_7 together with the carbon atom to which they are both attached form a 3- to 7-membered cycloalkyl ring that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl.

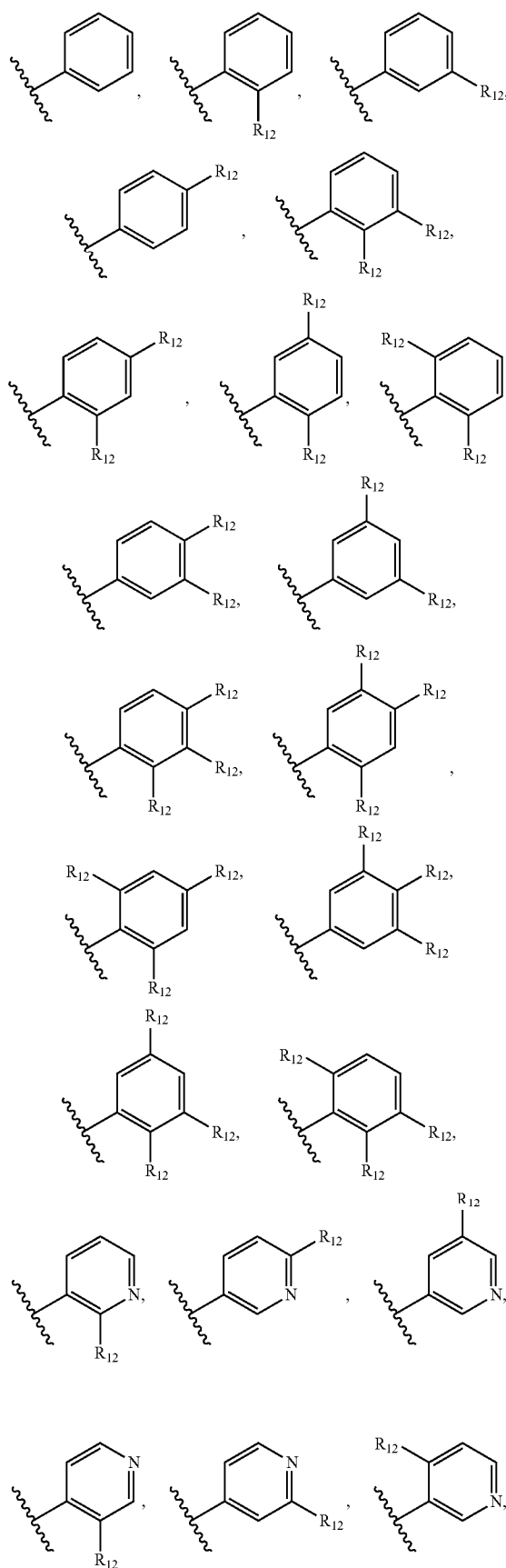
Embodiment 18 is the compound of any one of embodiments 1 to 17, wherein

R_1 is selected from the group consisting of phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, and 1,2,4-triazinyl; and is optionally substituted with 1, 2, or 3 R_{12} independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, and OR_{14} .

Embodiment 19 is the compound of embodiment 18, wherein R_1 is phenyl or pyridyl, each of which is substituted with 1 to 3 R_{12} .

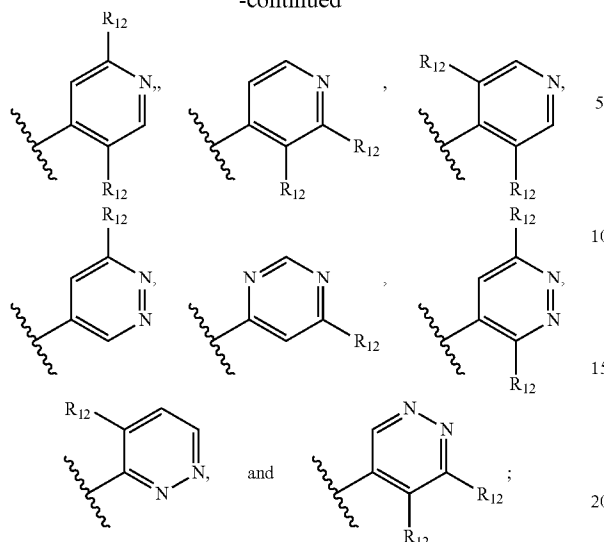
Embodiment 20 is the compound of any one of embodiments 1 to 18, wherein R_1 is selected from the group consisting of:

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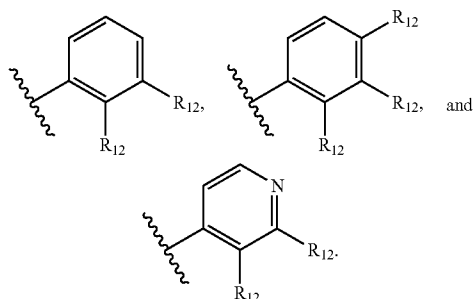
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each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

Embodiment 21 is the compound of embodiment 20, wherein R₁ is selected from the group consisting of:



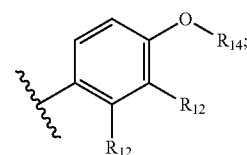
Embodiment 22 is the compound of any one of embodiments 1 to 21, wherein R₁₄ is selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl.

Embodiment 23 is the compound of embodiment 22, wherein R₁₀ is phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl.

Embodiment 24 is the compound of embodiment 1, wherein R₁₄ is selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of C₁₋₄alkylamido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

Embodiment 25 is the compound of embodiment 1, wherein R₁ is

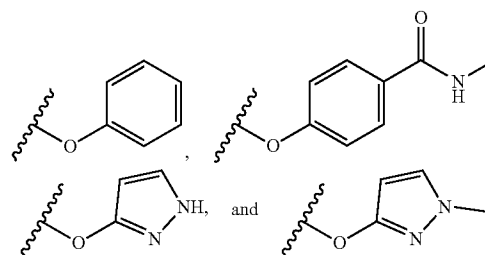
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each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and C₁₋₄aminoalkyl; and

R₁₄ is phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

Embodiment 26 is the compound of embodiment 1, wherein each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, CF₃,



Embodiment 28 is a compound of embodiment 1 or 2, for use as a medicament.

Embodiment 29 is a compound of embodiment 1 or 2, for use in the treatment of a disease driven by one or more PTPN11 mutations.

Embodiment 30 is a compound of embodiment 1 or 2, for use in the treatment of cancer.

Embodiment 31 is the compound of embodiment 30, wherein the cancer is selected from the group consisting of leukemia, melanoma, breast cancer, and colon cancer.

Embodiment 32 is a compound of embodiment 1 or 2, for use in the manufacture of a medicament for the prevention or treatment of a disease or condition ameliorated by the inhibition of PTPN11.

Embodiment 33 is a pharmaceutical composition comprising a compound of embodiment 1 or 2, together with a pharmaceutically acceptable carrier.

Embodiment 34 is a method of inhibition of PTPN11 comprising contacting PTPN11 with a compound of embodiment 1 or 2.

Embodiment 35 is a method of treatment of a PTPN11-mediated disease comprising the administration of a therapeutically effective amount of a compound of embodiment 1 or 2, to a patient in need thereof.

Embodiment 36 is the method of embodiment 35, wherein the disease is cancer.

Embodiment 37 is the method of embodiment 36, wherein the cancer is selected from the group consisting of breast cancer, colon cancer, leukemia, and melanoma.

Embodiment 38 is a method of treatment of a PTPN11-mediated disease comprising the administration of:

R₁ is selected from the group consisting of C₆₋₁₀aryl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R₁ is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O), R₁₄,

C(O)NR₁₅C R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄, R₂, R₃, R₁₀, and R₁₁ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and C₃₋₈cycloalkyl;

R₄, R₅, R₈, and R₉ are each independently selected from the group consisting of hydrogen, cyano, C₁₋₄alkoxy, amino, hydroxy, C₃₋₈cycloalkyl, halo, and C₁₋₄alkylamino;

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

R₇ is selected from the group consisting of hydrogen, halo, and hydroxy, or is selected from the group consisting of amido, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, and C₁₋₄aminoalkyl;

or R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated ring formed by R₆ and R₇ is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

any two groups of R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ and R₁₁ can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices selected from N, O and S;

any two groups of R₂, R₄, R₆, R₈ and R₁₀ can form a direct bond, or a 1 or 2 atom carbon bridge;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, —NH—NHR₁₉, —NHR₁₉, —OR₁₉, —NHC(O)R₁₉, —NHC(O)NHR₁₉, —NHS(O)₂NHR₁₉, —NHS(O)₂R₁₉, —C(O)OR₁₉, —C(O)NR₁₉R₂₀, —C(O)NH(CH₂)_qOH, —C(O)NH(CH₂)_qR₂₁, —C(O)R₂₁, —NH₂, —OH, —S(O)₂NR₁₉R₂₀, C₃₋₈cycloalkyl, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C₁₋₄alkyl, —OH, —NH₂, —OR₂₁, halo, cyano, and oxo;

R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl;

R₁₇ and R₁₈ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and CF₃;

R₁₉ and R₂₀ are each independently selected from the group consisting of hydrogen, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl; and

each R₂₁ is independently selected from the group consisting of hydrogen, —OH, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl.

3. The compound of claim 1, wherein subscripts a and b are each 1.

4. The compound of claim 1, wherein Y₁ is a direct bond.

5. The compound of claim 1, wherein Y₂ is C₁₋₄alkyl.

6. The compound of claim 5, wherein Y₂ is methyl.

7. The compound of claim 1, wherein R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, and C₁₋₆haloalkyl.

8. The compound of claim 7, wherein R₁₃ is hydrogen, Cl, Br, methyl, or CF₃.

9. The compound of claim 1, wherein

R₁ is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

10. The compound of claim 1, wherein R₂, R₃, R₄, R₅, R₈, R₉, R₁₀ and R₁₁ are hydrogen.

11. The compound of claim 1, wherein:

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino; and

R₇ is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6-membered heteroaryl, any of which is optionally substituted with one or two groups selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

12. The compound of claim 11, wherein

R₆ is amino or aminomethyl; and

R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, and C₁₋₄hydroxyalkyl.

13. The compound of claim 1, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

14. The compound of claim 13, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 4- to 6-membered saturated ring having 1 to 3 heteroatoms as ring vertices independently selected from N and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

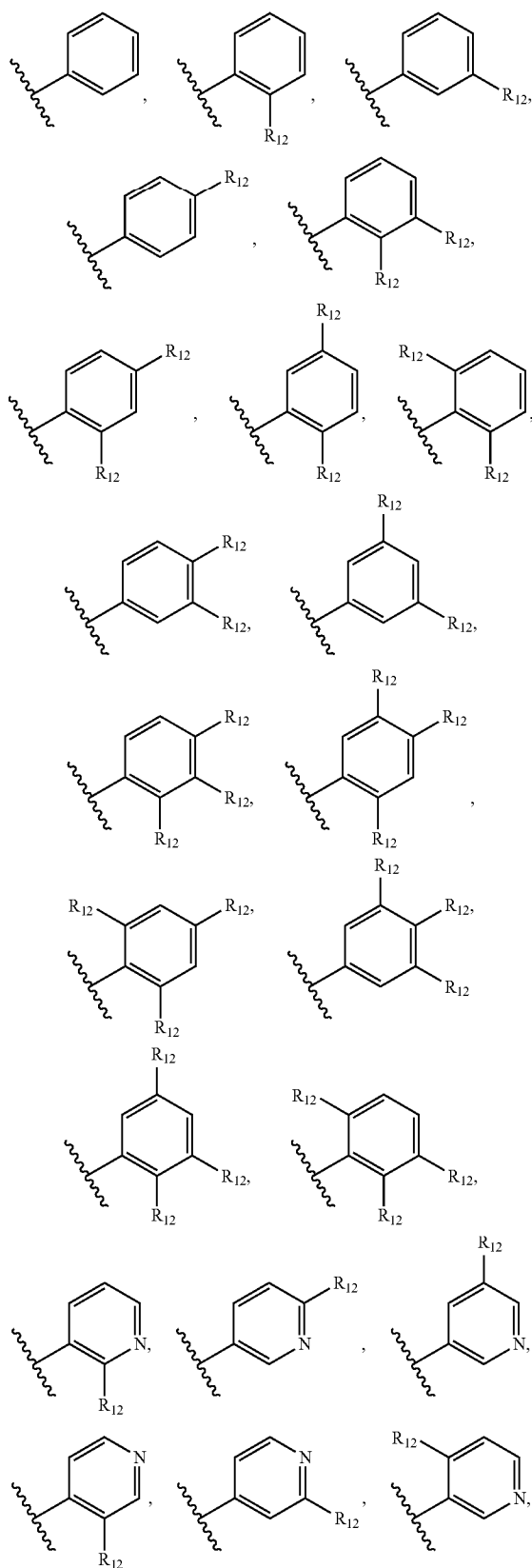
15. The compound of claim 1, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered cycloalkyl ring that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

16. The compound of claim 1, wherein R₁ is phenyl or pyridyl, each of which is substituted with 1 to 3 R₁₂ independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

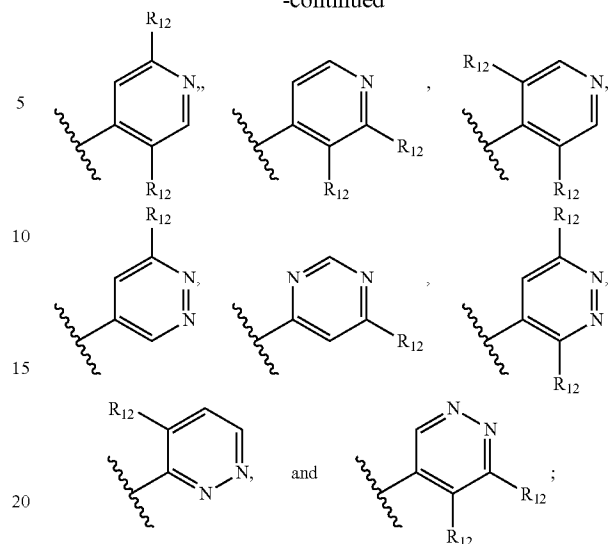
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17. The compound of claim 1, wherein R₁ is selected from the group consisting of:



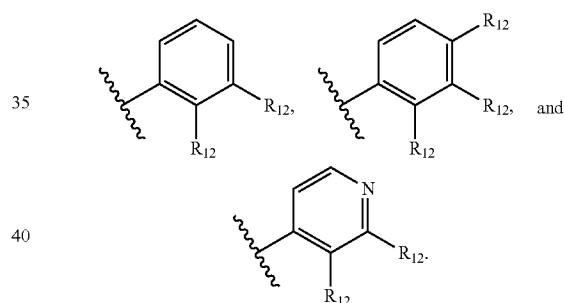
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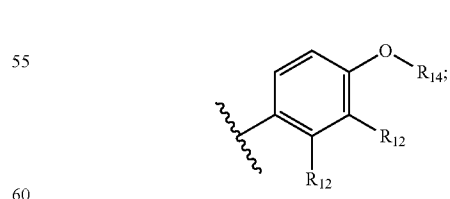
each R_{12} is independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} aminoalkyl, and OR_{14} .

18. The compound of claim 17, wherein R₁ is selected from the group consisting of:



19. The compound of claim 1, wherein R₁₄ is selected from the group consisting of C₆₋₁₀aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of C₁₋₄alkylamido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

20. The compound of claim 1, wherein R₁ is



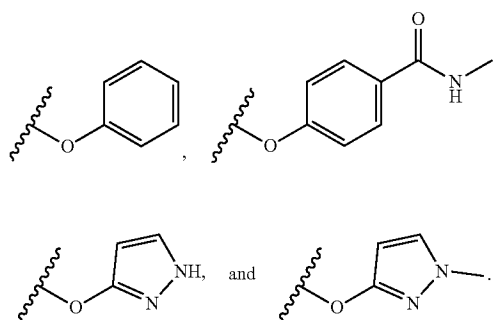
each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, and C₁₋₄aminoalkyl; and

R₁₄ is phenyl or a 5-6 membered heteroaryl having 1 to 4 heteroatoms as ring vertices independently selected

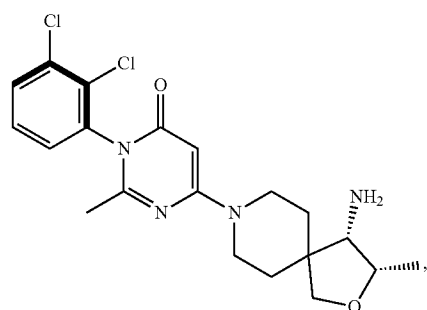
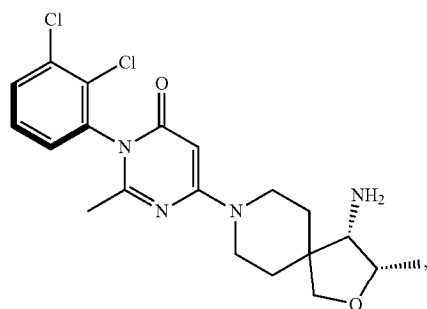
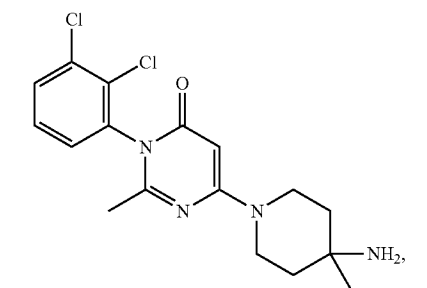
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from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C₁₋₄alkylamido, halo, hydroxy, cyano, and C₁₋₄alkyl.

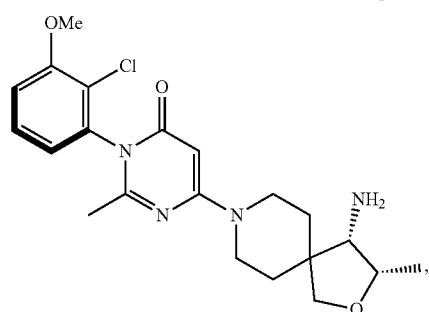
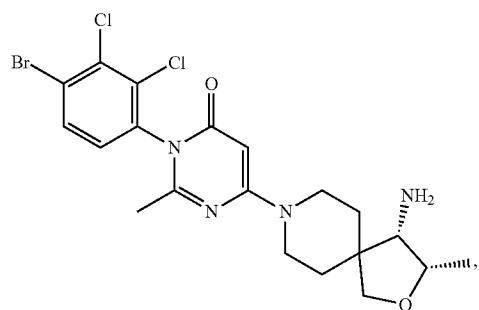
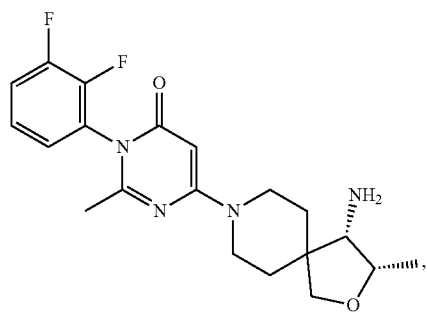
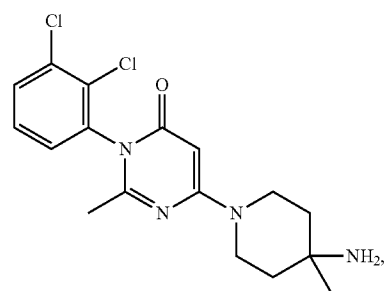
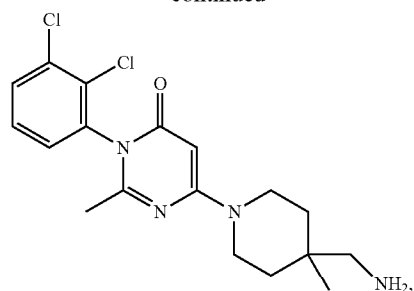
21. The compound of claim 1, wherein each R₁₂ is independently selected from the group consisting of F, Cl, Br, CH₃, OCH₃, CF₃,



22. The compound of claim 1, having a structure selected from the group consisting of:

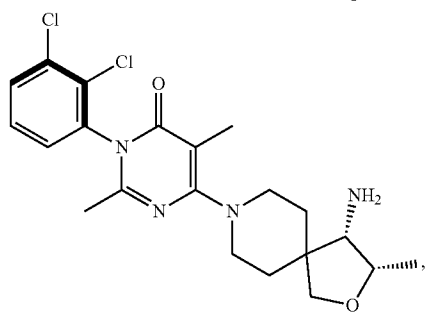
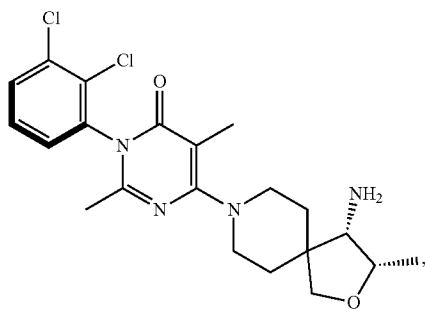
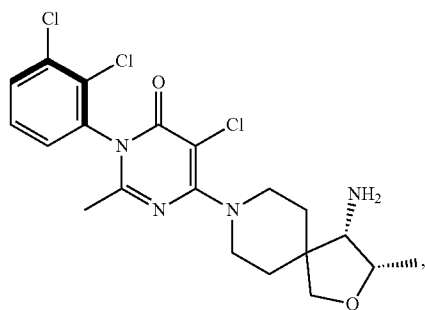
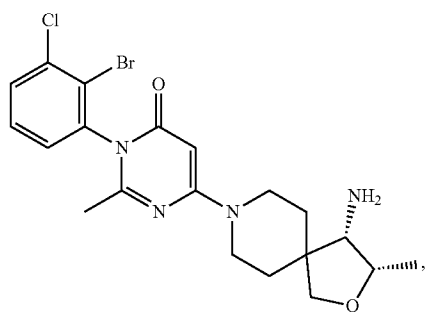
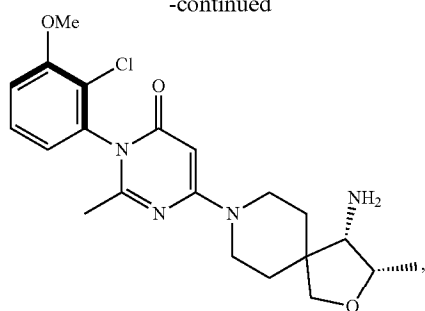
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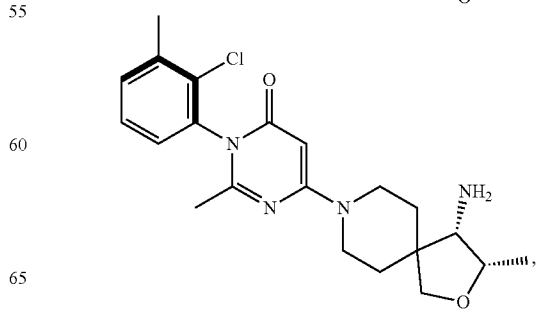
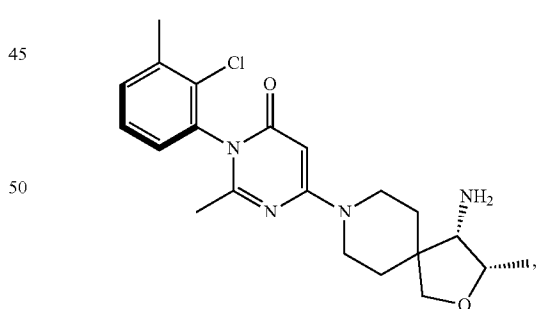
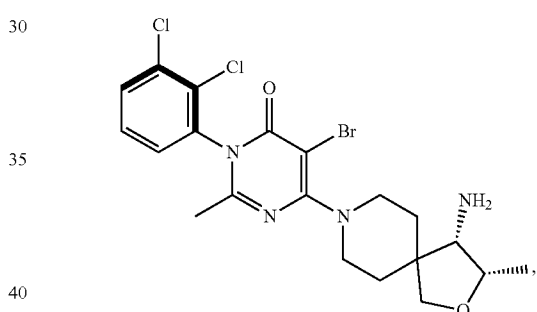
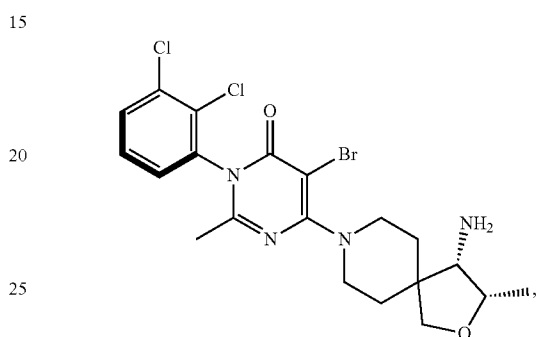
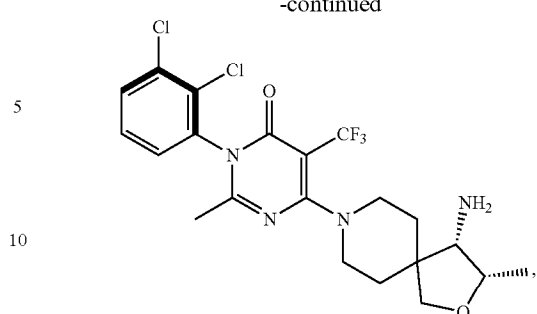


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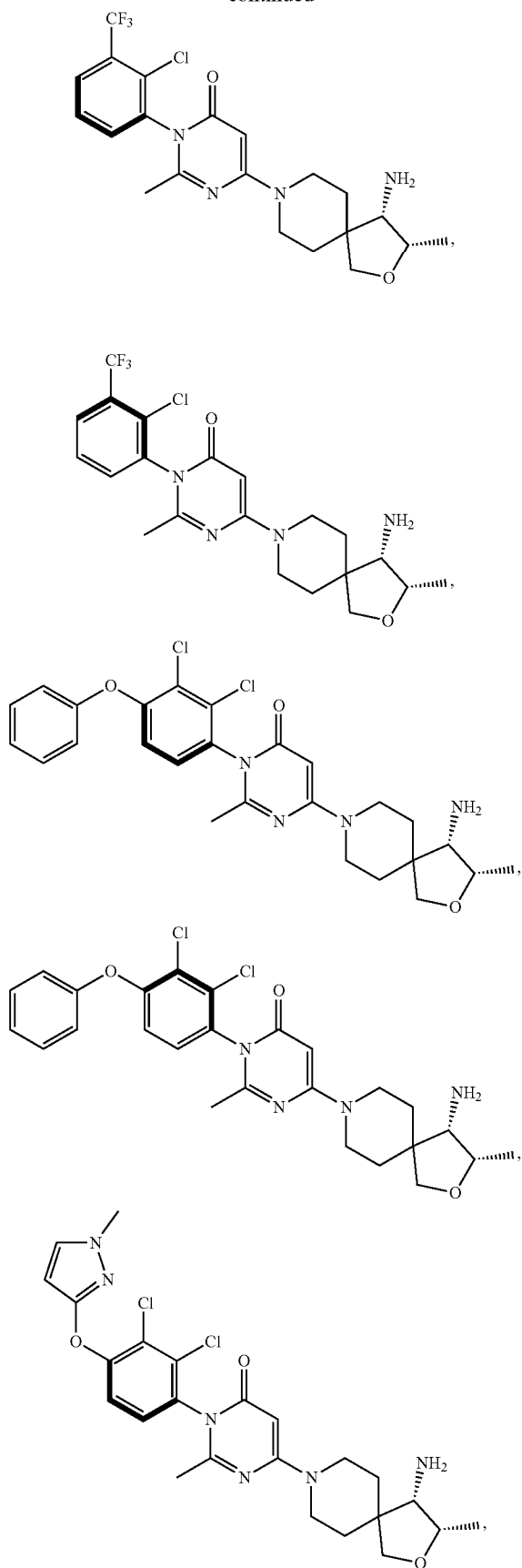
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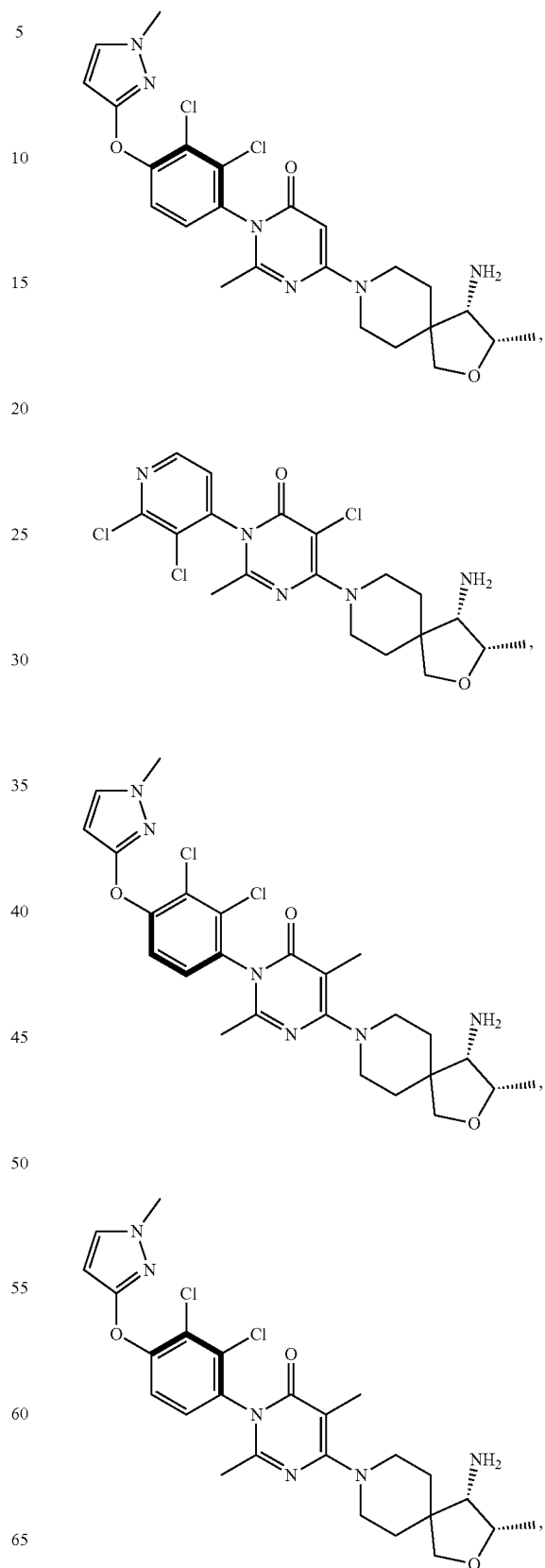


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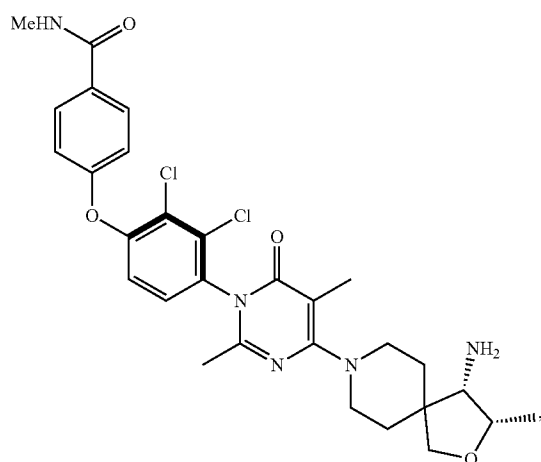
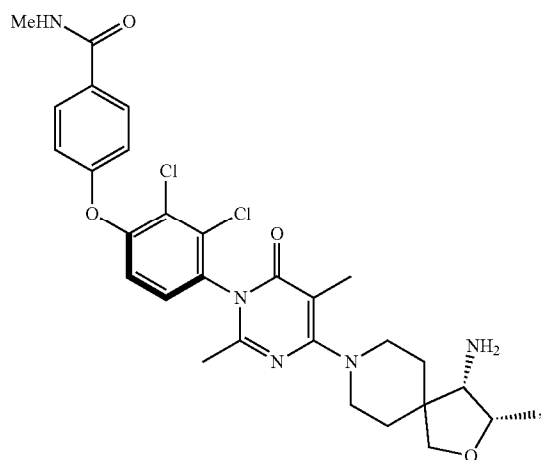
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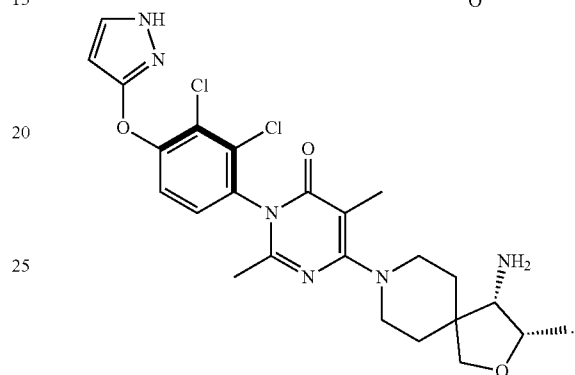
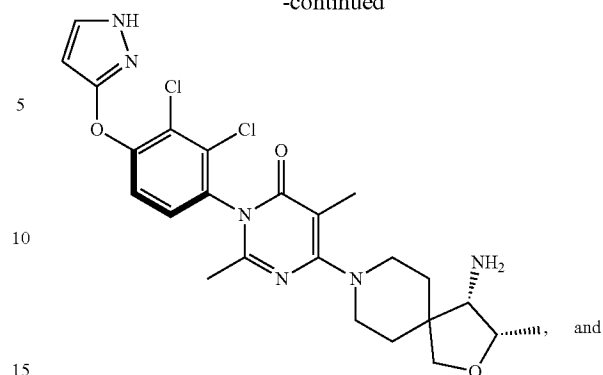


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23. A pharmaceutical composition comprising a compound of claim 1, together with a pharmaceutically acceptable carrier.

24. A method of treatment of a PTPN11-mediated disease comprising the administration of a therapeutically effective amount of a compound of claim 1, to a patient in need thereof, wherein the PTPN11-mediated disease is a Noonan Syndrome, LEOPARD Syndrome, or cancer selected from the group consisting of breast cancer, colon cancer, lung cancer, esophageal cancer, gastric cancer, leukemia, and melanoma.

* * * * *



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NOTICE OF ALLOWANCE AND FEE(S) DUE

176569 7590 04/22/2021
Mintz Levin/BridgeBio
One Financial Center
Boston, MA 02111

EXAMINER

WARD, PAUL V

ART UNIT

PAPER NUMBER

1624

DATE MAILED: 04/22/2021

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/536,923	08/09/2019	Philip JONES	052736-504001US	7575

TITLE OF INVENTION: PTPN11 INHIBITORS

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	\$600	\$0.00	\$0.00	\$600	07/22/2021

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

Notice of Allowability	Application No. 16/536,923	Applicant(s) JONES et al.	
	Examiner PAUL V WARD	Art Unit 1624	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 4/15/21.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some *c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. _____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____. 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____. 4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	5. ☐ Examiner's Amendment/Comment 6. ☒ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
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/PAUL V WARD/ Primary Examiner, Art Unit 1624	
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Continuation of 3. The allowed claim(s) is/are: 1-6,8-9,11-17,19-21,24-27,33 and 35

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance: the methods, compositions and compounds were not found to be obvious or anticipated by the prior art of record. The prior art does not teach or suggest the methods, compositions and compounds substituted in the manner claimed by the Applicant.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to PAUL V WARD whose telephone number is (571)272-2909. The examiner can normally be reached on M-F 9am to 5pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mark Shibuya can be reached on (571)272-0806. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

**/PAUL V WARD/
Primary Examiner, Art Unit 1624**

Notificación de Concesión	Solicitud No. 16/536,923	Solicitante(s) JONES et al.	
	Examinador PAUL V WARD	Unidad de Arte 1624	Estado AIA Sí

-- La FECHA DE ENVIO de esta comunicación aparece en la hoja de cubierta con la dirección de correspondencia--

Todas las reivindicaciones son concedidas, TRÁMITE SOBRE EL FONDO ESTÁ (O PERMANECE) CERRADO en esta solicitud. Si no se incluye acá (o enviada previamente), una Notificación de Concesión (PTOL-85) u otra comunicación adecuada será enviada en su debido momento. **ESTA NOTIFICACION DE CONCESION NO ES UNA CONCESION DE DERECHOS DE PATENTE.** Esta solicitud está sujeta a retiro de emisión a la iniciativa de la Oficina o a petición del solicitante. Ver 37 CFR 1.313 y MPEP 1308.

1. ☒ Esta comunicación responde a 4/15/21.

Una declaración bajo **37 CFR 1.130(b)** se presentó el_____.

2. ☐ Se hizo una elección por el solicitante en respuesta a un requerimiento de restricción definido durante la entrevista el ____; el requerimiento de restricción y la elección se han incorporado en esta acción.

3. ☒ Las reivindicaciones concedidas son Ver Hoja a Continuación. Como resultado de las reivindicaciones aceptadas, usted puede ser elegible del beneficio del programa de **Procedimiento Acelerado de Patentes** en una oficina de propiedad intelectual participante para la correspondiente solicitud. Para más información, por favor ver http://www.uspto.gov/patents/init_events/pph/index.jsp o enviar una pregunta a PPHfeedback@uspto.gov.

4. ☐ Se reconoce una reivindicación de prioridad extranjera bajo 35 USC sección 119(a)-(d) o (f).

Copias certificadas:

a) ☐ Todas b) ☐ Algunas c) ☐ Ninguna de las:

1. ☐ Copias certificadas de los documentos de prioridad se han recibido.

2. ☐ Copias certificadas de los documentos de prioridad se han recibido en la Solicitud No. ____.

3. ☐ Copias de las copias certificadas de los documentos de prioridad se han recibido en esta solicitud de fase nacional de la Oficina Internacional (Regla PCT 17.2 (a)).

* Copias certificadas no recibidas: _____.

El Solicitante tiene TRES MESES A PARTIR DE LA “FECHA DE ENVIO” de esta comunicación para presentar una respuesta que cumpla con los requerimientos indicados anteriormente. Falla en cumplir a tiempo resultará en el ABANDONO de esta solicitud.

ESTE PERIODO DE TRES MESES NO ES PRORROGABLE

5. ☐ Se deben presentar FIGURAS CORREGIDAS (como “hojas de reemplazo”).
- ☐ Incluyendo cambios requeridos por la Enmienda/Comentario del Examinador adjunto o en la Acción Oficial de Papel No./Fecha de Envío ____.

Los indicios de identificación tal como el número de solicitud (ver 37 CFR 1.84(c)) se deben escribir en las figuras en el frente (no atrás) de cada hoja. Las hojas de reemplazo deben estar etiquetadas como tal en el encabezado de acuerdo con 37 CFR 1.121(d).

6. ☐ Se debe presentar DEPOSITO DE y/o INFORMACIÓN acerca del depósito de MATERIAL BIOLOGICO. Note el comentario del Examinador adjunto con relación al REQUERIMIENTO PARA EL DEPOSITO DE MATERIAL BIOLOGICO.

Adjunto(s)

1. ☐ Notificación de Referencias Citadas (PTO-892)

2. ☐ Declaraciones de Divulgación de Información (PTO/SB/08), Papel No./Fecha Envío ____.

3. ☐ Comentario del Examinador con Relación a Requerimiento para Depósito de Material Biológico

4. ☐ Resumen de Entrevista (PTO-413), Papel No./Fecha Envío ____.
5. ☐ Enmienda/Comentario del Examinador

6. ☒ Declaración del Examinador de Razones para Concesión.

7. Otro ____.

/PAUL V WARD/
Examinador Principal, Unidad de Arte 1624

Continuación de 3. La(s) reivindicación(es) concedida(s) es/son: 1-6,8-9,11-17,19-21,24-27, 33 y 35

Aviso de estado Pre-AIA o AIA

La presente solicitud, presentada el 16 de marzo de 2013, se examinó en el primer inventor para presentar las disposiciones de la AIA.

Razones para la concesión

La siguiente es una declaración del examinador de las razones para la concesión: los métodos, composiciones y compuestos no resultaron obvios o anticipados por el estado de la técnica anterior registrada. La técnica anterior no enseña ni sugiere los métodos, composiciones y compuestos sustituidos de la manera reivindicada por el solicitante.

Cualquier comentario que el solicitante considere necesario debe enviarse antes del pago de la tasa de emisión y, para evitar retrasos en el procesamiento, preferiblemente debe acompañar a la tasa de emisión. Dichas presentaciones deben estar claramente etiquetadas como "Comentarios sobre la declaración de razones para la concesión".

Cualquier duda relacionada con esta comunicación o comunicaciones anteriores del examinador se debe dirigir a PAUL V WARD cuyo número de teléfono es (571)272-2909. El examinador puede ser encontrado normalmente de 9:00am - 5.00pm de lunes a viernes.

Las entrevistas con el examinador están disponibles por teléfono, en persona y por videoconferencia usando una herramienta de colaboración basada en la web suministrada por la USPTO. Para programar una entrevista, se recomienda al solicitante utilizar la Solicitud de Entrevista Automatizada (AIR) de la USPTO en <http://www.uspto.gov/interviewpractice>.

Si los intentos por encontrar al examinador por teléfono son fallidos, el supervisor del examinador, Elaine Gort se puede encontrar en (571)272-0806. El número de fax para la organización donde esta solicitud o procedimiento es asignado es 571-273-8300.

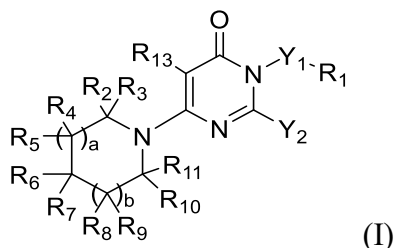
La información relacionada con el estado de una solicitud se puede obtener del sistema de Recuperación de Información de Solicitud de Patente (PAIR). La información de estado para solicitudes publicadas se puede obtener del PAIR Privado o el PAIR Público. La información de estado para solicitudes sin publicar está disponible a través del PAIR Privado únicamente. Para mayor información acerca del sistema PAIR, ver <http://pair-direct.uspto.gov>. Si tiene preguntas sobre el acceso al sistema PAIR Privado, contacte el Centro de Negocio Electrónico (EBC) en 866-217-9197 (totalmente gratis). Si desea asistencia de un Representante de Servicio al Cliente de la USPTO o acceso al sistema de información automatizado, llame a 800-786-9199 (EN USA O CANADA) o 571-272-1000.

/PAUL V WARD/

Examinador Principal, Unidad de Arte 1624

GRANTED CLAIMS

1. A compound represented by Formula I



or a salt thereof, wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y₁ is a direct bond or CR₁₇R₁₈;

Y₂ is selected from the group consisting of C₁₋₄alkyl, amino, C₁₋₄alkylC(O)O-,

C₁₋₄alkylamino and C₁₋₄aminoalkyl;

R₁ is selected from the group consisting of C₆₋₁₀aryl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R₁ is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄;

R₂, R₃, R₁₀, and R₁₁ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and C₃₋₈cycloalkyl;

R₄, R₅, R₈, and R₉ are each independently selected from the group consisting of hydrogen, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, amino, hydroxy, C₃₋₈cycloalkyl, halo, and C₁₋₄alkylamino;

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

R₇ is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6- membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino, and C₁₋₄aminoalkyl;

or R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated or unsaturated ring formed by R₆ and R₇ is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

any two groups of R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ and R₁₁ can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices selected from N, O and S;

any two groups of R₂, R₄, R₆, R₈ and R₁₀ can form a direct bond, or a 1 or 2 atom carbon bridge;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆ dihydroxyalkyl, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(CH₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, C₃₋₈cycloalkyl, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C₁₋₄alkyl, -OH, -NH₂, -OR₂₁, halo, cyano, and oxo;

R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group

consisting of amido, amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;
R₁₇ and R₁₈ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and CF₃;
R₁₉ and R₂₀ are each independently selected from the group consisting of hydrogen, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl; and
each R₂₁ is independently selected from the group consisting of hydrogen, -OH, C₁₋₆ alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl.

2. The compound of claim 1, wherein:

subscript a is 0 or 1;

subscript b is 0 or 1;

Y₁ is a direct bond or CR₁₇R₁₈;

Y₂ is selected from the group consisting of C₁₋₄alkyl, amino, C₁₋₄alkylC(O)O-, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

R₁ is selected from the group consisting of C₆₋₁₀aryl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, and a 5-10 membered heteroaryl group having 1 to 4 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S; said aryl or heteroaryl of R₁ is optionally substituted with 1 to 5 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄hydroxyalkyl, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, C₃₋₈cycloalkyl, C₃₋₈cycloalkenyl, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, and S(O)₂R₁₄;

R₂, R₃, R₁₀, and R₁₁ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and C₃₋₈cycloalkyl;

R₄, R₅, R₈, and R₉ are each independently selected from the group consisting of hydrogen, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, amino, hydroxy, C₃₋₈cycloalkyl, halo, and C₁₋₄alkylamino;

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino;

R₇ is selected from the group consisting of hydrogen, halo, and hydroxy, or is selected from the group consisting of amido, C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6- membered heteroaryl, any of which is optionally substituted with one to five groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino, and C₁₋₄aminoalkyl;

or R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring, having 0 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m;

subscript m is 0, 1, or 2;

said saturated ring formed by R₆ and R₇ is unsubstituted or substituted with 1 to 3 groups independently selected from the group consisting of amino, halo, hydroxy, cyano, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄haloalkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl;

any two groups of R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ and R₁₁ can form a 5 to 6 membered ring, having 0 to 2 heteroatoms as ring vertices selected from N, O and S;

any two groups of R₂, R₄, R₆, R₈ and R₁₀ can form a direct bond, or a 1 or 2 atom carbon bridge;

R₁₃ is selected from the group consisting of hydrogen, halo, cyano, C₁₋₆ alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₁₋₆dihydroxyalkyl, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(CH₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, C₃₋₈cycloalkyl, aryl, heterocyclyl having 1-5 heteroatoms as ring vertices selected from N, O, S and P, heteroaryl having 1-5 heteroatoms as ring vertices selected from N, O, S and P; wherein the subscript q is an integer of from 0 to 6; and wherein aryl, heteroaryl, heterocyclyl and cycloalkyl are substituted with 0 to 3 groups independently selected from the group consisting of C₁₋₄alkyl, -OH, -NH₂, -OR₂₁, halo, cyano, and oxo;

R₁₄, R₁₅ and R₁₆ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, C₃₋₈cycloalkyl, C₆₋₁₀aryl and 5-10 membered heteroaryl, each of which is

optionally substituted by one or more groups independently selected from the group consisting of halo, hydroxy, cyano, and C₁₋₄alkyl;

R₁₇ and R₁₈ are each independently selected from the group consisting of hydrogen, C₁₋₄alkyl, and CF₃;

R₁₉ and R₂₀ are each independently selected from the group consisting of hydrogen, C₁₋₆alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl; and

each R₂₁ is independently selected from the group consisting of hydrogen, -OH, C₁₋₆ alkyl, C₁₋₆haloalkyl, C₁₋₆hydroxyalkyl, C₂₋₆alkenyl, C₂₋₆alkynyl and C₃₋₆cycloalkyl.

3. The compound of claim 1, wherein subscripts a and b are each 1.
4. The compound of claim 1, wherein Y₁ is a direct bond.
5. The compound of claim 1, wherein Y₂ is C₁₋₄alkyl.
6. The compound of claim 5, wherein Y₂ is methyl.
7. The compound of claim 1, wherein R₁₃ is selected from the group consisting of hydrogen, halo, C₁₋₆alkyl, and C₁₋₆haloalkyl.
8. The compound of claim 7, wherein R₁₃ is hydrogen, Cl, Br, methyl, or CF₃.
9. The compound of claim 1, wherein R₁ is phenyl or a 5- to 6-member heteroaryl group having 1 to 4 heteroatoms as ring vertices independently selected from N, O, and S; and is optionally substituted with 1, 2 or 3 R₁₂ groups independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.
10. The compound of claim 1, wherein R₂, R₃, R₄, R₅, R₈, R₉, R₁₀ and R₁₁ are hydrogen.
11. The compound of claim 1, wherein:

R₆ is selected from the group consisting of amino, C₁₋₄aminoalkyl, and C₁₋₄alkylamino; and R₇ is selected from the group consisting of hydrogen, amido, cyano, halo, and hydroxy, or is selected from the group consisting of C₁₋₄alkyl, C₁₋₄hydroxyalkyl, C₃₋₆cycloalkyl, phenyl, and 5- or 6- membered heteroaryl, any of which is optionally substituted with one or two groups selected from the group consisting of amino, halo, hydroxy, cyano, trifluoromethyl, trifluoromethoxy, C₁₋₄alkyl, and C₁₋₄alkoxy.

12. The compound of claim **11**, wherein

R₆ is amino or aminomethyl; and

R₇ is selected from the group consisting of hydroxy, C₁₋₄alkyl, and C₁₋₄hydroxyalkyl.

13. The compound of claim **1**, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered saturated or unsaturated ring having 1 to 3 heteroatoms or groups as ring vertices independently selected from N, C(O), O, and S(O)_m, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

14. The compound of claim **13**, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 4- to 6-membered saturated ring having 1 to 3 heteroatoms as ring vertices independently selected from N and O, and that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

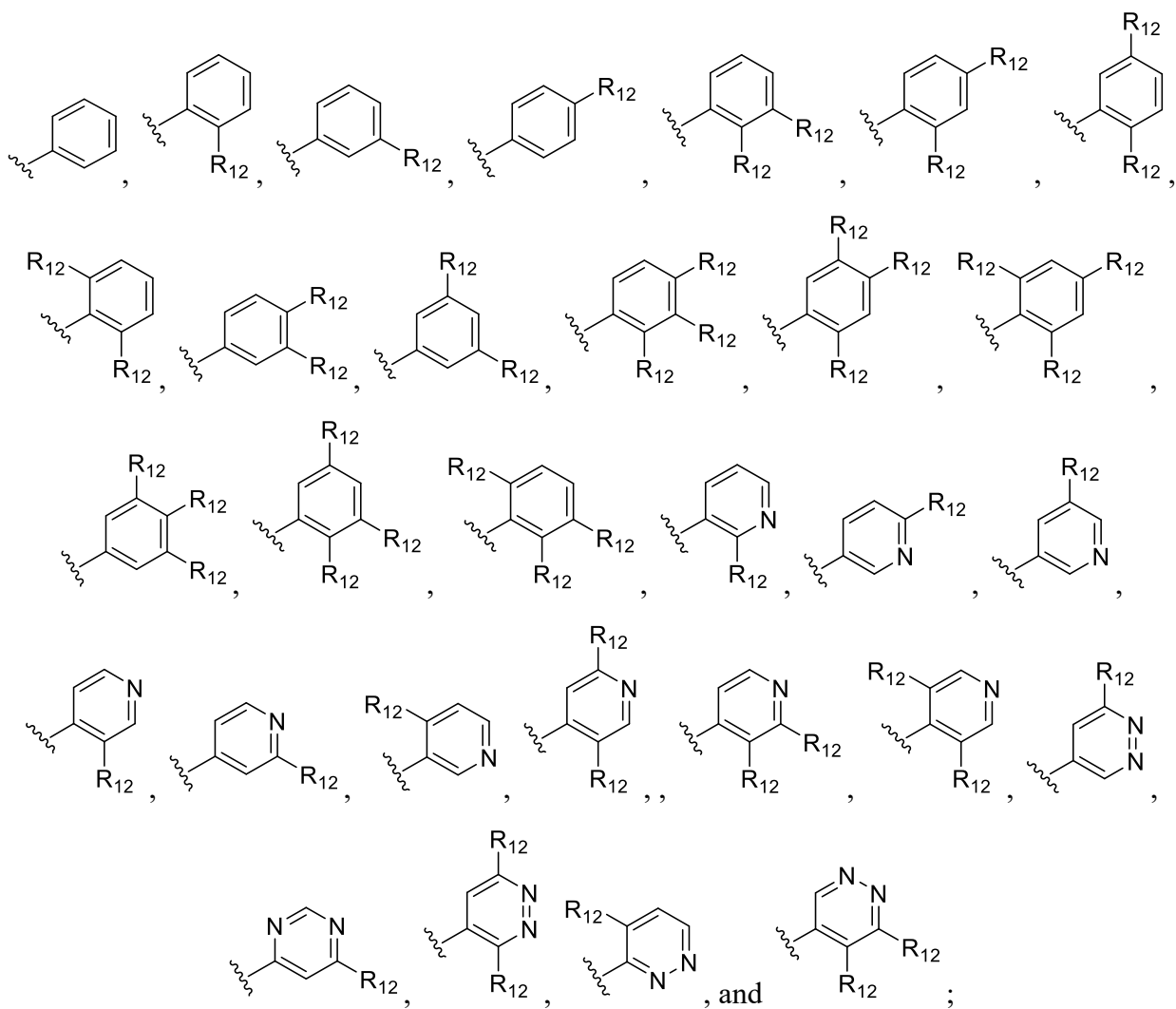
15. The compound of claim **1**, wherein

R₆ and R₇ together with the carbon atom to which they are both attached form a 3- to 7-membered cycloalkyl ring that is optionally substituted with one or two groups independently selected from the group consisting of amino, halo, hydroxy, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄alkylamino and C₁₋₄aminoalkyl.

16. The compound of claim **1**, wherein R₁ is phenyl or pyridyl, each of which is substituted with 1 to 3 R₁₂ independently selected from the group consisting of halo, hydroxy,

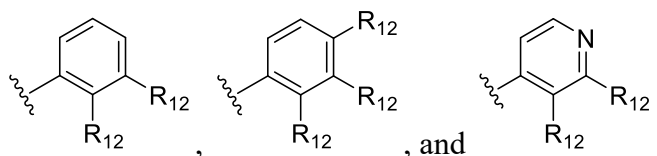
amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

17. The compound of claim **1**, wherein R₁ is selected from the group consisting of:



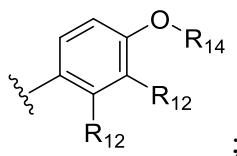
each R₁₂ is independently selected from the group consisting of halo, hydroxy, amino, C₁₋₄alkylamino, di(C₁₋₄alkyl)amino, C₁₋₄alkyl, C₁₋₄alkoxy, C₁₋₄haloalkyl, C₁₋₄aminoalkyl, and OR₁₄.

18. The compound of claim **17**, wherein R_1 is selected from the group consisting of:



19. The compound of claim **1**, wherein R_{14} is selected from the group consisting of C_{6-10} aryl and a 5-10 membered heteroaryl, each of which is optionally substituted by one or more groups independently selected from the group consisting of C_{1-4} alkylamido, amino, halo, hydroxy, cyano, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, C_{1-4} haloalkoxy, C_{1-4} alkylamino and C_{1-4} aminoalkyl.

20. The compound of claim **1**, wherein R_1 is

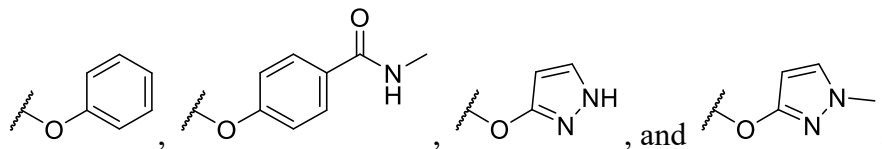


each R_{12} is independently selected from the group consisting of halo, hydroxy, amino, C_{1-4} alkylamino, di(C_{1-4} alkyl)amino, C_{1-4} alkyl, C_{1-4} alkoxy, C_{1-4} haloalkyl, and C_{1-4} aminoalkyl; and

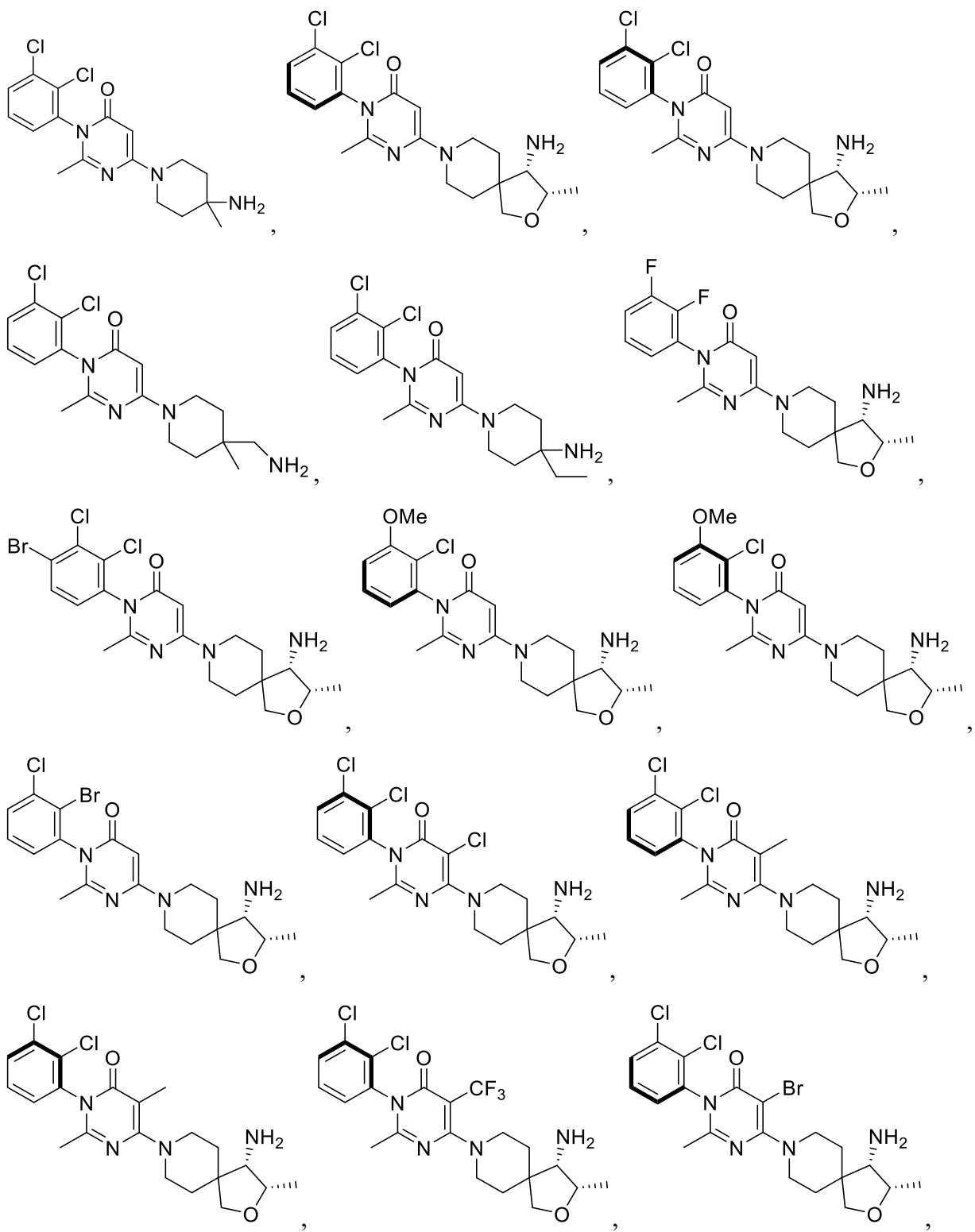
R_{14} is phenyl or a 5-6 membered heteroaryl having

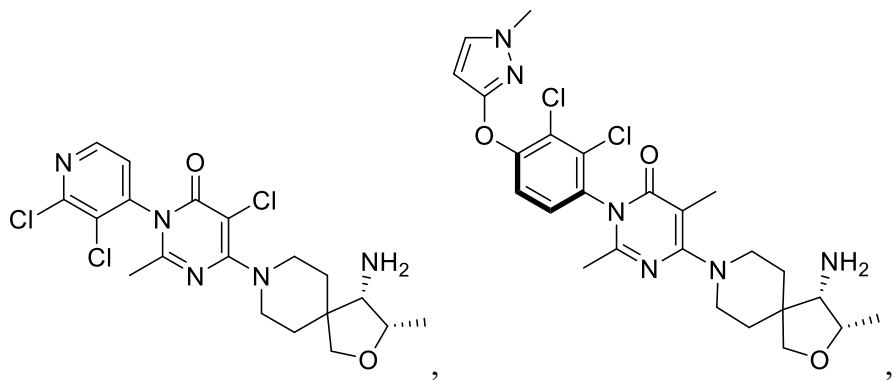
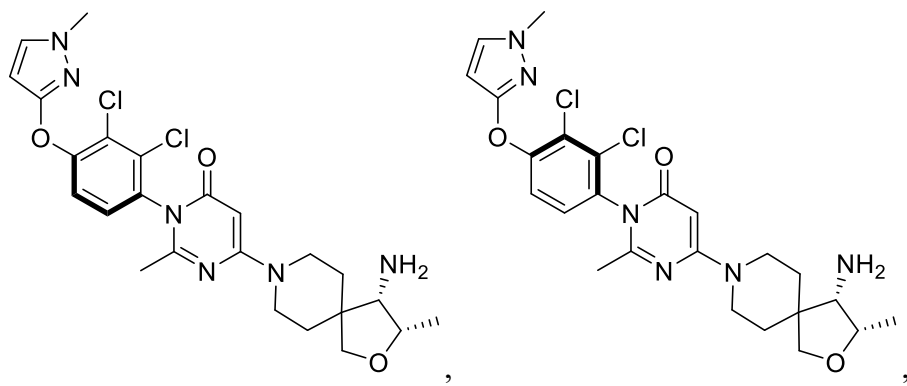
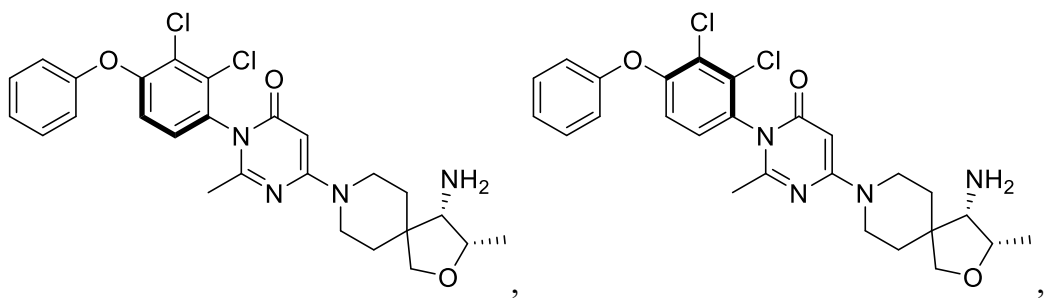
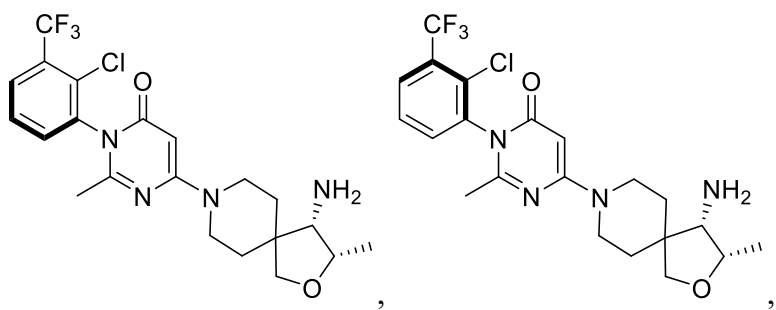
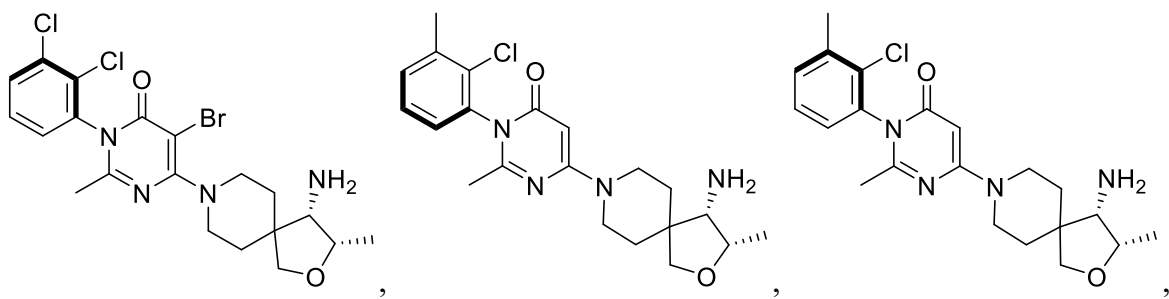
1 to 4 heteroatoms as ring vertices independently selected from N, O, and S, each of which is optionally substituted by one or two groups independently selected from the group consisting of C_{1-4} alkylamido, halo, hydroxy, cyano, and C_{1-4} alkyl.

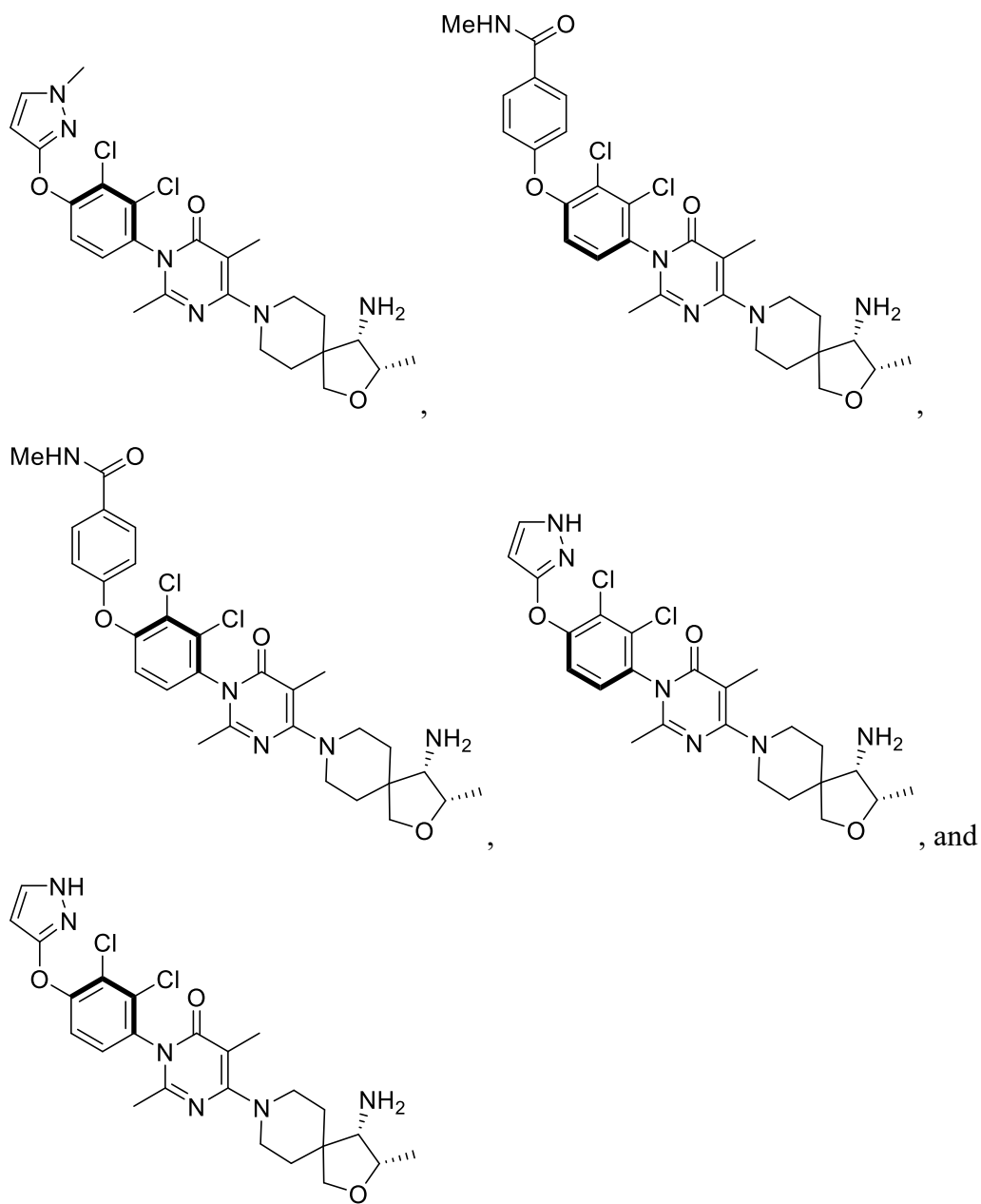
21. The compound of claim **1**, wherein each R_{12} is independently selected from the group consisting of F, Cl, Br, CH_3 , OCH_3 , CF_3 ,



22. The compound of claim **1**, having a structure selected from the group consisting of:





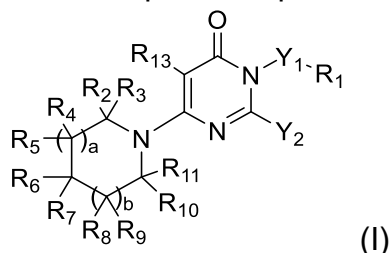


23. A pharmaceutical composition comprising a compound of claim **1**, together with a pharmaceutically acceptable carrier.

24. A method of treatment of a PTPN11-mediated disease comprising the administration of a therapeutically effective amount of a compound of claim **1**, to a patient in need thereof, wherein the PTPN11-mediated disease is a Noonan Syndrome, LEOPARD Syndrome, or cancer selected from the group consisting of breast cancer, colon cancer, lung cancer, esophageal cancer, gastric cancer, leukemia, and melanoma.

REIVINDICACIONES CONCEDIDAS PATENTE US 11,104,675

1. un compuesto representado por la Fórmula I



o una salde este, donde:

el subíndice a es 0 o 1

el subíndice b es 0 o 1;

Y₁ es un enlace directo o CR₁₇R₁₈;

Y₂ se selecciona del grupo que consiste en alquiloC₁₋₄, amino, alquiloC₁₋₄C(O)O-,
alquilaminoC₁₋₄ y aminoalquiloC₁₋₄;

R₁ se selecciona del grupo que consiste en ariloC₆₋₁₀, cycloalquiloC₃₋₈,
cicloalqueniloC₃₋₈, y

un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos
como vértices del anillo que se seleccionan independientemente de N, C(O), O y S; dicho
arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan
independientemente del grupo que consiste en halo, hidroxilo, amino, alquilaminoC₁₋₄,
di(alquiloC₁₋₄)amino, ciano,

alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄,

cicloalquiloC₃₋₈, cicloalqueniloC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆,
NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄,
C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄;

R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en
hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈;

R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en
hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxilo, cicloalquiloC₃₋₈, halo, y

alquilaminoC₁₋₄;

R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄;

R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxilo, o se selecciona del grupo que consiste en alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxilo, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄;

o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m;

el subíndice m es 0, 1 o 2;

dicho anillo saturado o insaturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxilo, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄;

dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se eligen de N, O y S;

dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos;

R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(CH₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P, heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice

q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH,

-NH₂, -OR₂₁, halo, ciano, y oxo;

R₁₄, R₁₅ y R₁₆ se seleccionan cada uno independientemente del grupo que consiste en hidrógeno,

alquilo C₁₋₄, cicloalquilo C₃₋₈, arilo C₆₋₁₀ y heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del grupo que consiste en amido, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄,

haloalquilo C₁₋₄, haloalcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄;

cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno,

alquilo C₁₋₄ y CF₃;

cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno,

alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alqueno C₂₋₆, alquino C₂₋₆ y cicloalquilo C₃₋₆; y

cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alqueno C₂₋₆, alquino C₂₋₆ y cicloalquilo C₃₋₆.

2. El compuesto de la reivindicación **1**, donde:

el subíndice a es 0 o 1

el subíndice b es 0 o 1;

Y₁ es un enlace directo o CR₁₇R₁₈;

Y₂ se selecciona del grupo que consiste en alquilo C₁₋₄, amino, alquilo C₁₋₄C(O)O-, alquilamino C₁₋₄ y aminoalquilo C₁₋₄;

R₁ se selecciona del grupo que consiste en arilo C₆₋₁₀, cicloalquilo C₃₋₈, cicloalqueno C₃₋₈, y

un grupo heteroarilo de 5 a 10 miembros que tiene de 1 a 4 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S; dicho arilo o heteroarilo de R₁ se sustituye opcionalmente con 1 a 5 grupos R₁₂ que se seleccionan independientemente del grupo que consiste en halo, hidroxilo, amino, alquilaminoC₁₋₄, di(alquiloC₁₋₄)amino, ciano,

alquiloC₁₋₄, alcoxiC₁₋₄, hidroxialquiloC₁₋₄, haloalquiloC₁₋₄, aminoalquiloC₁₋₄,

cicloalquiloC₃₋₈, cicloalquenoC₃₋₈, NR₁₅C(O)R₁₄, NR₁₅C(O)OR₁₄, NR₁₄C(O)NR₁₅R₁₆, NR₁₅S(O)R₁₄, NR₁₅S(O)₂R₁₄, C(O)NR₁₅R₁₆, S(O)NR₁₅R₁₆, S(O)₂NR₁₅R₁₆, C(O)R₁₄, C(O)OR₁₄, OR₁₄, SR₁₄, S(O)R₁₄, y S(O)₂R₁₄;

R₂, R₃, R₁₀, y R₁₁ se seleccionan independientemente del grupo que consiste en hidrógeno, alquiloC₁₋₄ y cicloalquiloC₃₋₈;

R₄, R₅, R₈, y R₉ se seleccionan independientemente del grupo que consiste en hidrógeno, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, amino, hidroxilo, cicloalquiloC₃₋₈, halo, y

alquilaminoC₁₋₄;

R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilaminoC₁₋₄;

R₇ se selecciona del grupo que consiste en hidrógeno, halo e hidroxilo, o se selecciona del grupo que consiste en amido, alquiloC₁₋₄, hidroxialquiloC₁₋₄, cicloalquiloC₃₋₆, fenilo y heteroarilo de 5 o 6 miembros, cualquiera de los cuales se sustituye opcionalmente con uno a cinco grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxilo, ciano, alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄;

o R₆ y R₇, junto con el átomo de carbono al que están unidos, forman un anillo saturado o insaturado de 3 a 7 miembros, que tiene de 0 a 3 heteroátomos o grupos como vértices del anillo que se seleccionan independientemente de N, C(O), O y S(O)_m;

el subíndice m es 0, 1 o 2;

dicho anillo saturado formado por R₆ y R₇ no se sustituye o se sustituye con 1 a 3 grupos que se seleccionan independientemente del grupo que consiste en amino, halo, hidroxilo, ciano,

alquiloC₁₋₄, alcoxiC₁₋₄, haloalquiloC₁₋₄, haloalcoxiC₁₋₄, alquilaminoC₁₋₄ y aminoalquiloC₁₋₄;

dos grupos cualquiera de R₂, R₃, R₄, R₅, R₇, R₈, R₉, R₁₀ y R₁₁ pueden formar un anillo de 5 a 6 miembros, que tiene de 0 a 2 heteroátomos como vértices del anillo que se seleccionan de N, O y S;

dos grupos cualquiera de R₂, R₄, R₆, R₈ y R₁₀ pueden formar un enlace directo, o un puente de carbono de 1 o 2 átomos;

R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, ciano, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, dihidroxialquilo C₁₋₆, -NH-NHR₁₉, -NHR₁₉, -OR₁₉, -NHC(O)R₁₉, -NHC(O)NHR₁₉, -NHS(O)₂NHR₁₉, -NHS(O)₂R₁₉, -C(O)OR₁₉, -C(O)NR₁₉R₂₀, -C(O)NH(CH₂)_qOH, -C(O)NH(CH₂)_qR₂₁, -C(O)R₂₁, -NH₂, -OH, -S(O)₂NR₁₉R₂₀, cicloalquilo C₃₋₈, arilo, heterociclilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P, heteroarilo que tiene de 1 a 5 heteroátomos como vértices del anillo seleccionados entre N, O, S y P; donde el subíndice q es un número entero de 0 a 6; y donde arilo, heteroarilo, heterociclilo y cicloalquilo están sustituidos con 0 a 3 grupos seleccionados independientemente del grupo que consiste en alquilo C₁₋₄, -OH,

-NH₂, -OR₂₁, halo, ciano, y oxo;

cada R₁₄, R₁₅ y R₁₆ se selecciona independientemente del grupo que consiste en hidrógeno,

alquiloC₁₋₄, cicloalquiloC₃₋₈, ariloC₆₋₁₀ y heteroarilo de 5 a 10 miembros, cualquiera de los cuales se sustituye opcionalmente por uno o más grupos que se seleccionan independientemente del grupo que consiste en halo, hidroxí, ciano, alquiloC₁₋₄;

cada R₁₇ y R₁₈ se selecciona independientemente del grupo que consiste en hidrógeno,

alquiloC₁₋₄ y CF₃;

cada R₁₉ y R₂₀ se selecciona independientemente del grupo que consiste en hidrógeno,

alquiloC₁₋₆, haloalquiloC₁₋₆, hidroxialquiloC₁₋₆, alquenoC₂₋₆, alquinoC₂₋₆ y

cicloalquilo C₃₋₆; y

cada R₂₁ se selecciona independientemente del grupo que consiste en hidrógeno, -OH, alquilo C₁₋₆, haloalquilo C₁₋₆, hidroxialquilo C₁₋₆, alquenilo C₂₋₆, alquinilo C₂₋₆ y cicloalquilo C₃₋₆.

3. El compuesto de la reivindicación **1**, donde los subíndices a y b son cada uno 1.

4. El compuesto de la reivindicación **1**, donde Y₁ es un enlace directo.

5. El compuesto de la reivindicación **1**, donde Y₂ es alquilo C₁₋₄.

6. El compuesto de la reivindicación **5**, donde Y₂ es metilo.

7. El compuesto de la reivindicación **1**, donde

R₁₃ se selecciona del grupo que consiste en hidrógeno, halo, alquilo C₁₋₆, y haloalquilo C₁₋₆.

8. El compuesto de la reivindicación **7**, donde R₁₃ es hidrógeno, Cl, Br, metilo, o CF₃.

9. El compuesto de la reivindicación **1**, donde

R₁ es fenilo o un grupo heteroarilo de 5 a 6 miembros que tiene de 1 a 4 heteroátomos como vértices del anillo seleccionados independientemente entre N, O y S; y está opcionalmente sustituido con 1, 2 o 3 grupos R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxilo, amino, alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄, y OR₁₄.

10. El compuesto de la reivindicación **1**, donde R₂, R₃, R₄, R₅, R₈, R₉, R₁₀ y R₁₁ son hidrógeno.

11. El compuesto de la reivindicación **1**, donde:

R₆ se selecciona del grupo que consiste en amino, aminoalquilo C₁₋₄, y alquilamino C₁₋₄; y

R₇ se selecciona del grupo que consiste en hidrógeno, amido, ciano, halo e hidroxilo, o se selecciona del grupo que consiste en alquilo C₁₋₄, hidroxialquilo C₁₋₄, cicloalquilo C₃₋₆, fenilo, y heteroarilo de 5 o 6 miembros, cualquiera de los cuales está opcionalmente

sustituido con uno o dos grupos seleccionados del grupo que consiste en amino, halo, hidroxilo, ciano, trifluorometilo, trifluorometoxi, alquilo C₁₋₄, y alcoxi C₁₋₄alkoxy.

12. El compuesto de la reivindicación **11**, donde

R₆ es amino o aminometilo; y

R₇ se selecciona del grupo que consiste en hidroxilo, alquilo C₁₋₄, e hidroxialquilo C₁₋₄.

13. El compuesto de la reivindicación **1**, donde

R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado o no saturado de 3 a 7 miembros que tiene de 1 a 3 heteroátomos o grupos como vértices del anillo seleccionados independientemente entre N, C(O), O y S(O)_m, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxilo, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.

14. El compuesto de la reivindicación **13**, donde

R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo saturado de 4 a 6 miembros que tiene de 1 a 3 heteroátomos como vértices del anillo seleccionados independientemente de N y O, y que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxilo, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.

9. El compuesto de la reivindicación **1**, donde

R₆ y R₇ junto con el átomo de carbono al que están unidos forman un anillo de cicloalquilo de 3 a 7 miembros que está opcionalmente sustituido con uno o dos grupos seleccionados independientemente del grupo que consiste en amino, halo, hidroxilo, alquilo C₁₋₄, alcoxi C₁₋₄, alquilamino C₁₋₄ y aminoalquilo C₁₋₄.

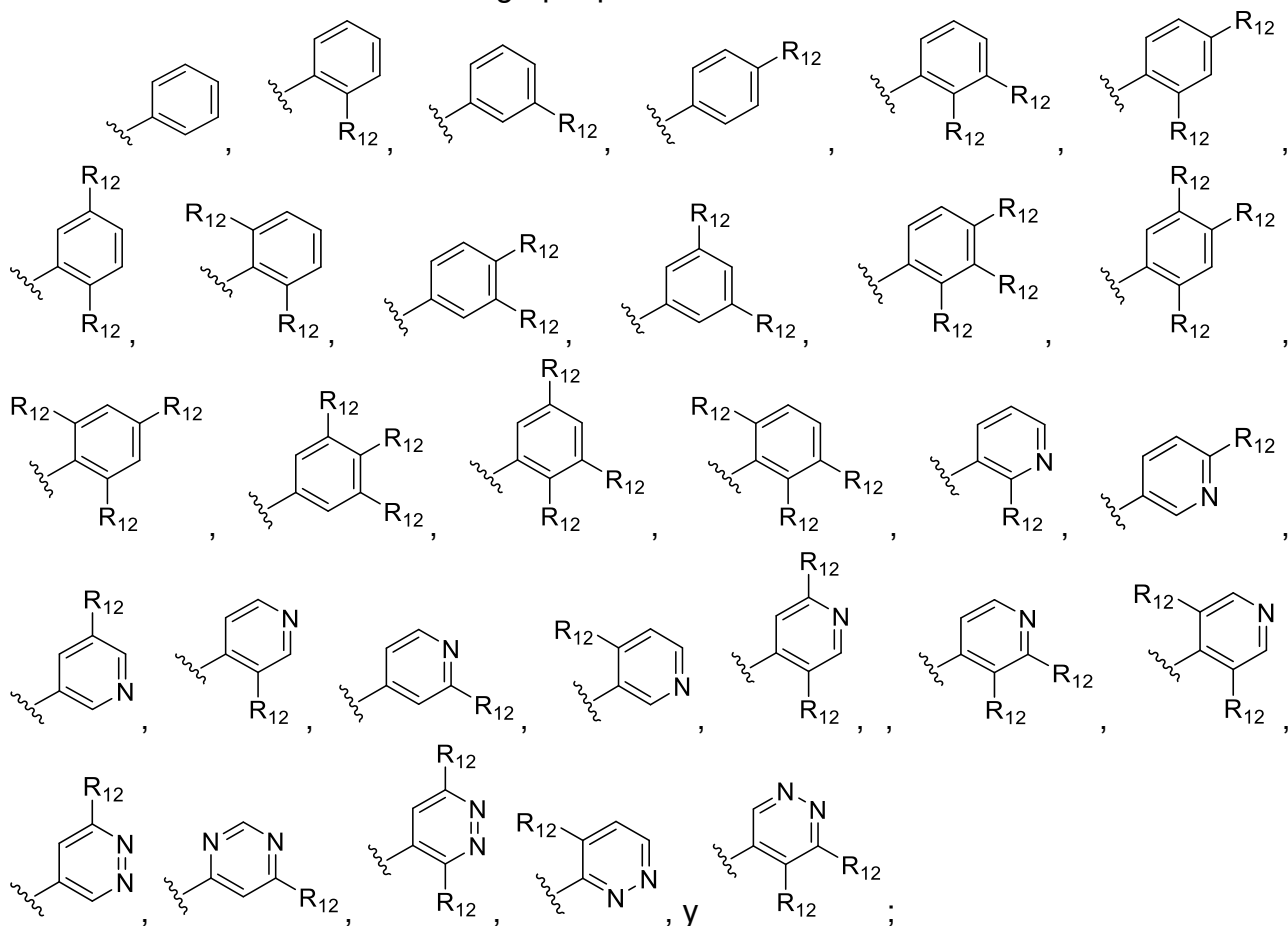
10. El compuesto de la reivindicación **1**, donde

R₁ se fenilo o piridilo cada uno de los cuales se sustituye con 1 a 3 R₁₂ seleccionados independientemente del grupo que consiste en halo, hidroxilo, amino,

alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, aminoalquilo C₁₋₄ y OR₁₄.

11. El compuesto de la reivindicación **1**,

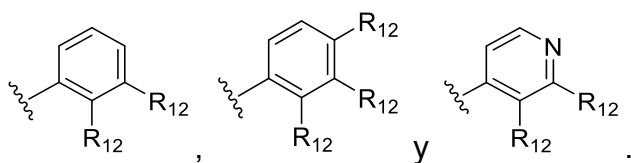
donde R_1 se selecciona del grupo que consiste en:



cada R_{12} se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino,

alquilamino C_{1-4} , di(alquilo C_{1-4})amino, alquilo C_{1-4} , alcoxi C_{1-4} , haloalquilo C_{1-4} , aminoalquilo C_{1-4} , y OR_{14} .

18. El compuesto de la reivindicación **17**, donde R_1 se selecciona del grupo que consiste en:

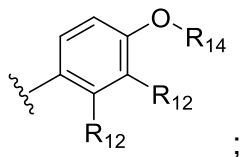


19. El compuesto de la reivindicación **1**, donde R_{14} se selecciona del grupo que consiste en arilo C_{6-10} y un heteroarilo de 5 a 10 miembros, cada uno de los cuales está opcionalmente sustituido por uno o más grupos seleccionados independientemente del

grupo que consiste en alquilamido C₁₋₄, amino, halo, hidroxilo, ciano, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, haloalcoxi C₁₋₄,

alquilamino C₁₋₄ y aminoalquilo C₁₋₄.

20. El compuesto de la reivindicación 1, donde R₁ es

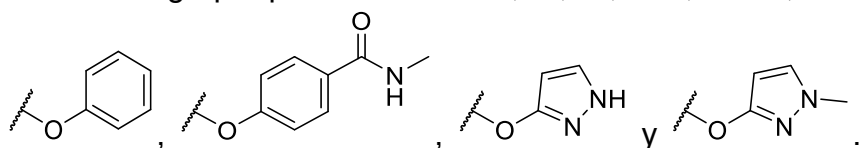


cada R₁₂ se selecciona independientemente del grupo que consiste en halo, hidroxilo, amino,

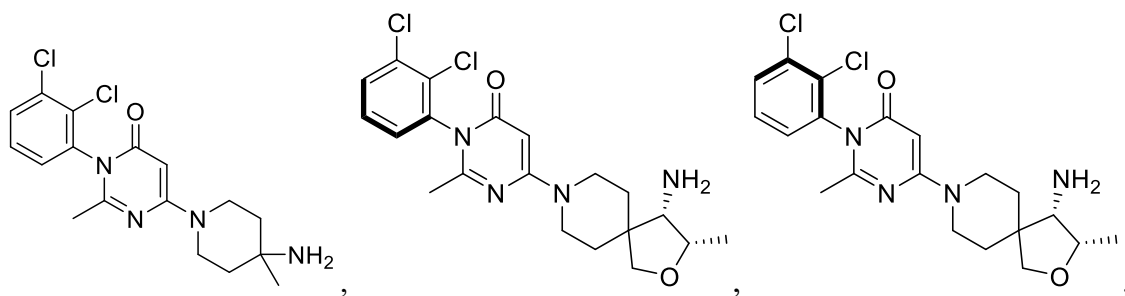
alquilamino C₁₋₄, di(alquilo C₁₋₄)amino, alquilo C₁₋₄, alcoxi C₁₋₄, haloalquilo C₁₋₄, y aminoalquilo C₁₋₄, y.

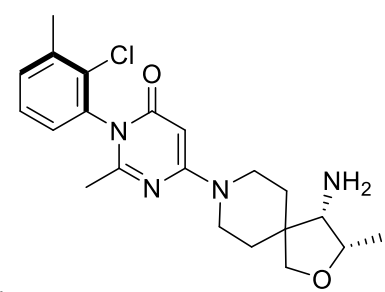
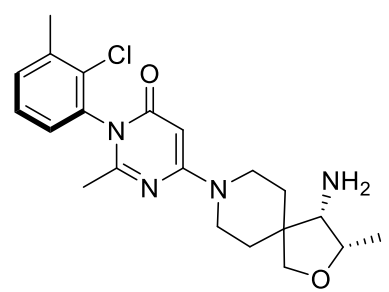
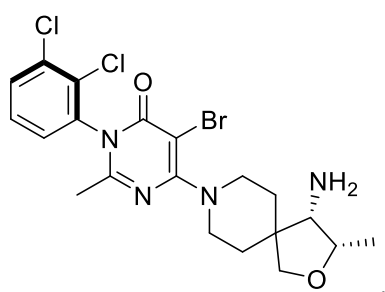
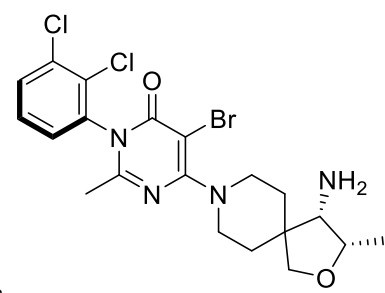
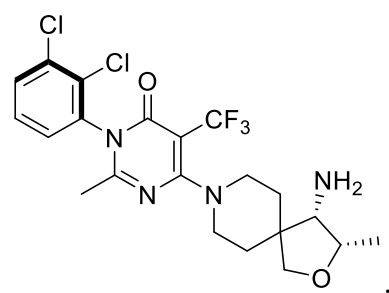
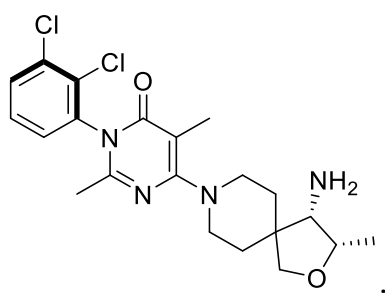
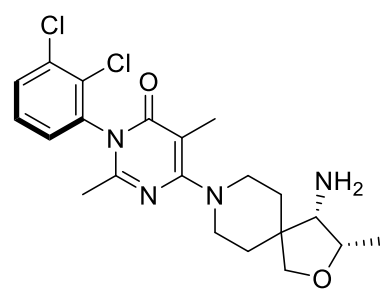
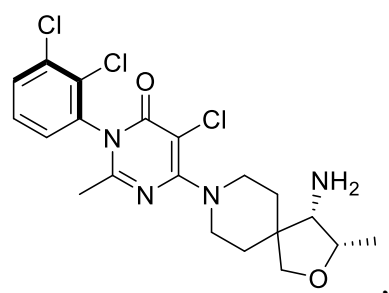
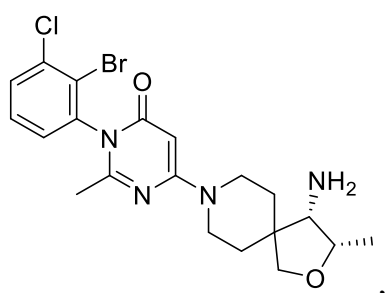
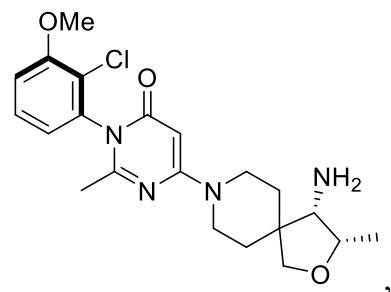
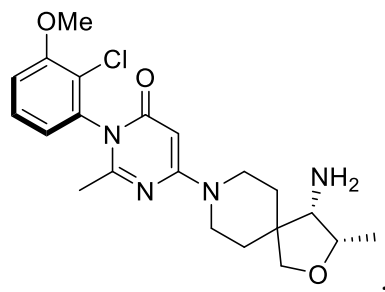
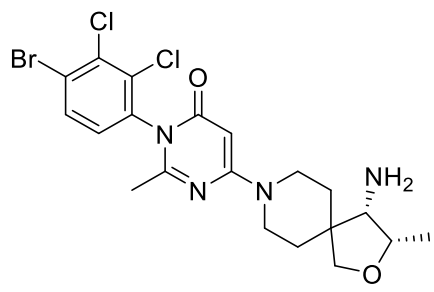
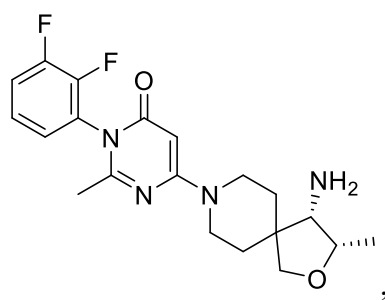
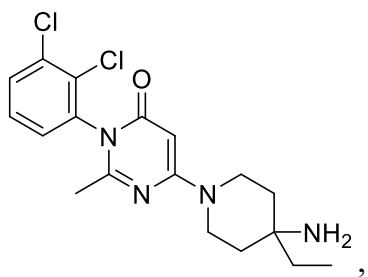
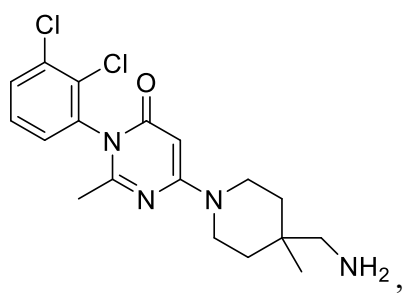
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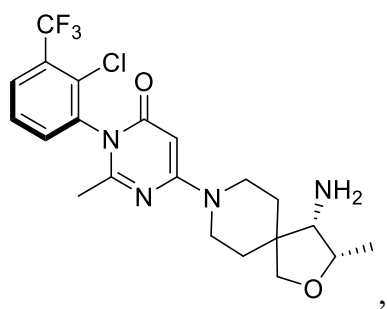
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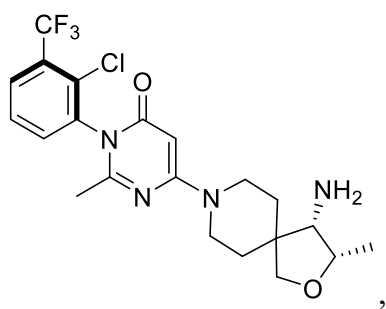
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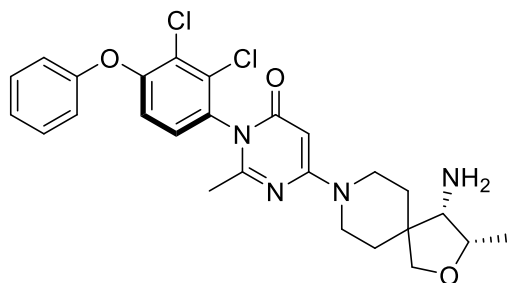




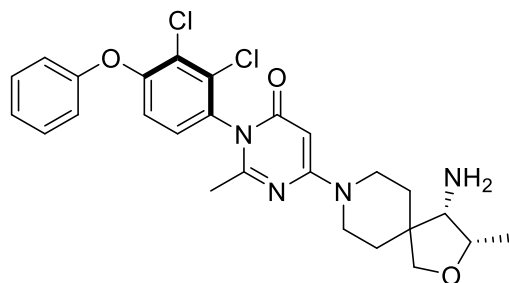
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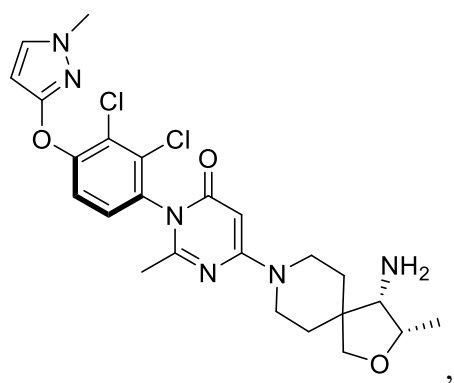
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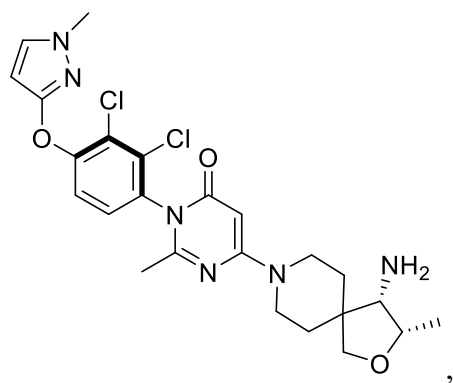
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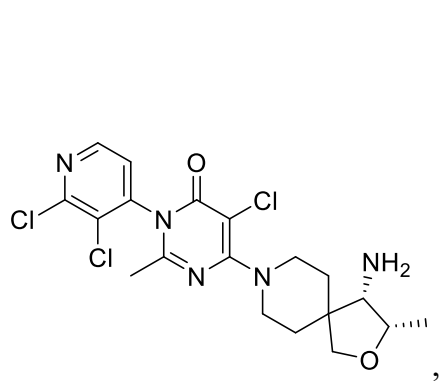
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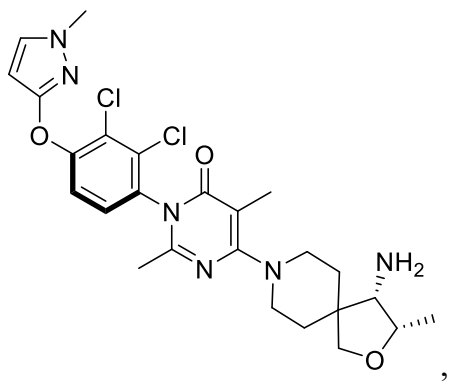
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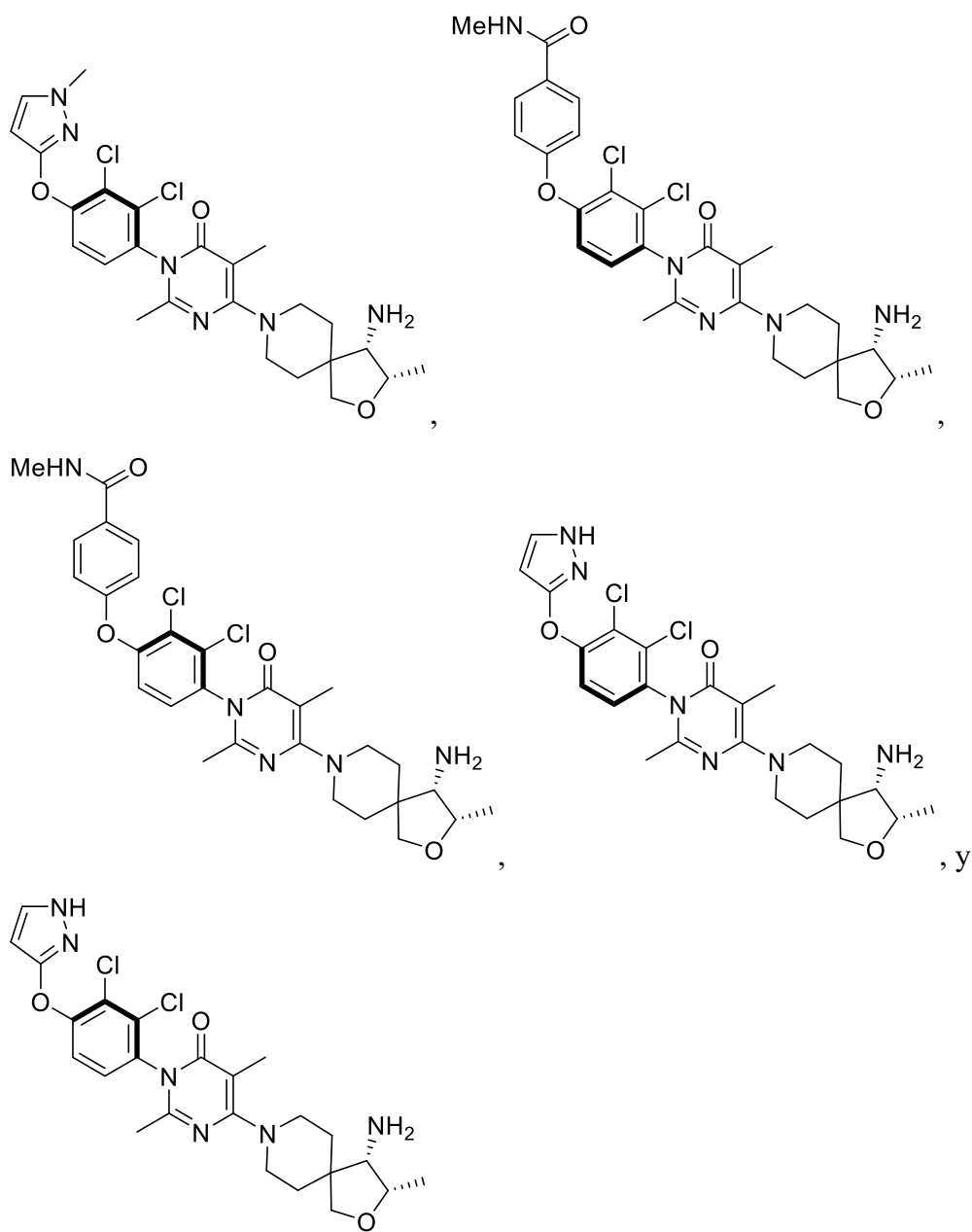
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23. Una composición farmacéutica que comprende un compuesto de la reivindicación **1**, junto con un portador farmacéuticamente aceptable.

24. Un método de tratamiento de una enfermedad mediada por PTPN11 que comprende la administración de una cantidad terapéuticamente eficaz de un compuesto de la reivindicación **1**, a un paciente que lo necesita, en el que la enfermedad mediada por PTPN11 es un síndrome de Noonan, un síndrome LEOPARD o un cáncer seleccionado del

grupo que consiste en cáncer de mama, cáncer de colon, cáncer de pulmón, cáncer de esófago, cáncer gástrico, leucemia y melanoma.

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Fifth Edition

Volume I: Principles and Practice

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9 Some Considerations for Prodrug Design

use of liver microsome preparations as shown for new dopaminergic compounds (245), and to include human microsomes in order to gain some insight into the situation potentially encountered in humans.

8.6 Validity of Classical Pharmacokinetic Concepts for Prodrug Design

As seen in the previous paragraphs, classical pharmacokinetic concepts can be used for the study of prodrugs despite the fact that they have essentially been derived from theoretical considerations related to the situation where the parent compound is the active moiety. As for metabolite kinetics, difficulties arise when the prodrug is not totally biotransformed to the "drug." There is one situation, however, in which it is questionable whether these concepts are fully appropriate, namely drug targeting. For example, if a prodrug is very specifically targeted to one type of cells or to one organ system, it might be necessary to define a "targeting index" to describe distribution of the prodrug and the active moiety, since a concept such as the apparent volume of distribution might only poorly describe the distribution properties of the therapeutic agent. The same restriction may apply to the concepts of systemic availability, systemic clearance and apparent half-life of elimination. This does not mean that classical pharmacokinetic concepts would become invalid, because they are robust and well validated, but that some creativity is probably necessary in order to improve the descriptive power of pharmacokinetics in order to allow comparisons of "drug" and prodrug or between prodrugs. Similar considerations also apply to the concepts basic to biopharmaceutic evaluations. If the necessity to define new pharmacokinetic parameters for the description of targeted prodrugs is accepted, it will be of utmost importance to define only concepts that can be quantified based

on experimental data. It is possible that development of imaging techniques such as PET- or NMR-scan will give an impetus to these foreseeable developments in pharmacokinetics.

A problem requiring special attention is the stereoselective *in vivo* activation of prodrugs derived from racemic mixtures, as exemplified by the stereoselective hydrolysis of *O*-acetyl-propanolol (246), for which it was also found that the selectivity of plasma enzyme urase differs from that of liver and intestine enzymes.

9 SOME CONSIDERATIONS FOR PRODRUG DESIGN

9.1: Rationale of Prodrug Design

The design of prodrugs in a rational manner requires, as stated by Bundgaard (9), that the underlying causes which necessitate or stimulate the use of the prodrug approach be defined and clearly understood. It may then be possible to identify the means by which the difficulties can be overcome. The rational design of the prodrug can thus be divided into three basic steps:

- Identification of the drug delivery problem.
- Identification of the physicochemical properties required for optimal delivery.
- Selection of a prodrug derivative that has the proper physicochemical properties and that will be cleaved in the desired biological compartment.

In this context it must be accepted that a very close collaboration is needed between the pharmaceutical chemists active in drug synthesis and those working in the area of xenobiotic metabolism. This is particularly important if more targeted prodrugs are designed in function of enzymes available at the right place, in the right amount and with the right prodrug specificity.

9.2 Practical Considerations

In the rational design and synthesis of prodrugs, several factors should be considered before starting the development of a new compound intended for large-scale production (247):

- The chemical intermediates or modifiers should be available in a high state of purity at reasonable cost.
- Complicated synthetic schemes should be avoided and purification steps should be efficient without markedly increasing production costs. The production should be easy to scale-up from the bench mark to industrial production.
- The prodrug should be stable in bulk form. This is of particular importance for substances like esters, which are likely to be degraded in the presence of even trace amounts of moisture.
- The *in vivo* lability should be efficient to permit release of the active moiety at a rate adequate to ensure its therapeutic activity. Regeneration can be either chemical (pH effects) and/or enzymatic.
- The prodrug and the "carrier/moiety" should be nontoxic. Relatively "safe" moieties include amino acids, short to medium length alkyl esters, and some of the macromolecules described previously.
- The pharmacokinetics of the active moiety should be well documented before starting prodrug synthesis, and at a later stage, prodrug kinetics should be thoroughly investigated in man.
- The biopharmaceutical consequences for prodrug formulations should be carefully evaluated.
- Last but not least, the prodrug should present some clinically relevant advantages over the active principle administered directly. In this context, it must be remembered that modification of one

Metabolic Considerations in Prodrug Design

pharmacokinetic property frequently alters other properties of the drug molecule and caution must thus be exercised when embarking on a program of this nature.

10 CONCLUSIONS

Although prodrug design started more than 30 years ago and many reviews have been written on this subject, very little information is available in official guidelines or pharmacokinetic textbooks on the regulatory requirements or data analysis for this type of compounds. This chapter is an attempt to gather and confront available information on the subject.

Some basic problems have, however, been left untouched. For example, the difficulty of extrapolating data from animal to humans encountered during toxicokinetic and toxicologic studies with drugs is amplified with prodrugs since not only metabolism of the active moiety might differ, but also its availability from the prodrug. As a matter of fact, there is presently no published rationale for the conduct of animal and human pharmacokinetic programs during prodrug research and development.

The authors concluded a review on prodrugs (7), quoting the question asked in 1985 (248) by Stella et al.: "Do prodrugs have advantages in clinical practice?" The opinion was the following: "Today, the answer is certainly YES in some particular cases, but for many drugs this aspect of drug design has received no clear and satisfactory solution. The main reason for this situation is that most prodrugs have been synthesized starting from valuable and well-known drugs. As a consequence, the potential advantage of the new chemical entity over its "seasoned precursor" has often been only marginal. It is thus important that in the future, drug design of new chemical entities should incorporate

Prodrug Design

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"delivery and/or targeting components" from the earliest stages of research and development. This strategy might help substances too toxic, or unable to show adequate pharmacologic effects in their basal form to go through primary and secondary screening, before successfully reaching human testing. It is evident that if such an approach were to become an integral part of basic drug design and not just a hindsight attempt to solve problems associated with older drugs, it would also be necessary to develop new biopharmaceutical and pharmacokinetic approaches to tackle the new challenges." After five years, the authors still believe that this is a valid statement.

After this chapter, which focused more on pharmacokinetic aspects than on chemical synthesis, we can conclude that, indeed, additional thinking on new ways to approach the toxicokinetic and the clinical pharmacokinetics of prodrugs and their active moiety is of paramount importance if prodrug design is to remain (or to become?) an important part for research and development of new therapeutic agents. In parallel, great efforts must be undertaken in order to better understand the molecular basis of xenobiotic metabolism. It should then be easier to synthesize compounds which would show the most appropriate physicochemical characteristics.

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B. Prodrugs

A *prodrug* is a compound resulting from chemical modification of a biologically active compound that will liberate the active form in vivo by enzymatic or hydrolytic cleavage. The primary purpose in forming a prodrug is to modify the physicochemical properties of the drug, usually to alter the membrane permeability of the parent compound. This change in physicochemical properties of the drug influences the ultimate localization of the drug. There are various reasons for formulating a prodrug system. If the parent compound is insoluble, this can be modified [62]. If it is easily degraded, modification can protect the parent compound from enzymatic or hydrolytic attack. Modifications can also reduce side effects, such as GI irritation [63]. Several drugs are now marketed in the form of a prodrug; for example, sulindac, a nonsteroidal anti-inflammatory agent, and numerous angiotension-converting enzyme (ACE) inhibitors. The necessary conversion of prodrug to parent can occur by a variety of reactions, the most common being hydrolytic cleavage [64]. The prodrug ester forms of a hydroxyl or carboxyl group of the parent compound can be readily cleaved by blood esterase. Other activation processes may include biochemical reduction or oxidation. However the conversion occurs, to achieve sustained drug action, the rate of conversion from prodrug to active compound should not be too high [65]. Site-specific, controlled delivery is achieved by the antiviral prodrug acyclovir, being converted to active form by a virus-specific enzyme [66]. Sustained release of steroid prodrugs, especially progestagens and progestagen-estrogen combinations, have seen a substantial amount of clinical experience, both as a means of birth control and as symptomatic menopausal treatment [67].

The concept of the double prodrug (proprodrug), may allow more controlled delivery of various prodrug compounds [68]. For example, if a prodrug that shows site-specific activation, but has poor transport properties or stability problems, it could be converted to a proprodrug that transported better or is more stable (Fig. 12). Prodrug systems have been taken even further by including as prodrugs, polymer prodrugs, in which a drug is covalently linked to a polymer backbone. This type of system could encompass a staggering number of possibilities. Encouraging results have been shown with mitomycin [69,70], for example.

The most serious disadvantage to the prodrug approach to controlled-sustained delivery is that extensive development must be undertaken to find the correct chemical modification for a specific drug. Additionally, once a prodrug is formed, it is a new drug entity and, therefore, requires extensive and costly studies to determine safety and efficacy.

C. Nanoparticles

Nanoparticles are solid colloidal particles ranging in size from 10 to 1000 nm. They can be used as drug carriers, with the drug encapsulated, dissolved, adsorbed, or covalently attached

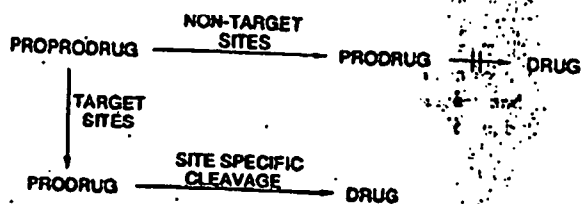


Fig. 12 Illustration of prodrug and proprodrug concept.

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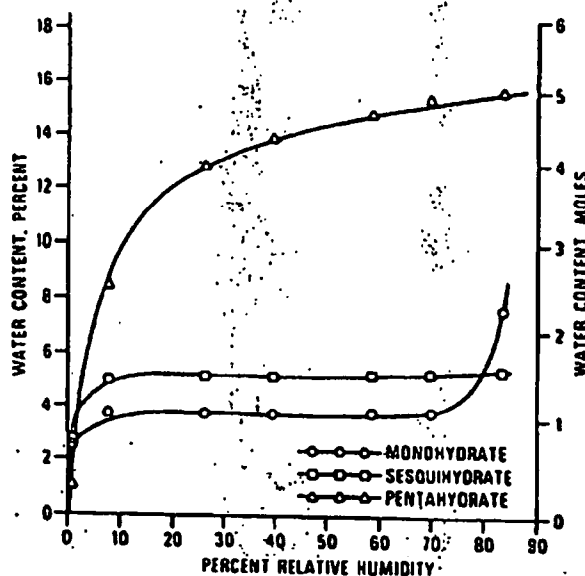


Fig. 8. Relative humidity versus water content of hydrate forms of sodium cefazolin. (From Ref. 5.)

enhance depot action, ease formulation difficulties, and possibly, decrease pain on injection. The molecularly modified drug that converts back to the active parent structure is defined as a *prodrug*. This conversion usually occurs within the body system or, for some drugs that are formulated as dry powders, occurs on reconstitution. The preparation of prodrugs is becoming a common practice with many types of drugs. Examples of antibiotic prodrugs include benzathine penicillin, procaine penicillin, metronidazole phosphate, and chloramphenicol sodium succinate.

The preparation of salts of organic compounds is one of the most important tools available to the formulator. Compounds for both IM and IV solutions require high solubility so that the drug may be incorporated into small volumes for IM administration and also be acceptable for IV use. Sodium and potassium salts of weak acids and hydrochloride and sulfate salts of weak bases are widely used in parenterals requiring highly soluble compounds, based on their overall safety and history of clinical acceptance.

If a drug's solubility is to be reduced to enhance stability or to prepare a suspension, the formulator may prepare water-insoluble salts. A classic example is procaine penicillin G, the decreased solubility (7 mg/ml) of which, when compared with the very soluble penicillin G potassium, is utilized to prepare stable parenteral suspensions. Another alternative to preparing an insoluble drug is to use the parent acidic or basic drug and to buffer the pH of the suspension in the range of minimum solubility.

Polymorphism

The literature lists numerous examples of polymorphism; that is, the existence of several crystal forms of a given chemical that exhibit different physical properties [6]. The conversion of one polymorph to another may cause a significant change in the physical properties of the drug.

Crystalline solids

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Abstract

Many drugs exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. Phase transitions such as polymorph interconversion, desolvation of solvate, formation of hydrate and conversion of crystalline to amorphous form may occur during various pharmaceutical processes, which may alter the dissolution rate and transport characteristics of the drug. Hence it is desirable to choose the most suitable and stable form of the drug in the initial stages of drug development. The current focus of research in the solid-state area is to understand the origins of polymorphism at the molecular level, and to predict and prepare the most stable polymorph of a drug. The recent advances in computational tools allow the prediction of possible polymorphs of the drug from its molecular structure. Sensitive analytical methods are being developed to understand the nature of polymorphism and to characterize the various crystalline forms of a drug in its dosage form. The aim of this review is to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, to address the current challenges faced by pharmaceutical scientists and to anticipate future developments. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Crystallinity; Polymorphs; Hydrates; Solvates; Formulation; Drug substance; Phase transformation; Characterization

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1. Introduction

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms. When applied to solids, the adjective, *crystalline*, implies an ideal crystal in which the structural units, termed *unit cells*, are repeated regularly and indefinitely in three dimensions in space. The unit cell has a definite orientation and shape defined by the translational vectors, *a*, *b*, and *c*, and hence has a definite volume, *V*, that contains the atoms and molecules necessary for generating the crystal. Each crystal can be classified as a member of one of seven possible crystal systems or crystal classes that are defined by the relationships between the individual dimensions, *a*, *b*, and *c*, of the unit cell and between the individual angles, α , β , and γ of the unit cell [1,2]. The structure of a given crystal may be assigned to one of the seven crystal systems, to one of the 14 Bravais lattices, and to one of the 230 space groups [1]. All the 230 possible space groups, their symmetries, and the symmetries of their diffraction patterns are compiled in the International Tables for Crystallography [3].

The common crystalline forms found for a given drug substance are polymorphs and solvates. Crystalline polymorphs have the same chemical composition but different internal crystal structures and, therefore, possess different physico-chemical properties. The different crystal structures in polymorphs arise when the drug substance crystallizes in different crystal packing arrangements and/or different conformations. The occurrence of polymorphism is quite common among organic molecules, and a large number of polymorphic drug compounds have been noted and catalogued [4–7].

Solvates, also known as pseudopolymorphs, are

crystalline solid adducts containing solvent molecules within the crystal structure, in either stoichiometric or nonstoichiometric proportions, giving rise to unique differences in the physical and pharmaceutical properties of the drug. If the incorporated solvent is water, a solvate is termed a hydrate. Adducts frequently crystallize more easily because two molecules often can pack together with less difficulty than single molecules. While no definite explanations can be given, possible reasons include adduct symmetry, adduct-induced conformation changes, and the ability to form hydrogen bonds through the solvent molecules [2,8,9]. Desolvated solvates are produced when a solvate is desolvated and the crystal retains the structure of the solvate [10]. Desolvated solvates are less ordered than their crystalline counterparts and are difficult to characterize, because analytical studies indicate that they are unsolvated materials (or anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived [11].

Because different crystalline polymorphs and solvates differ in crystal packing, and/or molecular conformation as well as in lattice energy and entropy, there are usually significant differences in their physical properties, such as density, hardness, tabletability, refractive index, melting point, enthalpy of fusion, vapor pressure, solubility, dissolution rate, other thermodynamic and kinetic properties and even color [12]. Differences in physical properties of various solid forms have an important effect on the processing of drug substances into drug products [13], while differences in solubility may have implications on the absorption of the active drug from its dosage form [14], by affecting the dissolution rate and possibly the mass transport of the molecules. These concerns have led to an increased regulatory

interest in understanding the solid-state properties and behavior of drug substances. For approval of a new drug, the drug substance guideline of the US Food and Drug Administration (FDA) states that “appropriate” analytical procedures need to be used to detect polymorphs, hydrates and amorphous forms of the drug substance and also stresses the importance of controlling the crystal form of the drug substance during the various stages of product development [11]. It is very important to control the crystal form of the drug during the various stages of drug development, because any phase change due to polymorph interconversions, desolvation of solvates, formation of hydrates and change in the degree of crystallinity can alter the bioavailability of the drug. When going through a phase transition, a solid drug may undergo a change in its thermodynamic properties, with consequent changes in its dissolution and transport characteristics [15].

Various pharmaceutical processes during drug development significantly influence the final crystalline form of the drug in the dosage form. The various effects of pharmaceutical processing on drug polymorphs, solvates and phase transitions have been described in detail by Brittain and Fiese [16] and will be discussed in later chapters. Briefly, processes such as lyophilization and spray drying may lead to the formation of the amorphous form of drug, which tends to be less stable and more hygroscopic than the crystalline product. Also, processing stresses, such as drying, grinding, milling, wet granulation, oven drying and compaction, are reported to accelerate the phase transitions in pharmaceutical solids. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. Keeping these factors in mind, it is desirable and usual to choose the most stable polymorphic form of the drug in the beginning and to control the crystal form and the distributions in size and shape of the drug crystals during the entire process of development. The presence of a metastable form during processing or in the final dosage form often leads to instability of drug release as a result of phase transformation [17].

Crystallization plays a critical role in controlling the crystalline form and the distribution in size and shape of the drug. The significance of crystallization

mechanisms and kinetics in directing crystallization pathways of pharmaceutical solids and the factors affecting the formation of crystals have been reviewed in detail by various researchers [12,18,19]. A crystalline phase is created as a consequence of molecular aggregation processes in solution that lead to the formation of nuclei, which achieve a certain size during the nucleation phase to enable growth into macroscopic crystals to take place during the growth phase. The factors affecting the rate and mechanisms by which crystals are formed are: solubility, supersaturation, rate at which supersaturation and desupersaturation occur, diffusivity, temperature, and the reactivity of surfaces towards nucleation. The various forces responsible for holding the organic crystalline solids together, such as nonbonded interactions and hydrogen bonding, have been discussed in detail by Byrn et al. [2] and Etter [20].

Various analytical methods are being currently used to characterize the crystalline form of the drug during the various steps of processing and development. These methods have been reviewed recently in detail by many authors [7,10,21–25]. The single most valuable piece of information about the crystalline solid, including the existence of polymorphs and solvates, is the molecular and crystalline structure, which is determined by single-crystal X-ray diffractometry [2]. Powder X-ray diffractometry provides a “fingerprint” of the solid phase and may sometimes be used to determine crystal structure. Once the existence of polymorphism (or solvate formation) is definitely established by single-crystal and powder X-ray diffractometry, spectral methods, such as Fourier transform infrared absorption (FTIR) spectroscopy, Fourier transform Raman scattering (FT Raman) spectroscopy, solid-state nuclear magnetic resonance (SSNMR) spectroscopy, ultraviolet and visible (UV–Vis) and/or fluorescence spectroscopy [23] may be employed for further characterization. Of special significance are thermal methods, such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and optical microscopy using a hot stage [24]. These methods are almost always employed for further characterization. Modulated (temperature) differential scanning calorimetry (MDSC) in combination with DSC and optical microscopy are able to identify the glass

transition of amorphous forms with much greater clarity and allow unique insights into the glass transitional and polymorphic behavior of drug substances [26].

Because solid-state NMR spectroscopy can be used to study crystalline solids, as well as pharmaceutical dosage forms, this powerful method is finding increasing application in deducing the nature of polymorphic variations [27], such as variations in hydrogen bonding network and molecular conformations among polymorphs [28,29] and for the determination of molecular conformations and mobility of drugs in mixtures and dosage forms [2]. Solid-state ^{13}C -NMR in conjunction with the techniques, known as high power proton decoupling, cross polarization (CP), and magic-angle spinning (MAS) offers information not obtained readily by other techniques. Recently, two-dimensional ^{13}C -solid-state NMR spectroscopy has been used to study the three conformational polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophenecarbonitrile [30]. Use of two-dimensional NMR and total suppression of spinning side bands (TOSS) pulse sequences allowed the separation of isotropic and anisotropic chemical shifts for the three forms. This is a very powerful method for analyzing differences in the chemical environment and is finding increased application in the study of conformational polymorphism.

With advances in analytical methods, the current focus of research in the solid-state area is to understand polymorphism and pseudopolymorphism at the molecular level. Knowledge of the crystal packing arrangements and the various intermolecular forces involved in the different packing arrangements will help in the prediction and preparation of the most stable polymorphs of a given compound well in advance, to avoid surprises during product development. A current emphasis is on the development of software to predict crystal structures of polymorphs from molecular structures. A thorough understanding of the physicochemical properties of polymorphs and solvates (hydrates) is of primary importance to the selection of a suitable crystalline form and development of a successful pharmaceutical product. Bray et al. [31] have shown that, by thorough characterization of four different crystalline forms of L-738,167, a fibrinogen receptor antagonist by various analytical

techniques, it was possible to determine the suitability of one or two forms for the development of pharmaceutical oral dosage forms.

The present review aims to emphasize the recent advances made in the area of prediction and characterization of polymorphs and solvates, attempts to address the current challenges and problems faced by pharmaceutical scientists and intends to anticipate future development. This review does not attempt to provide solutions to the problems but attempts to review comprehensively the advances made in recent years to help address these problems.

2. Recent advances in the identification, prediction and characterization of polymorphs

2.1. Types of polymorphism

Based on differences in the thermodynamic properties, polymorphs are classified as either enantiotropes or monotropes, depending upon whether one form can transform reversibly to another or not. In an enantiotropic system, a reversible transition between polymorphs is possible at a definite transition temperature below the melting point. In a monotropic system, no reversible transition is observed between the polymorphs below the melting point. Four useful rules have been developed by Burger and Ramburger [32,33] to determine qualitatively the enantiotropic or monotropic nature of the relationship between polymorphs. These rules are the heat of transition rule, heat of fusion rule, infrared rule and density rule.

If, by use of the above rules, it is established that the polymorphs of a particular drug are enantiotropic or monotropic, then the next goal is to define the thermodynamically stable (or metastable) domain of each crystalline phase of a substance as a function of temperature. The plot of the Gibbs free energy difference, ΔG , against the absolute temperature, T , gives the most complete and quantitative information on the stability relationship of polymorphs [22], with the most stable polymorph having the lowest Gibbs free energy. The ΔG between the polymorphs may be obtained using several techniques operating at

different temperatures, such as solubility [34] and intrinsic dissolution rate. Yu [35] has derived thermodynamic equations to calculate ΔG between two polymorphs and its temperature slope from the melting data. This method is essentially an extension of the heat of fusion rule, which is based on statistical mechanics. Extrapolating ΔG to zero gives an estimate of the transition temperature, from which the existence of monotropy or enantiotropy is inferred. The integration of different types of data provides the ΔG vs. T curve over a wide temperature range and allows the consistency between techniques to be checked [22]. Another approach to establish the order of stability among various polymorphs has been studied using pressure versus temperature plots, e.g., for sulfanilamide and piracetam [36]. This approach is based upon Ostwald's principle of least vapor pressure, according to which the stable polymorph exhibits the lowest vapor pressure. The accuracy of this approach to establish the stability hierarchy among the polymorphs has been shown to be very much dependent on the accuracy of the experimental data.

In recent years, the main focus of research has been the characterization of polymorphs arising from structural differences in the crystal lattice. It has been established for some time that organic molecules are capable of forming different crystal lattices through two different mechanisms. One of the mechanisms is termed *packing polymorphism*, and represents instances where conformationally relatively rigid molecules can be assembled into different three-dimensional structures through the invocation of different intermolecular mechanisms. The other mechanism is termed *conformational polymorphism* and arises when a nonconformationally rigid molecule can be folded into different arrangements, which subsequently can be packed into alternative crystal structures. The distinction between *packing polymorphism* and *conformational polymorphism* is somewhat artificial because different packing arrangements impose different conformations on the molecules, however slight, and different conformations will inevitably pack differently. The structural aspects associated with polymorphs have been reviewed recently [2], as have the analogous features of solvate and hydrate systems [9]. In the next

section, the results of some more recent investigations are discussed.

2.2. Packing polymorphism

An investigation into the structures and charge densities of two polymorphs of *p*-nitrophenol has been performed with the aim of deducing the different modes of inter-molecular hydrogen bonding that lead to the formation of the two structures shown in Fig. 1a and b [37]. A detailed analysis of the charge density of the two forms indicates charge migration from the benzene ring region to the nitro and hydroxyl groups that accompanies the transformation of one form into the other. In addition, polarization of the oxygen lone-pair electrons was found to be substantially larger in the crystal forms than in the free molecule, resulting in considerably larger dipole moments in the solid state.

During the study of a new crystal form (form I) of

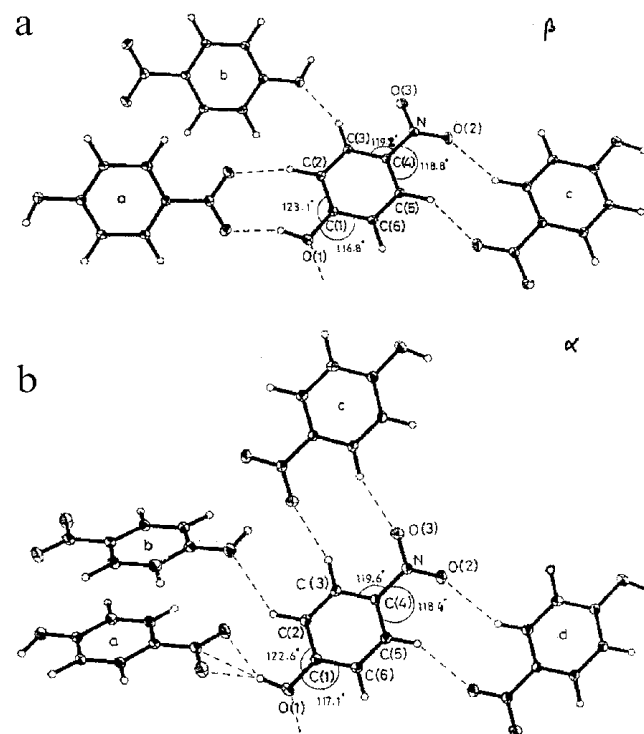


Fig. 1. Molecular packing diagrams of the (a) β polymorph of *p*-nitrophenol, (b) α polymorph of *p*-nitrophenol, showing 50% probability displacement ellipsoids ([37], reproduced with the permission of the American Chemical Society).

chlordiazepoxide, it was found that the heat of transition between the two forms (forms I and II) is rather modest, and kinetic factors permit the existence of the metastable phase [38]. Both structures contain four crystallographically independent molecules linked in dimers through hydrogen bonding, but the dimers are packed differently to yield the two crystal forms. Because the dimers in the fundamental units are spaced differently in the two forms, it was proposed that the solid-state enantiotropic transformation entailed rearrangement of the dimer units.

A different approach has been taken during an evaluation of the different structures formed by sulfathiazole [39]. Using a graph set approach to classify the known structural differences and similarities among the various forms, it became possible to identify packing motifs common to three of the four crystal structures. Fig. 2 shows the unit cells of the polymorphs I, II, III and IV, where molecules are paired as hydrogen-bonded dimers. At the end of the process, the authors were able to deduce possible links between the observed patterns of hydrogen bonding, processes of nucleation, and the crystal growth observed from a number of solvent systems. Interestingly, the analysis did not indicate a relationship between the appearance of a particular polymorph from solution and the growth of its fastest

growing surface. Rather, it appeared as if the different solvents affected the process of polymorph formation through their effects on nucleation of the various forms.

2.3. Conformational polymorphism

The conformational polymorphism of the two forms of piroxicam pivalate has been studied in detail [40]. This compound is distinctive in that the high-melting form (polymorph 1) contains an unanticipated array of associated molecules bound as centrosymmetric dimers through hydrogen bonding, with the amido nitrogen atom acting as the donor and the pyridine nitrogen as the acceptor (Scheme 1, structure I). The low-melting form (polymorph 2) contains molecules of two distinct conformational states coexisting in the same crystal (Fig. 3), but linked through different hydrogen bonding arrangements. This latter finding represents another unusual aspect of the crystallography of the substance.

The inclusion of different solvent molecules in a crystal lattice can lead to the existence of different packing patterns, and has also been found to influence the molecular conformation of paroxetine hydrochloride in two solvate forms [41]. One form

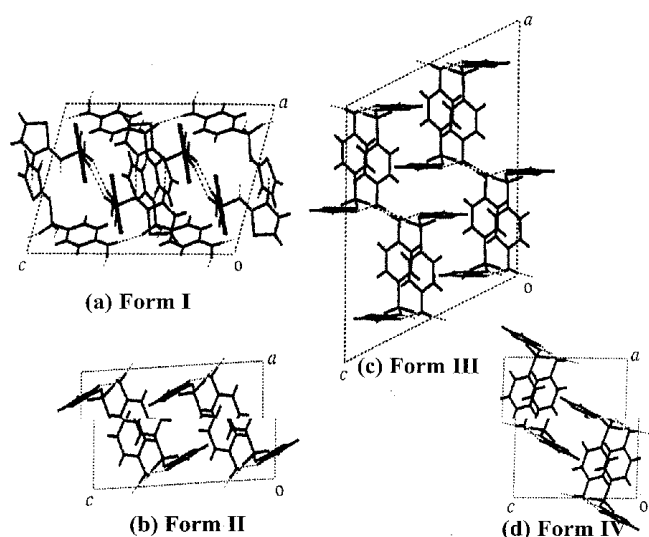
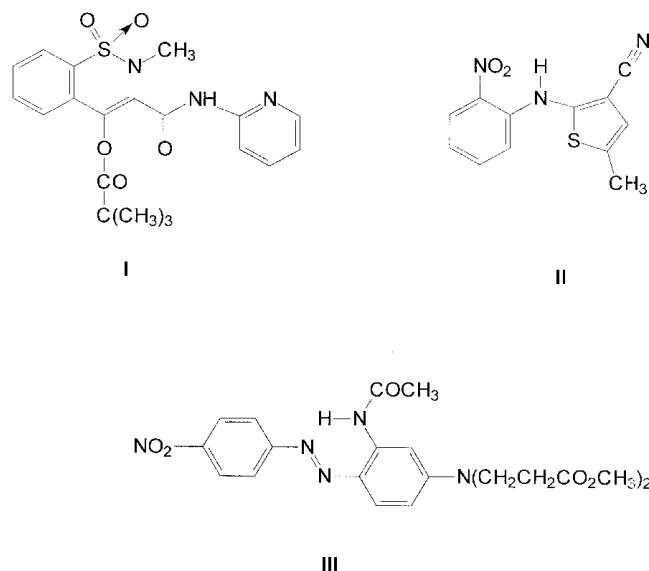
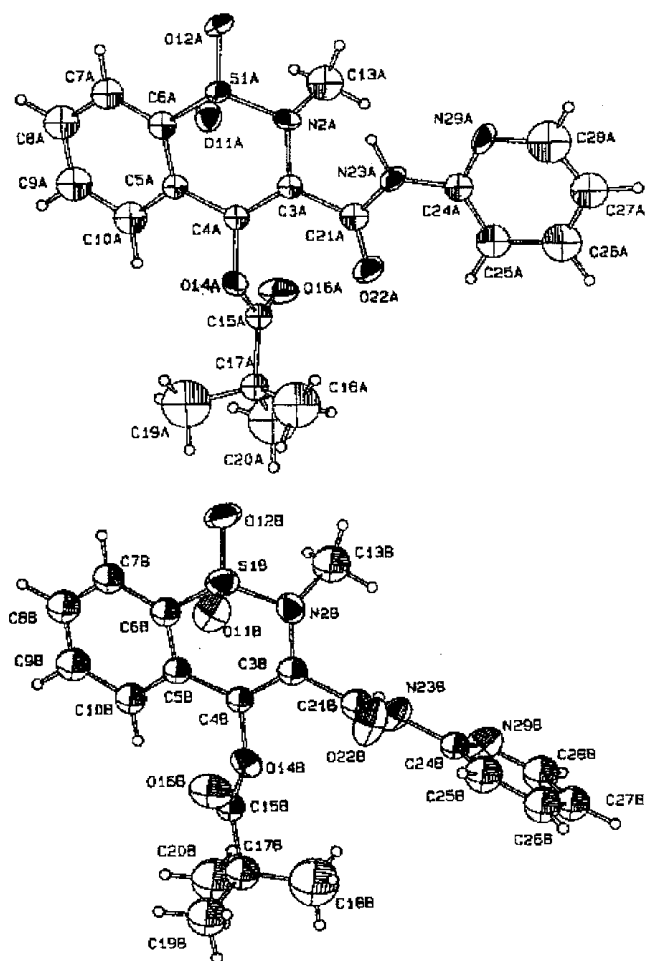


Fig. 2. Unit cells of four polymorphs (I, II, III and IV) of sulfathiazole showing hydrogen bonds, with the dimer structure clearly discernible ([39], reproduced with the permission of the Royal Society of Chemistry).



Scheme 1. Molecular structure of piroxicam pivalate (I) [40], 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II) [30], 2'-acetamido-4'-[N,N-bis(2-methylcarbonyl)ethyl]amino]-4-nitroazobenzene (III) [48].



was obtained as a hemihydrate, and the other as the solvate of isopropanol (2-propanol). In the unit cell of the hemihydrate, one finds two protonated paroxetine and two chloride ions together with one water molecule. Interestingly, the two paroxetine molecules are conformationally nonequivalent, and exhibit a number of different bond angles and torsion angles. In the other form, the unit cell contains one protonated paroxetine molecule, one chloride ion, and one isopropanol molecule disordered along a molecular channel. Furthermore, the conformation of the paroxetine molecule in the isopropanol solvate is different from either molecular conformation observed in the

In an impressive fundamental study, the polymorphism of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (Scheme 1, structure II) has been catalogued [42,43] and discussed in detail [2]. This compound was crystallized as six solvent-free polymorphs, each of which differed in the mode of packing and in molecular conformation. The different conformers yielded sufficient perturbations on the respective molecular orbital so that a variety of crystal colors (red, orange, and yellow) were observed. To obtain a more detailed evaluation of the relative stability, the authors considered a partitioning of polymorphic energy differences into lattice and conformational contributions, and were able to deduce general trends that appeared valid in the absence of hydrogen bonding. The act of crystallization was found to feature an interplay of opposing forces, with perpendicular molecular conformations being favored in fluid solutions, while a preference for planar/high dipole conformers existed in most crystal forms, as shown in Fig. 4 [42]. The unusual polymorphism displayed by this system may result from one or more of the following factors: the preference for perpendicular conformations in solutions, the preference for planar/high dipole conformers in crystals, the formation of inter- and intramolecular hydrogen bonds, and the thermodynamic tendency towards low energy and high entropy.

2.4. Phase transformations in the solid state

Studies of phase transformations in the solid state are important, because the sudden appearance or disappearance of a crystalline form can threaten process development, and can lead to serious pharmaceutical consequences if the transformation occurs in the dosage forms. Hence, an understanding of the kinetics and mechanism of phase transformations is of practical importance. The rearrangement of molecules into a new structure during phase transformation may or may not involve a solvent or vapor phase. To explain the mechanism of solid-solid

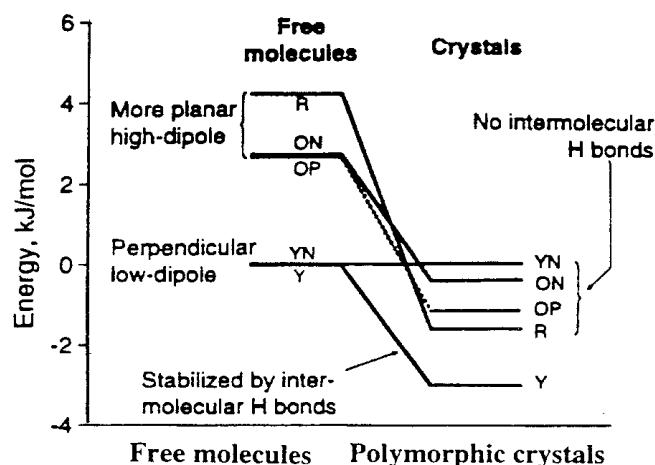


Fig. 4. Comparison of conformational energies and crystal energies of the various polymorphs of 5-methyl-2-[2-(nitrophenyl)amino]-3-thiophenecarbonitrile (II). Form R (red prisms, m.p. 106.2°C), form Y (yellow prisms, m.p. 109.8°C), form OP (orange plates, m.p. 112.7°C), form ON (orange needles, m.p. 114.8°C), form YN (yellow needles, m.p. not measurable) ([42], reproduced with the permission of the American Chemical Society).

physical transition, four steps have been proposed: (a) molecular loosening in the initial phase; (b) formation of an intermediate solid solution; (c) nucleation of the new solid phase and (d) growth of the new phase [2]. In an interesting study, Skwierczynski [44] has proposed a two-environment model to describe the decomposition reaction kinetics of a crystalline solid, aspartame. The decomposition reaction of aspartame is a simple unimolecular thermally-induced aminolysis and the reaction proceeds under anhydrous conditions, i.e., water is not a reactant [45]. This model links the chemistry of the solid-state reaction with the molecular mobility of the reactant as the reaction proceeds. The advantage of this model is that it can be used to determine the shelf life of a product from kinetic data gathered at elevated temperatures. Apart from solid–solid physical transformations, solution-mediated physical transformations among polymorphs are also known to occur in processes, such as wet granulation and during dissolution testing.

While the majority of studies have probed the equilibrium properties of polymorphic and solid-state solvated systems, relatively few have been concerned with the dynamics of phase transformation. Byrn et

al. [2] have reviewed briefly the aspect of polymorphic interconversions and the factors affecting the transformations. In one study, the contribution of hydrogen bonding to the $\alpha \rightarrow \beta$ phase change of resorcinol has been detailed [46]. The α form is more stable than the β form at room temperature, but is less dense than the β form. The transition of $\alpha \rightarrow \beta$ at an estimated transition temperature of 337 ± 1 K is accompanied by an increase in crystal density, with the structure shifting from an open array of molecules (linked through hydrogen bonding) to a denser structure resembling molecular crystals. Through the use of a simple potential model, it was concluded that, during the phase transformation, the energy of the hydrogen bonds decreases along with the extent of such bonding. The energy liberated by this process is almost offset by the enhanced Van der Waals energies associated with the increase in crystal density, and consequently the transition enthalpy is rather small. Accompanying the shifts in hydrogen bonding is a number of effective proton transfers, altering the covalent and ionic portions of the crystal. It was also learned that the increase in entropy produced from the redistribution of protons was of the same order of magnitude as the entropy of the phase transition.

A number of spectroscopic techniques have been used to study the processes associated with a polymorphic transition of 2-(2,4-dinitrobenzyl)-3-methylpyridine [47]. The two interconverting structures coexisted over a temperature range of at least 8–9°C. The phase change was associated with a molecular tautomerization that translated through the collective changes of a large number of molecules, yielding domains having definite short-range order. The slowly evolving spectroscopy that took place above the transition temperature was interpreted as the annealing of domains into a long-range ordered system. The process of phase transformation appeared to consist of an initial fast redistribution of the mole ratio of the coexisting phases, followed by a much slower process involving a macroscopic relaxation of the system. Although local thermodynamic equilibrium was thought to exist in individual domains, the magnitude noted for the temperature range of the phase transition was proposed to arise from nonequilibrium conditions existing among the various types of domain.

A combination of solid-state ^{15}N -NMR spectroscopy and X-ray crystallography was used to study polymorphic transitions in an azobenzene dyestuff, 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonyl)amino]-4-nitroazobenzene (Scheme 1, structure III) [48]. This work established that the structure of one polymorph was disordered, and that the process of phase transformation entailed a crankshaft-type motion of the azo linkage. The ORTEP plots of the two molecular conformations for the X-ray structure determination of structure III at 293 K are shown in Fig. 5. Selective polarization inversion and band shape-fitting experiments were used to deduce the thermodynamic parameters of the exchange process.

Raman spectroscopy was used to study the effect of pressure on the phase transitions in hexamethylbenzene and hexa(methyl- d_3)benzene [49]. The form II $\rightarrow$ form III transition of the partially deuterated substance was found to take place at a lower pressure relative to that of the analogous hexamethylbenzene compound, which was attributed to differences in the energies of the intramolecular methyl torsional vibration in the two crystal forms. In another study performed by the same group, the effects of both temperature and pressure on the phase transitions of tetrafluoro-1,4-benzoquinone were considered [50]. In this system, the changes in en-

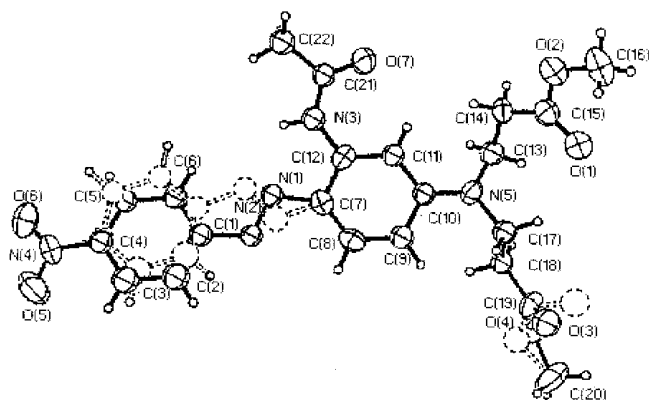


Fig. 5. ORTEP plots of the two molecular conformations (1 and 2) for the X-ray structure determination of 2'-acetamido-4'-[*N,N*-bis(2-methoxycarbonyl)amino]-4-nitroazobenzene (Scheme I Structure) at 293 K. Thermal ellipsoids are shown at 30% for clarity, with conformer 2 being represented by the solid lines and Conformer I by the dotted lines ([48], reproduced with the permission of the American Chemical Society).

vironmental conditions were found to influence a number of intermolecular and intramolecular vibrational modes, yielding conformational changes that in turn produced the observed phase transitions.

2.5. Prediction of polymorphs

The main challenge in managing the phenomenon of multiple solid forms of a drug is the inability to predict the number of forms that can be expected in a given case. This prediction would involve quantification of the myriad intermolecular forces within any proposed crystal structure as well as the ability to postulate the likely packing modes for a given molecule in all its configurations [10]. Accurate theoretical prediction of polymorphs from studies of molecular dynamics and crystal structure generation would be of outstanding importance in drug research [36].

More research is now being directed towards developing computational tools to understand the nature of polymorphism and to predict polymorphic forms at an early stage in the drug development process. The recent developments in computational chemistry allow the prediction of possible polymorphic forms based only on the molecular structure of the drug. The Polymorph Predictor, from Molecular Simulations, is currently the only commercial software package that can predict the possible polymorphs of an organic compound from its molecular structure [51]. The package developed by Karfunkel and co-workers [52–54] uses a Monte Carlo simulated annealing approach to generate thousands of possible crystal packing alternatives for a given molecule. Each of the unique crystal structures is then subjected to a lattice energy minimization to obtain the relative stability ranking of the various packing possibilities and the resulting lowest-energy structures are the potential polymorphs. This method has been successfully employed to generate known polymorphs of primidone (Fig. 6A and B) and progesterone, starting from the molecular structures alone [55]. It has also been used to predict polymorphs for a range of small molecules and to predict unknown polymorphic structures of 4-amidinoin-danone guanyldiazide, a selective inhibitor of *S*-adenosylmethionine decarboxylase [56], and of aspirin [57].

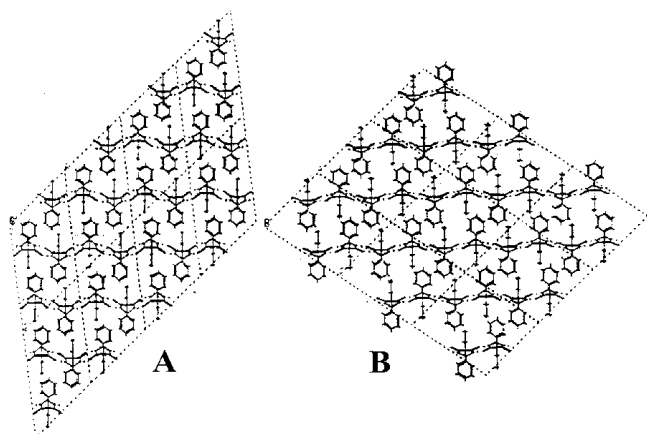


Fig. 6. Comparison of the crystal structure of primidone A (A) versus the most likely packing arrangement, frame 7 (B) ([55], reproduced with the permission of Elsevier Science).

The theoretical predictions of lattice energies, entropies, morphologies and polymorphs should stimulate experimental activities and vice versa. The current crystal-modeling efforts have the potential of producing more quantitative tools for bridging structures and properties, which could help in creating solid forms with desired properties [22]. There are many limitations in using computational methods for predicting polymorphs theoretically. The first limitation is that the *ab initio* screening is useful only for nonionic rigid molecules. For more complex systems, the method is very useful for generating plausible crystal structures, but it is not accurate enough to determine which of these possible structures can actually be crystallized [58]. In addition, the limitations in computer power can restrict the use of this method for predicting polymorphs of complex molecules. An issue of concern is that the existing methods only predict the lattice energies, which relate to internal energies or enthalpies of the crystals. However, the relative thermodynamic stability of polymorphs is determined by the Gibbs free energy, which is a linear function of both enthalpy and entropy. Predictions of the relative stability of polymorphs will be more accurate when the entropies, as well as lattice energies, are considered. Application of molecular dynamics may enable the entropies to be calculated. Hence, no general method is currently available for the prediction or interpretation of the properties of complicated polymorphic or pseudopolymorphic systems.

2.6. Directing the crystallization of specific polymorphs

Complementing the different computational methods for predicting the stable polymorphs of a given compound, various experimental methods are also being employed extensively to control the type of polymorph formed during the crystallization process. Many studies have reported the role of additives in controlling the outcome of the crystallization process. Some of the preselected additives are capable of inhibiting the nucleation and/or growth of the unwanted polymorphs. For the first time, the role of reaction by-products in controlling polymorph appearance of a drug has been reported [59]. This drug is sulfathiazole that is known to exist as polymorphs, forms I, II, III and IV, that differ in the hydrogen-bond network. Form I was found to be different from the other three forms as a result of a different hydrogen bonding at the aniline moiety of the molecule. From studies of the hydrogen-bonding pattern, it was predicted that the ethamido derivative of sulfathiazole could selectively control the formation of form I over other forms by entering the growing face of form I without disrupting the structure (Fig. 7a). Because a similar effect was not possible with the other forms, incorporation of the ethamido derivative in the other forms should inhibit their growth (Fig. 7b). Experimentally, it was shown that the ethamido by-product stabilized form I over the other polymorphs. This study clearly shows that the combination of crystal morphology and the hydrogen-bond network analysis of the different polymorphs offer a new and powerful approach to understanding and controlling polymorph appearance and stability in the presence of additives.

A similar approach was also applied to stabilize a metastable α conformational polymorph of L-glutamic acid using additives [60]. Methods such as DREIDING and TRIPOS force fields were used to select appropriate additives which could mimic the α and β conformations. Four additives were chosen for this study of which two were present exclusively in the β conformation and theoretically should selectively inhibit the crystallization of the β phase and thus stabilize the metastable α phase. Experimentally, it was proven that the additives, by virtue of their conformation, were able to selectively inhibit the

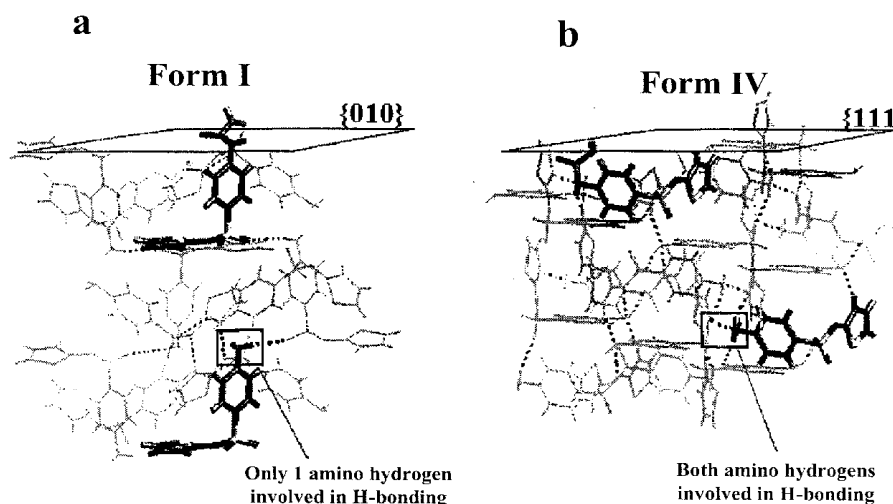


Fig. 7. Possible binding interaction of ethamidosulfamide in the fastest growing faces of (a) form I and (b) form IV of sulfathiazole ([59], reproduced with the permission of Elsevier Science).

appearance of the stable β polymorph of L-glutamic acid by interfering with either the nucleation rates or the growth rates and thus stabilize the metastable form. These studies demonstrate clearly that the molecular packing and intermolecular hydrogen bonds are the main features, which make possible the conformational discrimination. The use of conformational mimicry to stabilize the metastable structures of conformational polymorphs now offers a powerful tool for the prediction and development of robust processes for the control of polymorphic systems.

2.7. Characterization of polymorphs using a combination of analytical techniques

The common techniques often fail to differentiate definitively between two structurally similar polymorphs. Hence more advanced techniques or a combination of techniques need to be used to avoid errors of interpretation and in the identification of polymorphs [24]. Combinations of techniques are being employed currently for the characterization of crystalline pharmaceutical solids. For example, conventional single-crystal X-ray diffractometry and polarized microscopy were of no use in distinguishing the two forms I and II of roxifiban, a very promising cardiovascular drug, because of the relatively small crystallite sizes of the polymorphs. Hence, transmission electron microscopy (TEM) and

synchrotron X-ray diffraction techniques were employed to characterize the unit cells of the two forms. By coupling the highly resolved synchrotron powder X-ray diffraction data shown in Fig. 8, with in-

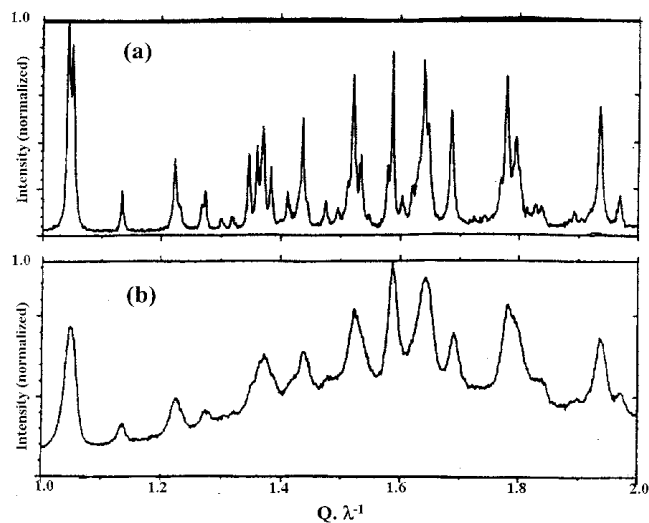


Fig. 8. A partial comparison of (a) a synchrotron pattern of polymorph II of roxifiban, collected using a wavelength of 1.00006 Å with (b) a conventional X-ray diffraction pattern using CuK α radiation in a region where there are many overlapping peaks. The patterns are plotted as a function of $Q = 2\pi/d = 4\pi \sin \theta/\lambda$ to remove the effects of different wavelengths ([61], reproduced with the permission of the American Pharmaceutical Association).

formation obtained from TEM diffraction patterns, the unit cell parameters of the two forms of roxifiban were determined [61]. Similarly, the three modifications, I, II and III, of the nonsteroidal antiinflammatory drug, tiaprofenic acid, could not be distinguished by the two traditional spectroscopic methods, FTIR and FT-Raman spectroscopy. The modifications can only be distinguished by a combination of thermoanalytical and powder X-ray diffractometric methods [62].

Another example, to which a combination of techniques has been successfully applied to identify the various conformational polymorphs of a drug, is the characterization of the solid forms of neotame [29]. Neotame, *N*-(3,3-dimethylbutyl)-L-aspartyl-L-phenylalanine methyl ester, a new high-potency sweetener exists in the following phase-pure crystalline forms: monohydrate, the most stable crystalline form of neotame under ambient conditions, a methanol + water solvate [63], a methanol solvate [64], an amorphous anhydrate [29] and a crystalline anhydrate (form A; [65]). The authors conducted a systematic study of the conversion of the monohydrate under vacuum to a mixture of anhydrate forms followed by the reconversion of the anhydrate to the monohydrate upon exposure to moisture under ambient conditions. No significant changes were observed in the powder X-ray diffraction patterns during part of the reconversion process, suggesting that no change in lattice structure had occurred. However, the solid-state ^{13}C -CP-MAS NMR spectra, indicated the presence of several forms of neotame during the reconversion (Fig. 9). This discrepancy in the results between the two techniques was attributed to the conformational change of neotame molecules during reconversion, without significant change in unit cell parameters. This example indicates that both solid-state ^{13}C -CP-MAS NMR spectroscopy and powder X-ray diffractometry are needed to analyze mixtures of solid forms of conformationally flexible molecules, such as neotame.

A combination of solid-state ^{13}C -NMR spectroscopy and single crystal X-ray diffractometry also has been used to examine the solid-state tautomerism of acetohexamide [66,67]. Polymorphism of the anti-diabetic drug acetohexamide has been investigated by numerous techniques. On the basis of FTIR data, form A of acetohexamide has been proposed to exist

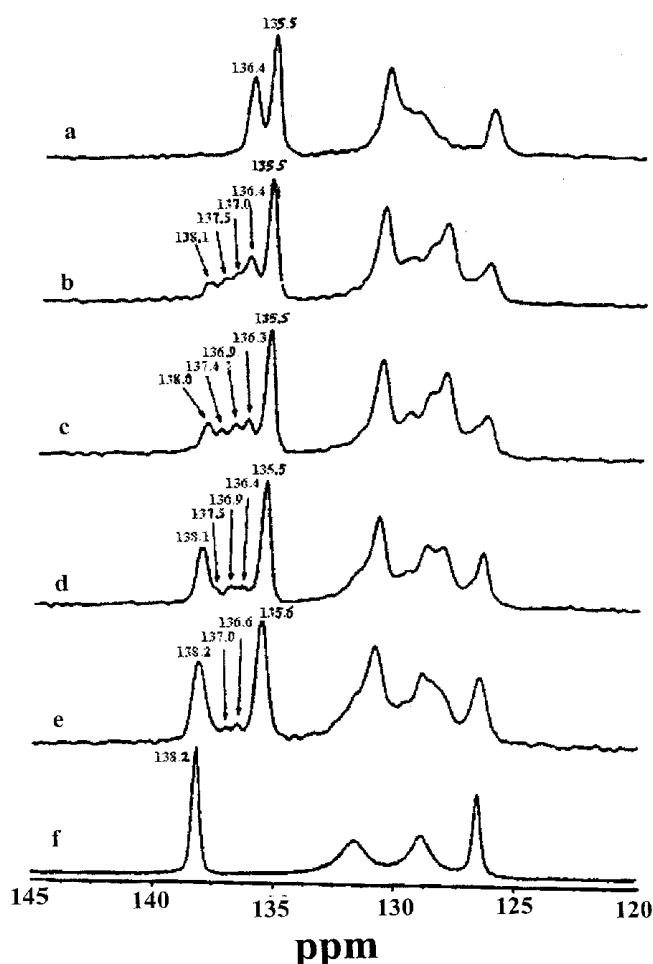


Fig. 9. Resonance signal of the phenyl carbon (C-15) attached to the side chain in the ^{13}C -CP-MAS NMR spectra of neotame anhydrate: (a) sample generated by placing the original monohydrate under vacuum (~ 1 Torr) for 3 days; (b–e) sample after being sealed in a jar for 2, 4, 6 and 8 days, respectively; (f) sample after being exposed to a relative humidity environment of 84% for 12 days ([29], reproduced with the permission of the American Chemical Society).

in the enol-tautomeric state, whereas form B has been proposed to be in the keto-tautomeric state. Using NMR and crystal structure data it was firmly established that both these acetohexamide polymorphic forms are present in the keto-form. Hence the combination of solid-state NMR spectroscopy and X-ray crystallography provided strong evidence that both forms of acetohexamide exist in the keto-tautomeric state and are truly polymorphic.

3. Recent advances in the identification and characterization of hydrates and solvates

3.1. Introduction to solvates and hydrates

It has been estimated that approximately one-third of the pharmaceutically active substances are capable of forming crystalline hydrates [68]. The water molecule, because of its small size, can easily fill structural voids and because of its multidirectional hydrogen bonding capability, is also ideal for linking a majority of drug molecules into stable crystal structures [2]. The mere presence of water in a system is not a sufficient reason to expect hydrate formation, because some compounds, though they are soluble in water, do not form hydrates. It is the activity of water in the medium that determines whether a given hydrate structure will form. Solvates may be formed when a pure organic solvent or a mixture of solvents is used as the solvent for crystallizing the compound. Guillory [69] has discussed the various methods of preparation of hydrates and solvates in detail. Because solvates behave similarly to hydrates, common analytical techniques can be used for characterization of solvates and hydrates.

3.2. Structural aspects

Crystalline hydrates, based on their structure may be classified into three categories. The first category (class I) are the isolated site hydrates, where the water molecules are isolated from direct contact with other water molecules by intervening drug molecules, e.g., cephadrine dihydrate. The second category (class 2) are channel hydrates where the water molecules included in the lattice lie next to other water molecules of adjoining unit cells along an axis of the lattice, forming channels through the crystal, e.g., ampicillin trihydrate. The channel hydrates can be subclassified into two subcategories. One category comprises the expanded-channel or nonstoichiometric hydrates, which may take up additional moisture in the channels when exposed to high humidity and for which the crystal lattice may expand or contract as the hydration or dehydration proceeds effecting changes in the dimensions of unit cells, e.g. cromolyn sodium. The other subcategory comprises

the planar hydrates, which are channel hydrates in which water is localized in a two-dimensional order, or plane, e.g., sodium ibuprofen. The third category (class 3) of crystalline hydrates are the ion-associated hydrates, in which the metal ions are coordinated with water, e.g., calteridol calcium [8,9].

In this section, some examples of nonstoichiometric hydrates and their characterization will be discussed in detail because these forms pose a special challenge in dosage form development due to unpredictability of water content in the crystals. Following the work of Cox et al. [70] the unusual water uptake and formation of nonstoichiometric hydrates of cromolyn sodium was reinvestigated using single crystal X-ray diffractometry, PXRD, as well as by molecular modeling [71]. Cromolyn sodium, an antiasthmatic drug, exists as two liquid crystalline phases and a crystalline hydrate phase that sorbs and liberates water continuously and reversibly to give a continuous range of nonstoichiometric hydrates [70]. The changes in the PXRD patterns of the crystalline hydrate phase of cromolyn sodium in response to the surrounding relative humidity (RH) were explained in the light of the molecular and crystal structure of cromolyn sodium. Single crystal X-ray diffractometry indicated the space group for cromolyn sodium as *P*1, a chiral space group, even though the molecule itself is achiral. The crystal structure of cromolyn sodium with five or six water molecules per cromolyn sodium molecule, solved at room temperature by Hamodrakas et al. [72], revealed the positions of only one sodium ion and two water molecules and showed that the second sodium ion and the other water molecules are disordered. Recently, the single crystal structure of cromolyn sodium at 76% RH, with 6.44 molecules of water was solved at 173 K by Chen et al. [71]. This work showed that the second undetermined sodium ion is disordered over three sites and that four of the eight water positions are partially occupied. Comparison of the crystal structures determined by Hamodrakas et al. [72] and Chen et al. [71] indicated that the cromolyn anion is flexible. In particular, the bond and torsional angles of the 2-hydroxypropane linking the two cyclic moieties, changed to accommodate lattice expansion or contraction resulting from water sorption and desorption by the crystals. As water is taken up, the relative occupancies of the sites of the

second sodium ion and that of water molecules change. As a result, the triclinic structure with $\alpha > 90^\circ$ approaches the monoclinic form with $\alpha \approx 90^\circ$. To summarize, the presence of large water channels, the flexibility of the 2-hydroxypropane link, the disorder of the second sodium ion (Fig. 10) and the disorder of the surrounding water molecules in the crystal lattice explain the reversible and nonstoichiometric water sorption and desorption by cromolyn sodium. This study emphasizes the importance of the detailed single crystal structure in explaining many unusual physico-chemical properties of drug hydrates.

The muscarinic agonist, LY297802 tartarate {i.e., (+)-3-[3-(butylthio)-1,2,5-thiadiazol-4-yl]-1-azabicyclo[2.2.2]octane monohydrogentartrate}, was also found to exhibit an unusual tendency to form nonstoichiometric hydrates of variable, but specific composition, ranging from 0 to 0.5 mol of water [73]. Solid-state ^{13}C -NMR spectroscopy, in conjunction with moisture sorption analysis and X-ray crystallography was used to provide unique insights into nonstoichiometric moisture sorption behavior. The PXRD patterns of the drug exposed to different RH values (0 to 75%), indicated neither a peak shift nor the presence of any new peaks, suggesting that

the anhydrous and the hydrated forms of the drug are isomorphic. Fig. 11 shows the significant changes in the SSNMR peaks on exposure to different relative humidities and temperature, indicating that water incorporated into the crystal lattice changes the local chemical environment and causes the observed NMR changes. The incorporation of water into the crystal lattice of the drug was also confirmed by X-ray crystallography. The considerable hygroscopicity of the drug was rationalized in terms of the similar crystal structures of the hydrated and nonhydrated forms, and hence no significant structural modifications are needed for the reabsorption of water into the solids. The rates of dehydration and rehydration are largely determined by the size of the water channels and the strength of the hydrogen-bonding interactions that bind the water molecules in the channels.

Another interesting study with different solvated forms of L-lysine monohydrochloride (LH) was conducted by Bandyopadhyay et al. [74]. LH was found to form: a pure methanol solvate at water activity, $a_w < 0.34$, with methanol activity, $a_m > 0.7$; a dihydrate at $a_w > \sim 0.65$ with $a_m < 0.45$; and mixed solvates at intermediate values of a_w and a_m . It was found that the dihydrate and the mono-

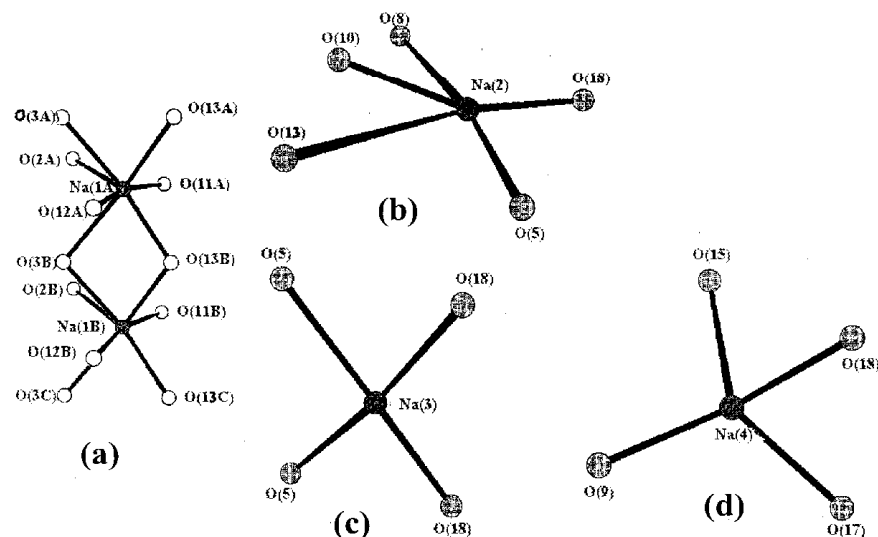


Fig. 10. In hydrated cromolyn sodium, coordination environment of: (a) the first (ordered) sodium ion, Na(1), shown in two neighboring unit cells (A and B); and the second (disordered) sodium ion at the three partially occupied sites, (b) Na(2), (c) Na(3), and (d) Na(4). The striped circles represent the sodium sites. The open circles represent the oxygen atoms coordinated to Na(1). The dotted (gray) circles represent the oxygen atoms coordinated to Na(2), Na(3), or Na(4) ([71], reproduced with the permission of the American Pharmaceutical Association).

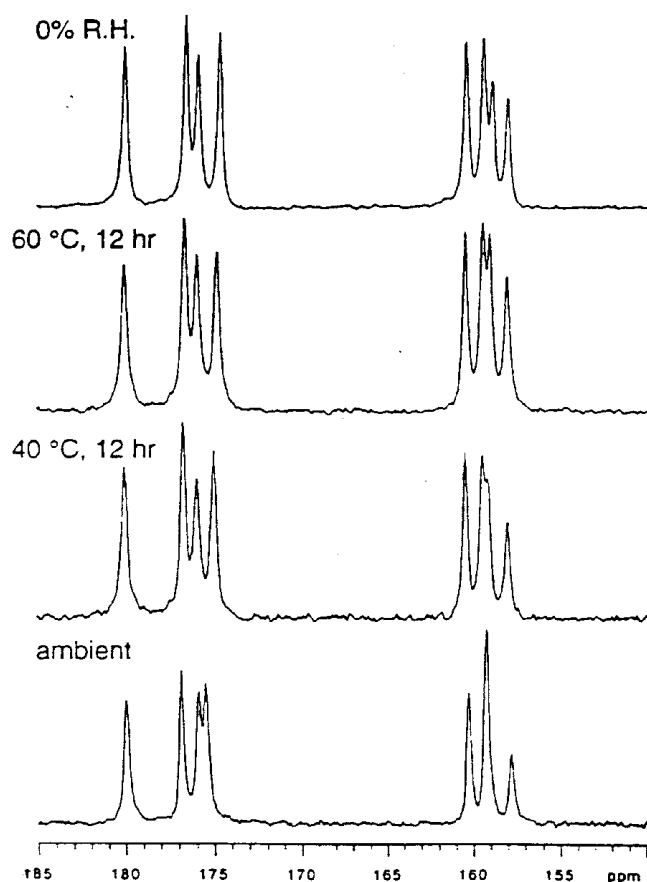


Fig. 11. Solid-state ^{13}C -NMR spectra of LY297802 tartrate $\{(+) \text{-} 3\text{-}[3\text{-}(\text{butylthio})\text{-}1,2,5\text{-thiadiazol-4-yl}]\text{-}1\text{-azabicyclo[2.2.2]octane monohydrogentartrate}\}$ after storage at 0% humidity and before and after drying at elevated temperatures ([73], reproduced with the permission of the American Pharmaceutical Association).

methanol solvated forms of LH possessed similar crystal structures, similar PXRD patterns, but differed in the crystal habit. The crystal structures of LH hydrate and methanol solvate indicate that one molecule of methanol in the methanol solvate occupies approximately the same volume as the two water molecules in the dihydrate. During dehydration in the presence of methanol in the crystallization medium, the loss of one or more water molecules from the crystal lattice was compensated by the gradual uptake of methanol into the crystal structure to satisfy the hydrogen bonding pattern within the lattice with minor rearrangements, giving rise to mixed solvates. The similarities of the crystal lattices of the dihydrate and monomethanol solvate explain the similarity of the PXRD patterns.

3.3. Phase transformation of hydrates and solvates

Phase changes due to hydration/dehydration and solvation/desolvation of pharmaceutical compounds during processing or in the final product may result in an unstable system that would effect the bioavailability of drug from solid dosage forms. Various types of phase changes are possible in solid-state hydrated or solvated systems in response to changes in environmental conditions, such as relative humidity, temperature and pressure. For example, some hydrated compounds may convert to an amorphous phase upon dehydration and some may convert from a lower to a higher state of hydration yielding forms with lower solubility. Alternatively, a kinetically favored but thermodynamically unstable form may be converted during pharmaceutical processing to a more stable and less soluble form [8]. The phase transitions in hydrates and solvates can occur at various stages of dosage form development. Morris [9] has discussed the behavior of hydrates during processing, handling and storage of formulations in detail.

The phase transformations associated with exposure to water, such as during solubility measurements, wet granulation processes, dissolution studies and accelerated stability tests are likely to occur via solution mediation. Solution mediated phase transformations depend upon the solution phase to provide the mobility necessary to rearrange in the most stable form and hence are much faster than solid-state transformations. The rate of a solution-mediated transformation is proportional to the solubility of the species involved. Temperature, pressure and relative humidity may increase the rate of phase transformation of hydrates by inducing mobility in the system.

Solution-mediated phase transformations have been reported for many hydrate systems, such as theophylline crystals [17], eprosartan mesylate [75] and nedocromil sodium [76]. Ghosh and Grant [77] have addressed a common problem associated with the characterization of solvates which centers around the determination of solubilities of solvates and of nonsolvates that undergo phase transformation in the presence of an interacting solvent, such as solvation of nonsolvates in the solvent of crystallization or the desolvation of solvates in water. A thermodynamic cycle analogous to Hess's law but based on free

energies has been developed to predict the theoretical solubilities of 1,2-dialkyl-3-hydroxy-4-pyridones, which form 1:1 formic acid solvates in the presence of formic acid, and of the 1:1 formic acid solvates which produce the corresponding unsolvated compounds in the presence of water. A good correlation was obtained between the solubility values measured by the standard extrapolation method and that calculated by means of the thermodynamic cycle.

Apart from identifying and characterizing the phases during various stages of drug development, it is very important to gain an understanding of the dehydration/hydration mechanisms and kinetics. Many models have been developed to account for the dehydration kinetics of the crystalline hydrates [78]. Nucleation is the most significant phenomenon in determining the transformation kinetics, that is, the rate of formation of a new phase [8]. The dehydration kinetics to some extent will also depend upon the class of the hydrate system to which the drug belongs, particle size and morphology. The practical applications of understanding the dehydration kinetics, as indicated by Morris [9], are mainly the determination of the conditions for allowable exposure of bulk drug substances during development and processing, proper packaging, allowable temperature ranges for shipping, storage, and labeling of the final product, and the initial selection of a form for development.

3.4. Prediction of the formation of hydrates and solvates

Predicting the formation of solvates or hydrates of a compound and the number of molecules of water or solvent incorporated into the crystal lattice of a compound is complex and difficult. Each solid compound responds uniquely to the possible formation of solvates or hydrates and hence generalizations cannot be made for a series of related compounds. Certain molecular shapes and features favor the formation of crystals without solvent; these compounds tend to be stabilized by efficient packing of molecules in the crystal lattice, whereas other crystal forms are more stable in the presence of water and/or solvents. There may be too many possibilities so that no computer programs are currently available

for predicting the crystal structures of hydrates and solvates.

3.5. Characterization of hydrates and solvates

The common methods for the characterization of hydrates and solvates are polarized light microscopy and hot stage microscopy, DSC, TGA, Karl Fischer titrimetry, single-crystal X-ray diffractometry, powder X-ray diffractometry, and infrared spectroscopy. These methods have been reviewed in detail [21] and will also be discussed in detail in later chapters.

Pressure DSC is gaining increasing popularity in the study of solvates and hydrates where dehydration reactions occur above or near the boiling point of water. Using conventional DSC, it is very difficult to measure the heats of dehydration and heat of vaporization separately, but if one conducts DSC experiments at elevated pressures, the two processes may be completely separated. The advantage of using pressure DSC is that the pressure can be precisely controlled and the solids can be subjected to a controlled temperature program while under substantially elevated temperatures. The influence of elevated pressures on the solid-state behavior of carbamazepine dihydrate was studied by Han and Suryanarayanan [79]. In Fig. 12 it is shown that pressure DSC can separate the dehydration and vaporization endotherms of carbamazepine dihydrate during its conversion to the anhydrate form. Also the technique permitted the water liberated on dehydration to remain in intimate contact with the anhydrous phase formed which could significantly influence its solid-state properties.

The combined physical analytical techniques of thermogravimetry and infrared spectroscopy (TG/IR) can permit identification of the solvent incorporated into the crystal lattice. This combined technique has been used to study formulated products, such as capsules and tablets [80].

4. Current challenges and future directions

4.1. Origins of the challenges

A series of flow charts and decision trees have been presented and discussed [11,22] that can be

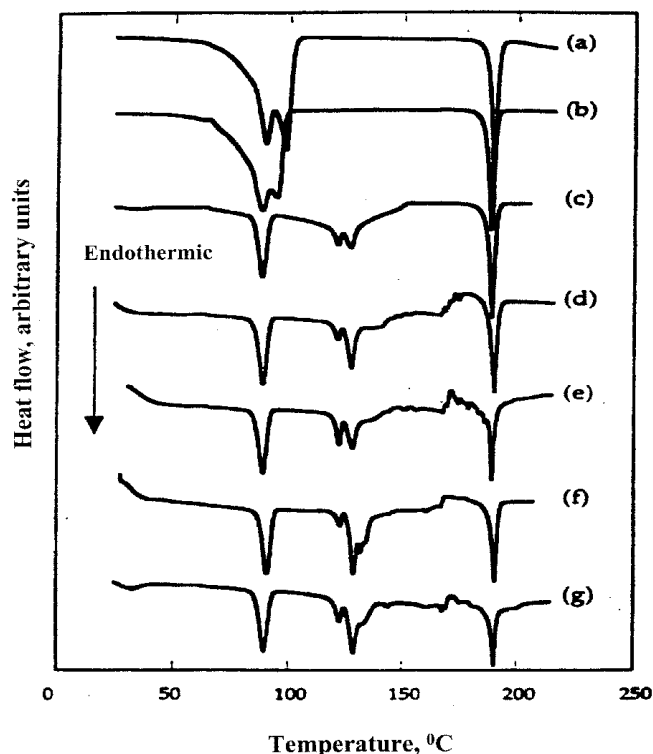


Fig. 12. Differential scanning calorimetry (DSC) curves of carbamazepine dihydrate at different pressures: (a) at atmospheric pressure in a conventional DSC cell, (b) at atmospheric pressure in a pressure DSC (PDSC) cell, (c) 100 p.s.i., (d) 200 p.s.i., (e) 300 p.s.i., (f) 400 p.s.i., and (g) 600 p.s.i. (1 p.s.i. = 6.9 kPa) ([79], reproduced with the permission of Elsevier Science).

used by investigators to characterize the polymorphs and solvates of compounds under development or for registration with regulatory authorities. Due to the complex and nonconventional behavior of various organic drug molecules, there are many opportunities for research and development in the area of characterization of polymorphs and solvates. Some of the problems which are commonly encountered during characterization of crystalline solids and which need to be addressed are: disorder in the crystal lattice due to pharmaceutical processing leading to conversion of a crystalline phase to an amorphous material or phase conversion from one form to the other; quantitating the amount of single polymorph in a mixture of polymorphs; identifying the solid form of the active ingredient in the formulated product, particularly when the drug is a minor component in the presence of numerous other materials (excipi-

ents); and the issues of disappearing polymorphs and the appearance of new polymorphs. In the following sections we will address some of these issues and some of the studies that have addressed these problems.

4.2. Phase transformations during processing

The effects of pharmaceutical processing on the crystalline state of drug polymorphs and solvates have been discussed recently by Brittain and Fiese [16]. Exposure to changes in temperature, pressure, relative humidity and comminution are encountered during processes such as drying, granulation, milling and compression. The stresses applied to crystals during pharmaceutical processing can cause defects in their crystal lattices, and contribute to lattice disorder, thus affecting the physical properties of the resulting powder [81]. This problem has been discussed in detail by Byrn et al. [10]. Arising from different degrees of crystalline disorder, the difficulty in reproducing materials with the same properties is a major concern in the pharmaceutical industry.

Milling, the last processing step in the production of bulk drug substance to reduce particle size, is often accompanied by a decrease in crystallinity due to the creation of lattice defects, beginning at the surface. The defects created by mechanical activation of the solid on the surface can migrate, transform, and change their number and nature. If the defects in the mechanically activated crystal heal to produce a crystal lattice different from the initial lattice, then a polymorphic transformation has taken place. Milling-induced polymorphic changes have been observed for many small drug molecules, such as fostedil, chloramphenicol palmitate, indomethacin and phenylbutazone [16]. Polymorphic transformation of the dipeptide sweetener, aspartame hemihydrate, can occur during milling [82]. Polymorph II of aspartame hemihydrate was found to transform to form I during ball-milling or on heating for 30 min at 160°C in the presence of steam as shown in the X-ray diffraction pattern (Fig. 13). The susceptibility of form II of aspartame hemihydrate to transform to form I has been attributed to the less symmetric crystal structure of form II compared to that of form I as studied by spectroscopic methods.

Some authors, such as Hüttenrauch [81] have

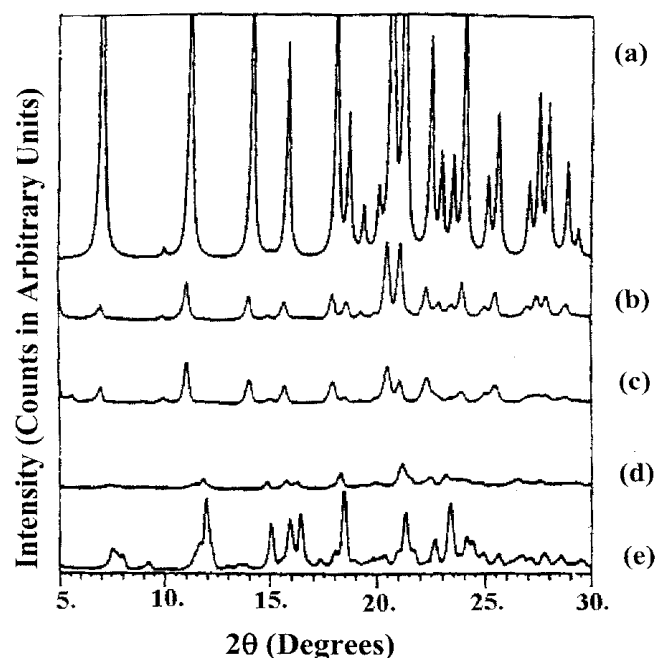


Fig. 13. Powder X-ray diffraction patterns of aspartame hemihydrate: (a) theoretical powder pattern calculated from the crystal structure of aspartame hemihydrate (form I) determined by Hatada et al., (1985) [99], (b) experimental powder pattern of the ball-milled aspartame hemihydrate (form I), (c) experimental powder pattern of aspartame hemihydrate that had been heated for 30 min at 160°C in the presence of steam (form I), (d) experimental powder pattern of aspartame hemihydrate after compression at 250 MPa for 1 min (form II), and (e) aspartame hemihydrate as received (form II) ([82], reproduced with the permission of the American Pharmaceutical Association).

suggested that the trauma to crystals during grinding may lead to a decrease in crystallinity, which should improve the compression capacity and dissolution rate of the drug molecules. This hypothesis was tested by studying the morphology, crystalline state, compression capacity and dissolution properties of native and ground crystals of aspirin and lactose monohydrate [83]. No significant increase in compression capacity was observed when native and ground crystals were compared. Only a slight increase in the dissolution rate was observed for ground aspirin crystals which was attributed to surface defects due to grinding resulting in improved crystal wetting.

The effect of roller compaction on lattice defects and phase change has been examined for aspirin [84]. The water vapor sorption isotherm obtained for aspirin on roller compaction indicated much more

water uptake than had been reported previously for other crystalline samples of aspirin under similar conditions. Various possibilities were suggested for the unusual water uptake of aspirin on roller compaction, such as formation of a polymorphic form of aspirin with a much greater affinity for water, formation of a crystalline hydrate in the aspirin sample, or significant reduction in particle size of the aspirin particles thereby providing an increased specific surface area for water vapor adsorption. It is also possible that roller compaction disrupted the crystalline order of some part of the aspirin crystals forming amorphous regions, which then take up relatively large quantities of water into their bulk structure. This example clearly indicates that processes, such as roller compaction, can introduce considerable disorder in the surface of crystals leading to a marked increase in the tendency to sorb water vapor.

Thermal activation, like mechanical activation during processing, also results in a high-energy state of crystals that may reorganize into a different lattice arrangement resulting in a phase change. The thermal stability of drug substances is important, because formulations are often dried at elevated temperatures after wet granulations so that the tablets may contain small regions of high temperature (hot spots) during compression. Various examples have shown that a change of temperature may influence the stability of drug molecules [16]. The effect of low temperatures, such as during freeze-drying, on the crystalline form of the drug has also been studied. The formation of a new mannitol hydrate during freeze-drying has been reported [85]. The formation of a crystalline hydrate by an excipient during freeze-drying may have several practical consequences, such that the difficulty of removing bound water from the crystal lattice can significantly limit the drying rate, while the residual water that is not removed by freeze-drying may be a potential threat to product stability if it is released during storage. The mannitol hydrate formed during freeze-drying survived the typical drying cycle and converted to the anhydrous polymorph of mannitol upon heating.

Spray drying has also been shown to lead to loss of crystallinity in materials, by a combination of processes involving rapid solidification of dissolved material and solid-state transitions due to milling effects in the atomiser. Spray drying leads to conver-

sion of a crystalline phase to an amorphous state and, because the amorphous state is metastable with respect to the crystalline form, phase transformations are likely to occur within the shelf life of the pharmaceutical product, resulting in loss of quality and potency in the product [86].

In view of the significant effects that the state of disorder in crystalline solids caused by pharmaceutical processing can have on the properties of pharmaceutical solids, it is important to be able to assess the extent of disorder in a solid quantitatively down to very low levels. Various methods have been used to measure the percent disorder, such as using predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization which revealed limits of detectability down to about 10%. Using water vapor sorption measurements under very carefully controlled conditions, it was possible to detect disorder as low as 1% in milled samples of sucrose [87]. A comparison of the four methods mentioned above for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous content were recognized and taken into account.

4.3. Degree of crystallinity

The previous section has emphasized that many pharmaceutical processes lead to a decrease in crystallinity of drug phases. Various studies have concluded that the formation of amorphous material during processing is highly undesirable. The amorphous material, being in a thermodynamically metastable state, is susceptible to reconversion to the crystalline state, affecting many physico-chemical characteristics of the drug. A later chapter provides detailed coverage of amorphous materials. An estimation of the degree of crystallinity of a sample before and after processing poses one of the larger challenges facing the pharmaceutical field. Powder X-ray diffractometry is still the commonly used method for determining the degree of crystallinity, though this method suffers from some limitations due to peak broadening, amorphous halo, and preferred orientation which make interpretation and quantitation difficult. DSC may not be a sensitive method for measuring crystallinity due to crystalliza-

tion of the amorphous content at elevated temperatures and the effects of differences in heat capacity. Solution calorimetry has been proposed as an accurate method for analysis of percent crystallinity [11,88,89]. A decrease in the endothermic enthalpy of solution indicates a decrease in the crystallinity of the sample. However, differences in surface area produced by grinding or by other processing techniques can also result in changes in the heat of wetting of a sample. Judicious choice of solvent can be employed to reduce such surface effects, which themselves contribute to the observed crystallinity of the sample.

Near infrared (NIR) spectroscopy is another technique being used to measure the degree of crystallinity, and has also proved useful in studies of the polymorphism and water content of sugars. The NIR spectrum of a sample contains both physical and chemical information. Being noninvasive, nondestructive and operable at room temperature, the method is a valuable tool with which to assess changes in the amorphous and crystalline state of lactose [90]. NIR has been used to follow the changes in the amorphous state, the onset of crystallization, and the changes between α - and β -lactose, which accompany the onset of crystallization. In another study, the nucleation and crystallization kinetics of amorphous lactose was investigated by gravimetry in an automated vacuum moisture balance. The combination of isothermal and nonisothermal activation energies allowed the investigation of both crystal growth and nucleation mechanisms and led to the separation of activation energies for nucleation and growth [91].

4.4. Characterization of mixtures of polymorphs

Another common problem encountered during drug development is quantitative control of the proportion of polymorphic forms present in a mixture. According to the US FDA regulations, the method of analysis for the proportion of forms must be validated, and also the proportion of forms must remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product. This is a very onerous requirement, especially if the forms have a tendency to interconvert. Byrn et al. [11] suggested that the best way to deal with this problem is probably by

developing methods to prepare only one crystal form and maintaining this form throughout processing. Powder X-ray diffractometry is often a useful method to determine the percentages of polymorphs in a mixture. However, the detection limit is variable from case to case, and is sometimes as high as 15%. It is therefore important to develop sensitive analytical methods with a lower limit of detection.

Attenuated total reflectance (ATR) FTIR spectroscopy has been shown to be valuable for the quantitative analysis of the polymorphic content of bulk pharmaceutical materials. The feasibility of using ATR-FTIR for the qualitative and quantitative analysis of mixtures of pharmaceutical polymorphs has been studied using three known polymorphs of ganciclovir as a model compound [92]. Definitive identification and quantitation of all three polymorphs could be achieved using ATR-FTIR spectroscopy in conjunction with partial least-squares modeling. This technique has many advantages, such as speed, nondestructiveness, relative ease of use, and most important, no sample pretreatment before measurement.

Raman spectroscopy is another technique that is being widely used to quantitatively estimate the percentage of one polymorph in a mixture of polymorphs. FT-Raman spectrometry offers many advantages, the most prominent being, minimal sample preparation, sensitivity to polymorphism, and noninterference from water. Two polymorphs of fluconazole were characterized using FT-Raman spectroscopy and principal components regression using cross validation provided quantitative analysis of the percentage of one polymorphic form in the mixture of other forms [93]. A novel sample holder was developed whereby the sample is held in an NMR tube which is rotated around its axis and at the same time moved up and down. This method of sample presentation leads to a large increase in the volume studied and is important for inhomogeneous samples for which sub-sampling is a problem. Possible degradation of the sample through heating by the laser can also be avoided [94].

X-Ray powder diffractometry is still the common method for the quantitative estimation of polymorphs in a mixture of polymorphs. This method requires that at least one high-intensity peak unique to each form is available for intensity measurements and that

the plot of the peak intensity ratio as a function of the weight ratio of the components should result in a straight line. Modern computer controlled X-ray powder diffractometers now permit quantitative analysis of multicomponent mixtures using the complete powder diffraction profile rather than a limited amount of low-angle integrated intensity data. Artificial neural networks (ANNs) in quantitative X-ray powder diffractometry were used successfully to identify and quantify the two known modifications of ranitidine hydrochloride even when the weight fraction of one polymorph in the mixture was as low as 0.01 [95]. ANNs have been used mostly in problems of pattern recognition and modeling, and is therefore useful in deciphering the pattern in diffraction data from polymorphic mixtures. The ANNs model predicted concentration precisely, accurately, and with minimal bias through a wide range of ratios of the two known ranitidine hydrochloride polymorphic forms in a mixture (Fig. 14). This method minimizes the problems associated with preferred orientation and overlapping X-ray lines. The same group of researchers has shown the potential of ANNs in combination with DRIFT spectroscopy to analyze the polymorphic purity of crystalline ranitidine hydrochloride as a bulk drug and as an active ingredient of

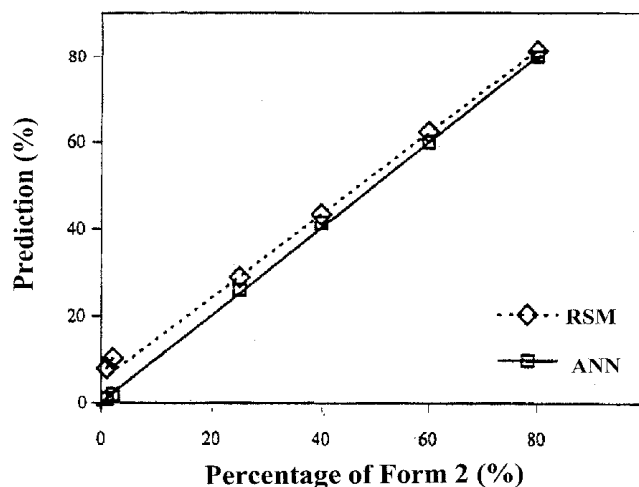


Fig. 14. Predicted concentrations of the two polymorphic forms (form 1 and 2) of ranitidine hydrochloride by the response surface methodology (RSM), a statistical modeling method, and by the artificial neural networks (ANNs) method, plotted against measured percentage of form 2 ([95], reproduced with the permission of Elsevier Science).

a tablet formulation. Simultaneous identification and quantification of all the ingredients in the tablet formulation was possible. This study has shown that the complex problem of quantifying a drug in mixtures containing two or more components with overlapping spectra can be solved by the DRIFT-ANNs technique [96].

Another technique, which is gaining popularity in the quantitative analysis of mixtures of polymorphs, is solid-state ^{13}C -CP-MAS NMR spectroscopy. This method was preferred for quantitative analysis of polymorphic mixtures of the herbicide, pendimethalin, which exists as two polymorphs with different colors and crystal habits [27]. ^{13}C -NMR provided the most sensitive and definitive evidence of the transition from the yellow to the orange form. This method enabled as little as 2% of orange pendimethalin to be determined in a sample consisting mostly of the yellow polymorph. It is the least invasive of the instrumental methods and can be used to detect the ratio of the two polymorphs in solid formulations.

A related challenge faced by the pharmaceutical industry is the determination of the polymorphic nature of the drug in the presence of excipients in a dosage form especially when the active drug is present as a low percentage of the overall formulation [97]. This problem can be addressed by developing sensitive techniques with lower limits of detection or by using a combination of techniques.

5. Conclusions

In order to save time and cost it is very important to choose the most suitable form of the crystalline drug in the initial stages of drug development. In recent years a good deal of research has been directed towards achieving this goal. Systematic isolation and early characterization of the largest number of possible forms of a drug reduces the chances of surprises at the late production stage due to identification of a new crystalline form or phase change. With the development of more sophisticated computational tools, the main focus of many investigators is to be able to predict all the possible forms of a drug from its molecular structure. Understanding the origins of the multiple solid forms of a drug

molecule, either due to differences in packing arrangement or conformation of the molecules, becomes the first step in prediction. Single crystal X-ray diffractometry and solid-state NMR spectroscopy are two techniques that are gaining increased application in determining the various crystal structures and the origins of polymorphism and pseudo-polymorphism of a particular drug. When crystal structures can be calculated with certainty, it will be possible to predict the various polymorphs of a compound and this information could be used to guide experimental studies. This goal may be difficult to achieve owing to the complex molecular structures of new organic molecules and the presence of several molecules in each asymmetric unit, but the future development of improved force fields and increased computational speeds, may make it achievable.

Improved experimental methods leading to more accurate and detailed phase diagrams are also finding increased use in determining the stability of various polymorphs. It is important to make every effort to prepare and to identify the most stable polymorph in order to guide the selection of the optimal form for development. The emergence of sensitive methods and the use of combination techniques, facilitate the identification and the more accurate characterization of the various polymorphs of a drug molecule. One of the main analytical challenges faced by the pharmaceutical analyst is the development of better quantitative methods for identifying a single polymorph in a mixture of polymorphs and for determining the percentages of amorphous or crystalline content of the drug. More and more sensitive methods are being developed to address this problem.

An increased understanding of the phenomenon of polymorphism should enable pharmaceutical scientists to gain control over the crystallization process in order to selectively obtain the desired polymorph or suppress the growth of an undesired one. Phase changes during processing and scale-up are a problem, which may be avoided by carefully designed initial small-scale studies. The availability of detailed structural data, combined with strategic design of substrates and additives, has led to significant advances in the control over the polymorphs obtained in a particular crystallization [98]. With all the information available from these initial studies, it

should be possible to design and to select processing conditions which would give a desired polymorph and maintain the desired form throughout the various stages of drug processing and manufacture.

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VEGF Receptor Signaling in Tumor Angiogenesis

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Key Words. Angiogenesis · Vascular endothelial growth factor (VEGF) · Tyrosine kinase receptors · Tyrosine kinase inhibitors

ABSTRACT

The growth of human tumors and development of metastases depend on the de novo formation of blood vessels. The formation of new blood vessels is tightly regulated by specific growth factors that target receptor tyrosine kinases (RTKs). Vascular endothelial growth factor (VEGF) and the Flk-1/KDR RTK have been implicated as the key endothelial cell-specific factor signaling pathway required for pathological angiogenesis, including tumor neovascularization. Inhibition of the VEGF

tyrosine kinase signaling pathway blocks new blood vessel formation in growing tumors, leading to stasis or regression of tumor growth. Advances in understanding the biology of angiogenesis have led to the development of several therapeutic modalities for the inhibition of the VEGF tyrosine kinase signaling pathway. A number of these modalities are under investigation in clinical studies to evaluate their potential to treat human cancers. *The Oncologist* 2000;5(suppl 1):3-10

INTRODUCTION

Angiogenesis, the process by which capillaries sprout from preexisting blood vessels, is tightly regulated by a large number of proangiogenic and antiangiogenic factors. Tumor cells have an absolute requirement for a persistent supply of new blood vessels to nourish their growth and to facilitate metastasis. Thus, tumor vascularization is a vital process for the progression of a neoplasm from a small localized tumor to an enlarging tumor with the ability to metastasize [1, 2]. The angiogenic cascade leading to tumor vascularization can be divided into two general phases, the prevascular phase (referred to as the "angiogenic switch") and the vascular phase [3, 4]. Once tumor cells undergo the transformation to an angiogenic phenotype, these malignant cells are capable of inducing phenotypic changes in endothelial cells as well as in other cell types [5, 6]. At that point, avascular tumors can acquire their own blood supply, which permits a rapid rate of growth. While tumors lacking adequate vasculature become necrotic [7] or apoptotic [8], tumors that have undergone neovascularization may not only enter a phase of rapid growth but may also have increased metastatic potential.

ANGIOGENIC FACTORS

A large number of proangiogenic factors and their cognate receptors have been identified, including basic fibroblast

growth factor [9], platelet-derived growth factor (PDGF) [10], platelet-derived endothelial cell growth factor [11], fibroblast growth factor [12], angiopoietin-1 [13], transforming growth factor beta-1 (TGF- β 1) [14], transforming growth factor alpha (TGF- α), and epidermal growth factor (EGF) [15]. Perhaps the best characterized of the proangiogenic factors is vascular endothelial growth factor ([VEGF] also known as vascular permeability factor), which is relatively unique among growth factors in terms of its specificity for the vascular endothelium [16-18]. The VEGF family currently includes six known members: VEGF, placenta growth factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E [19-21]. These are secreted as dimeric glycoproteins, all of which contain the characteristic regularly spaced eight-cysteine residues referred to as the "cysteine knot" motif [20, 22, 23]. These glycoproteins belong to a structural superfamily of growth factors that also includes PDGF-BB and TGF- β 2 [24].

VEGF, the most potent direct-acting angiogenic protein known [25, 26], is a diffusible endothelial cell-specific mitogen and angiogenic factor that also increases vascular permeability. It elicits a pronounced angiogenic response in a variety of in vivo models [27-31]. Endothelial cell survival in newly formed vessels is VEGF-dependent [32]. VEGF overproduction has been identified as a major factor underlying pathological angiogenesis in vivo in conditions such as psoriasis, macular degeneration, and tumor proliferation

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[33]. Malignant transformation of cultured cells often results in an induction of VEGF expression. For example, *Kieser et al.* [34] reported that mutant murine *p53* tumor-suppressor gene induced expression of VEGF mRNA in transient transfection assays. Oncogenic forms of the tumor-suppressor genes, Ras and Raf, have also been shown to upregulate VEGF expression [35]. Recently, constitutive expression of mRNA and proteins for VEGF and its cognate receptors was observed in most primary and metastatic melanoma cell lines and in SV40T-transformed melanocytes [36]. Neonatal melanocytes did not express VEGF or VEGF receptors, and VEGF expression could not be induced by exogenous growth factors [36].

ROLE OF VEGF IN ANGIOGENESIS

Hypoxia appears to be an important stimulus for VEGF production in both malignant and normal cells [37, 38]. The induction of VEGF gene expression by hypoxia in tumor cells involves both an increase in the rate of gene transcription, mediated by the transcription factor hypoxia-inducible factor-1 [39], and an enhancement of the stability of VEGF mRNA [40]. This mechanism is discussed in more detail in a subsequent chapter on the role of VEGF in von Hippel-Lindau Syndrome by *Adrian Harris*. In addition to its effect in tumors, hypoxia-induced VEGF is capable of stimulating angiogenesis in a number of other sites, including endothelial cells [41], retinal pericytes [42], and the myocardium [43-46].

Transcription of VEGF mRNA is also induced by a variety of growth factors and cytokines, including PDGF, EGF, tumor necrosis factor alpha, TGF- β 1, and interleukin 1-beta [19, 25]. In addition to its role in the paracrine stimulation of angiogenesis, VEGF may also have an autocrine stimulatory effect on tumor cells [47]. These autocrine and paracrine effects are summarized in Figure 1. The initial event of hypoxia-mediated transcription and factor secretion by the

growing tumor and the stromal tissue leads to an upregulation and activation of growth factor receptors. This results in endothelial sprouting, increased vascular permeability, the expression of tissue matrix metalloproteinases (MMPs), and eventually the digestion of matrix, which is required for the endothelial cell to move. The increased endothelial cell mitogenesis and spread and activation of other factors lead to the formation and movement of endothelial cells, including other supporting cells like pericytes, and eventually lead to vessel extension, increased capillary integrity, differentiation of microvessel support cells, and formation of the vascular network. VEGF plays a role in the earliest events in this process. Recent evidence suggests that VEGF may not only play a role in inducing angiogenesis but also is important in promoting the survival of new vessels formed in tumors. *Benjamin et al.* [48] demonstrated that downregulating VEGF transgene expression using a tetracycline-regulated expression system results in the selective obliteration of immature blood vessels that have not yet recruited periendothelial cells in a human glioma xenograft model. Similar results were observed when the constitutive production of VEGF by the glandular epithelium was suppressed as a consequence of androgen-ablation therapy in human prostate cancer. These results underscore the pivotal role of VEGF in the stimulation and maintenance of newly formed vessels in tumors.

VEGF AND FLK-1/KDR SIGNALING PATHWAY

Three high-affinity cognate endothelial receptors for VEGF have been identified: VEGFR-1/Flt-1, VEGFR-2/Flk-1/KDR, and VEGFR-3/Flt-4. These receptors function as signaling molecules during vascular development [49]. VEGFR-1 and VEGFR-2 are cell surface receptor tyrosine kinases (RTKs), which are localized on endothelial cells during embryogenic development. The coordinated patterns of expression of the genes for VEGF and its recep-

tors suggest that these proteins participate in vascular development during embryogenesis [50, 51]. As shown in Figure 2, VEGF RTKs are single-pass transmembrane receptors that possess intrinsic cytoplasmic enzymatic activity, catalyzing the transfer of the gamma-phosphate of ATP to tyrosine residues in protein substrates

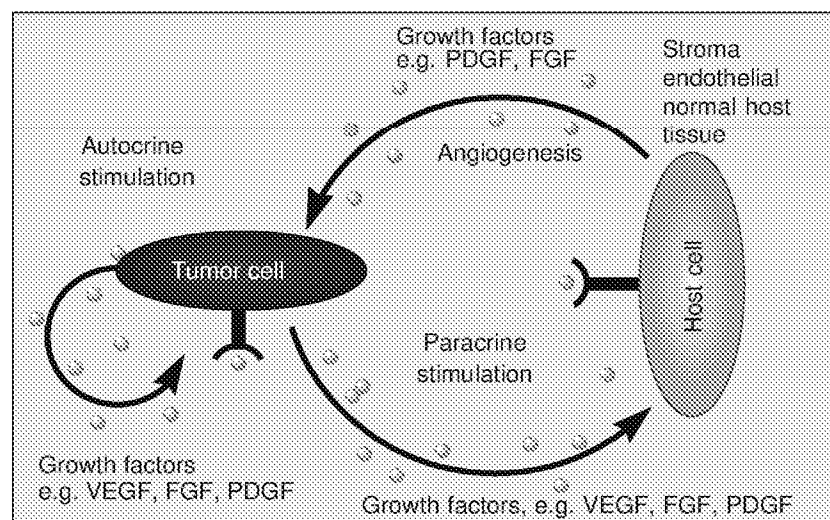


Figure 1. Paracrine and autocrine stimulation by angiogenic growth factors. VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor.

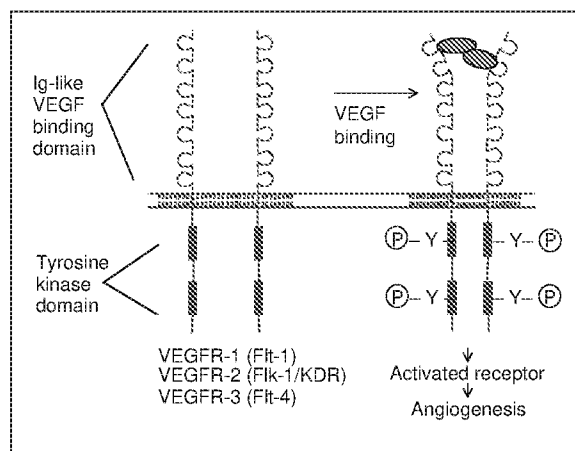


Figure 2. Representative structure of vascular endothelial growth factor (VEGF) tyrosine kinase receptors. The VEGF receptor family is represented by seven immunoglobulin-like loops in the extracellular domain, which binds VEGF. Two VEGF receptors form a dimer to activate autophosphorylation of tyrosine residues on the cytoplasmic domain. Ig = immunoglobulin; VEGF = vascular endothelial growth factor; Y- (P) = phosphorylated tyrosine residues.

[52]. VEGF RTKs, members of a large family of RTKs, are essential components of signal transduction pathways that affect cell proliferation, differentiation, migration, and metabolism. Activation of VEGF RTKs occurs through ligand binding, which facilitates receptor dimerization and autophosphorylation of tyrosine residues in the cytoplasmic portion. The phosphotyrosine residues either enhance receptor catalytic activity or provide docking sites for downstream signaling proteins [52, 53].

VEGFR-2 is exclusively expressed in endothelial cells and appears to play a pivotal role in endothelial cell differentiation and vasculogenesis [54, 55]. Many studies using molecular techniques have provided evidence for the role of VEGFR-2 in tumor vascularization, growth, and metastasis. For example, the manipulation of the cloned receptor to create a "dominant negative" mutation is one experimental technique that helps establish the relevance of Flk-1 to tumor

angiogenesis. Millauer *et al.* [56] used a retrovirus encoding a dominant-negative mutant of the VEGF-2 receptor to prevent the growth of a transplanted glioblastoma tumor, demonstrating the biological relevance of the VEGF-2/Flk-1/KDR receptor/ligand system for tumor-associated angiogenesis *in vivo*. Recently, Bernatchez [57] used antisense oligomers directed against Flk-1 and Flt-1 to show that VEGF stimulation of endothelial cell proliferation, migration, and platelet-activating factor synthesis is Flk-1 dependent, whereas inhibition of Flt-1 expression failed to affect VEGF ability to modulate these activities. These studies have validated targeting of the VEGFR-2 signaling pathway for the development of antiangiogenic agents.

THERAPEUTIC STRATEGIES FOR INHIBITING VEGF PATHWAY

VEGF and its receptors have been implicated in the angiogenesis that occurs in many solid tumors including breast cancer [58], colon cancer [59], hepatoma [60], bladder cancer [61], gastric cancer [62], and prostate cancer [63]. Since formation of solid tumors is angiogenesis dependent, several strategies have been developed for targeting the VEGF pathway as part of anticancer therapy (Table 1) [59, 64-75]. Potential approaches for blocking VEGF action include inhibiting secretion of endogenous tumor VEGF, neutralizing VEGF in the microcirculation, and preventing VEGF binding and subsequent signal transduction. A number of these strategies for inhibiting tumor angiogenesis by selectively targeting the VEGF signaling pathway are currently being tested in early phase I/II clinical trials.

Oligonucleotides

Secretion of VEGF can be inhibited by antisense oligonucleotides or expression constructs specific for VEGF, which have been successfully used in models of thyroid carcinoma [76], glioma [77], and melanoma [78]. Ribozymes are RNA molecules that possess the dual properties of RNA sequence-specific recognition and site-specific cleavage of other RNA molecules. Ribozymes that target the VEGF receptor mRNAs

Table 1. Therapeutic agents that have been developed to specifically target the VEGF pathway

Agent	Target	Reference
Angiozyme™ (Ribozyme Pharmaceuticals; Boulder, CO)	VEGFR	[64]
rhumAb-anti-VEGF Antibody (Genentech; South San Francisco, CA)	VEGF	[65-67]
Soluble Flt-1 (Genentech)	VEGF	[68]
Anti-Flk Antibody (Imclone; Somerville, NJ)	VEGFR-2	[69]
SU5416 (SUGEN; South San Francisco, CA)	VEGFR	[59, 70, 71]
ZD4190 (AstraZeneca; Macclesfield, UK)	VEGFR	[72-74]
PTK787/ZK222584/CGP 41251 (Novartis AG; Basel, Switzerland)	VEGFR	[75]

were developed, and their biological activities in cell culture and in an animal model were assessed [64]. Ribozymes targeting Flt-1 or KDR mRNA sites reduced VEGF-induced proliferation of cultured human vascular endothelial cells and specifically lowered the level of Flt-1 or KDR mRNA present in the cells. Anti-Flt-1 and KDR ribozymes also exhibited antiangiogenic activity in a rat corneal pocket assay of VEGF-induced angiogenesis [64].

Anti-VEGF Monoclonal Antibodies

Several anti-VEGF monoclonal antibodies have been developed and have demonstrated efficacy in a wide variety of human tumors in xenograft models [25, 65-67, 79-81]. Initial studies by Kim *et al.* [65] demonstrated treatment with a murine monoclonal antibody specific for VEGF (mumAb VEGF A.4.6.1) potently suppressed angiogenesis and growth in a variety of human tumor cell lines transplanted in nude mice. Subsequently, a recombinant humanized mAb VEGF version of this antibody, rhumAb VEGF, was developed for clinical evaluation in the treatment of solid tumors and other disorders [82]. A phase I clinical trial of rhumAb VEGF in 25 cancer patients showed that multiple doses of anti-VEGF were safe and well tolerated [83]. In a phase II trial of rhumAb VEGF in 15 patients with hormone refractory prostate cancer, administration of 10 mg/kg of rhumAb VEGF intravenously every two weeks resulted in three possible mixed responses [84].

Similarly, Prewett *et al.* [69] demonstrated that treatment with an anti-Flk-1 mAb significantly suppressed the growth of primary murine Lewis lung, 4T1 mammary, and B16 melanoma tumors and the growth of Lewis lung metastases. This antibody also completely inhibited the growth of established epidermoid, glioblastoma, pancreatic, and renal human tumor xenografts. Histological examination of anti-Flk-1 mAb-treated tumors showed evidence of decreased microvessel density, tumor cell apoptosis, decreased tumor cell proliferation, and extensive tumor necrosis. These findings support the conclusion that anti-Flk-1 mAb treatment inhibits tumor growth by suppressing tumor-induced neovascularization and demonstrate the potential for therapeutic application of anti-VEGF receptor antibody in the treatment of angiogenesis-dependent tumors [69]. Based on these results, the anti-Flk-1 antibody has been introduced into the clinic.

Soluble VEGF Receptor

An alternative approach would be to use a soluble recombinant Flt-1 receptor to inhibit the VEGF signaling pathway. Using this approach, a soluble full-length recombinant Flt-1 could be used to bind to circulating VEGF [68]. Another approach would be to produce tumor cells

transfected with cDNA encoding the native soluble Flt-1 (sFlt-1) truncated VEGF receptor, which can function by sequestering VEGF or by forming inactive heterodimers with membrane-spanning VEGF receptors in a dominant negative fashion [85]. Both of these techniques have demonstrated preclinical efficacy. Although VEGF has been successfully neutralized using these strategies in preclinical models, their potential application may be limited by the significant amount of protein that would be needed to treat humans. Therefore, preventing RTK signal transduction may be a more appropriate means of tumor control in humans.

Small Molecule Inhibitors

The 3-substituted indolinone compound, SU5416, is a specific and potent catalytic inhibitor of VEGFR protein kinases [86] (Fig. 3). It inactivates Flk-1/KDR by binding in the adenine-binding pocket (Fig. 4). It is a specific VEGFR inhibitor that has virtually no inhibitory activity against serine threonine protein kinases and tyrosine kinases, such as Src, FGF receptor, Met, and Abl and has little activity against PDGF receptor [70]. At present, SU5416 is the most clinically advanced VEGF RTK-selective tyrosine kinase inhibitor being developed for antiangiogenic treatment of cancer [87]. As would be expected by its mechanism of action, SU5416 inhibits tumor growth in vivo in a dose-dependent manner, whereas it has no effect on tumor cells in vitro [70]. SU5416 has shown activity in a large number of tumor xenografts in nude mice including melanoma, glioma, fibrosarcoma, and lung, epidermoid, mammary, and prostate carcinomas [70] as well as in neurogenic sarcoma xenografts [71]. Recently, Shaheen *et al.* [59] evaluated the effect of SU5416 on tumor angiogenesis and metastasis in a human colon cancer xenograft model. In this study, SU5416 inhibited tumor metastases, microvessel formation, and cell

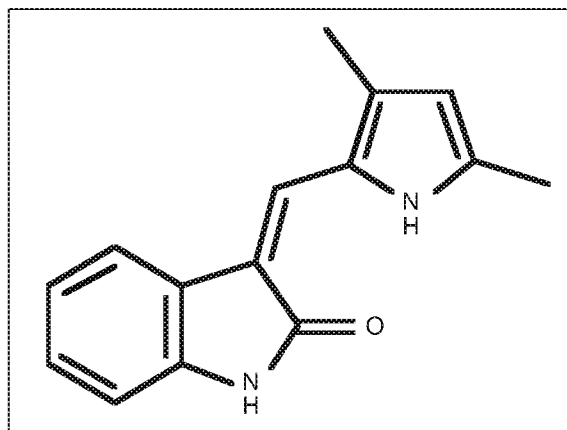


Figure 3. Structural formula of SU5416. The chemical name of the agent is 3-[(2,4-dimethylpyrrol-5-yl) methylidene]-indoline-2-one.

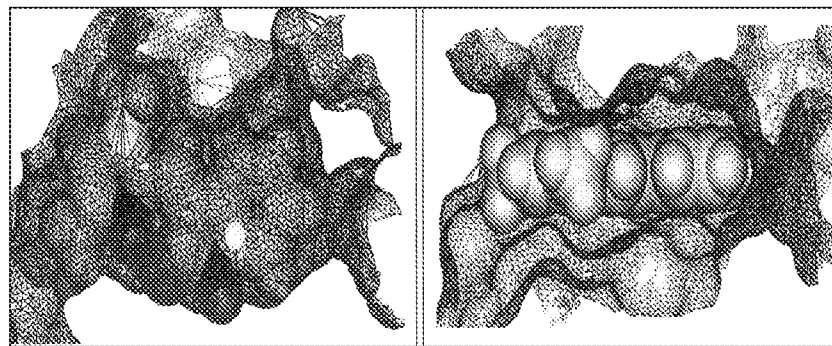


Figure 4. Binding site of SU5416 in the adenine-binding pocket of VEGFR. SU5416 is shown modeled in the adenosine triphosphate binding site of the Flk-1/KDR intracellular catalytic core. (Two views are shown.)

proliferation [59]. These findings indicate that targeting the VEGF receptor/ligand system with SU5416 decreases tumor vascularity and vessel density and increases tumor cell apoptosis and is a rational approach to inhibiting tumor growth. In a phase I clinical trial with SU5416 after enrollment of 69 patients, the drug was well tolerated at dose levels of 4.4-145 mg/m²/day, and stable disease was seen in patients with Kaposi's sarcoma and in patients with non-small cell lung, colorectal, and basal cell cancers [88, 89].

Another substituted indolin-2-one inhibitor of RTKs, SU6668, inhibits the signaling of the VEGF receptor (Flk-1/KDR) and also targets the PDGF and fibroblast growth factor receptors [86, 90, 91]. This novel drug is believed to inhibit tumor growth by preventing angiogenesis and by its direct effects on the tumor cells and the surrounding stromal cells, which support tumor cell growth. SU6668 has recently entered phase I human trials for the treatment of solid tumors.

Several other VEGF RTK inhibitors have shown preclinical efficacy. AstraZeneca Pharmaceuticals (Macclesfield, UK) have identified a series of substituted 4-anilinoquinazolines that are potent inhibitors of VEGFR-1 and VEGFR-2. Of these, ZD4190 is an orally active inhibitor of Flt-1 and Flk-1/KDR that prevented VEGF-mediated proliferation of endothelial cells in vitro [72]. Following chronic oral administration, this compound significantly inhibited the growth of various human tumor xenografts in vivo including breast, colon, lung, ovarian, and prostate carcinomas [73]. ZD4190 has also been shown to reduce significantly vascular endothelial permeability in experimental models [74]. Two

additional oral inhibitors of VEGF RTK activity, ZK222584 and CGP 41251, are under development by Novartis Pharma (Basel, Switzerland). Originally identified as an inhibitor of protein kinase C, CGP 41251 has been shown to inhibit the ligand-induced autophosphorylation of VEGF-R2/Flk-1/KDR without affecting the activity of other RTKs such as VEGF-1/Flt-1 and FGF [92]. CGP 41251 has been shown to have a broad antiproliferative effect in vitro and inhibits the angiogenic response to VEGF in vivo [92]. Similarly, ZK222584 has been shown to inhibit angiogenesis and growth of human ovarian carcinomas in vivo in a dose-dependent manner [75]. In mice, this compound was associated with increased survival time, decreased tumor weight, and decreased ascites volume when administered orally [75].

CONCLUSION

The future of RTK inhibitors as therapeutic agents will be clearer over the next several years as results of the current clinical trials become available and as the factors regulating angiogenesis and the interactions between these factors are more fully elucidated. Drug resistance is a major problem with chemotherapy agents because many tumors are genetically unstable. Since cytotoxic and antiangiogenic therapies are aimed at different cellular targets, clinical strategies to combine antiangiogenic agents with cytotoxic therapy are being devised. It is hoped that combining these agents will be more effective in controlling tumor growth; cytotoxic agents will reduce the tumor burden of already vascularized tumors, while antiangiogenic agents will prevent neovascularization and growth of small and occult metastatic foci as well as the formation of new metastatic lesions.

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INTRODUCTION

Translational Research: The Role of VEGF in Tumor Angiogenesis

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Angiogenesis is defined as the formation of new blood vessels from preexisting vasculature. Preclinical studies have shown the major role of angiogenesis in tumor growth and metastasis formation, and therefore, inhibiting tumor angiogenesis may be a promising therapeutic modality [1, 2]. Paracrine stimuli derived from tumor cells are the main promoters of angiogenesis. Once activated by these stimuli, endothelial cells begin to proliferate and migrate, subsequently resulting in new tube formation and blood flow. This complex process involves numerous biological activities. Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors of tumor-induced angiogenesis [3, 4]. Originally identified for its ability to induce vascular permeability and stimulate endothelial cell growth, VEGF is now recognized as a key factor required for growth of tumors [5]. The clinical importance of VEGF for tumor growth is supported by the fact that most tumors produce VEGF and that inhibition of VEGF-induced angiogenesis significantly inhibits tumor growth in vivo [6-8]. In addition to decreasing the number of vessels, antiangiogenic drugs may also decrease the interstitial tumor pressure. This reduction in interstitial pressure may result from the ability of these agents to block VEGF-induced permeability. VEGF expression has been shown to correlate with microvessel density in a number of solid malignancies including carcinomas of the breast, and tissue concentrations of this growth factor appear to be predictive of mortality associated with breast cancer [9, 10]. Similar results have been obtained in studies of solid malignancies in various organs including the lung, prostate, and colon [11-13]. These preclinical and clinical findings support VEGF as a promising target for anticancer therapy.

In patients with cancer, antiangiogenic agents, used alone or combined with chemotherapy, may inhibit tumor growth, reduce metastasis formation, prolong survival, and improve quality of life. Clinicians need to make a major shift in their thinking if the beneficial effects of these novel agents are to be recognized. Appropriate schedules of such combinations need to be developed so that the mechanism of action (i.e., antiangiogenic or cytotoxic) is fully considered. The concern that concomitant administration of antiangiogenic drugs and chemotherapy will impair cytotoxic drug delivery is precluded by evidence from animal studies. *Teicher et al.* [14-16] clearly demonstrated that combination therapy with angiogenesis inhibitors has synergistic antitumor effects in the Lewis lung carcinoma xenograft model. In these studies, addition of the antiangiogenic agents to the cytotoxic therapies reduced not only the number of lung metastases formed from the primary tumor but also the number of large metastases. These results provide evidence that antiangiogenic therapies can potentiate the efficacy of standard anticancer therapies.

This special issue of *The Oncologist* presents a diversity of preclinical and clinical studies about anti-VEGF strategies for cancer treatment. *McMahon* gives an excellent overview of the role of VEGF tyrosine kinase receptors in tumor angiogenesis and the strategies being developed to inhibit this signaling pathway. Subsequently, *Ellis* presents preclinical data that show the role of VEGF in the growth and metastasis of colon cancer. He also presents evidence that antiangiogenic therapy with VEGF receptor inhibitors SU5416 and SU6668 inhibits the growth of liver metastases. The study by *Vajkoczy* and coworkers strongly supports the idea that regional differences in Flk-1 activity in vivo may significantly affect tumor

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susceptibility to compounds that target the VEGF-Flk-1/KDR. Their study evaluated regional VEGF-Flk-1/KDR activities in vivo using SU5416 as measured by intravital fluorescence videomicroscopy. *Rosen* presents an overview of clinical studies of antiangiogenic therapy. *Arastéh* and *Hannah* review evidence that anti-VEGF treatment might also benefit patients with AIDS-related Kaposi's sarcoma. This is followed by an overview by *Harris* on the role of the von Hippel-Lindau (VHL) tumor-suppressor gene in regulating VEGF expression

and the rationale for the use of SU5416 treatment in VHL patients. *Gasparini* reviews the importance of VEGF tissue levels in treatment planning for breast cancer. Another promising application for anti-VEGF treatment is presented by *Verheul* and coworkers, who propose SU5416 as a treatment for ascites and pleural effusion formation in patients with advanced-stage cancer. Finally, *Carter* addresses the challenging question of the selection of strategies for clinical development of angiogenesis inhibitors.

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