(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 September 2021 (30.09.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/195279 A3

(51) International Patent Classification:
C12Q 1/68 (2018.01) *G01N 33/68* (2006.01)
A61K 31/335 (2006.01)

(21) International Application Number:
PCT/US2021/023981

(22) International Filing Date:
24 March 2021 (24.03.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/994,259 24 March 2020 (24.03.2020) US
63/139,394 20 January 2021 (20.01.2021) US

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE** [US/US]; 1800 Grant Street, Denver, Colorado 80203 (US).

(72) Inventors: **LABARBERA, Daniel V.**; 27604 E. Euclid Drive, Aurora, Colorado 80016 (US). **ABBOTT, Joshua M.**; 9890 E. Colorado Avenue, Apt. 108, Aurora, Colorado 80247 (US). **ZHOU, Qiong**; 27604 E. Euclid Drive, Aurora, Colorado 80016 (US). **ABRAHAM, Adedoyin D.**; 962 Crystal Way, #202, Aurora, Colorado 80012 (US). **ESQUER, Hector**; 2200 N. Ursula Street, Aurora, Colorado 80045 (US). **PRIGARO, Brett Joseph**; 13621 Navajo Street, Broomfield, Colorado 80023 (US).

(74) Agent: **SULLIVAN, Sally A.** et al.; 4940 Pearl East Circle, Suite 200, Boulder, Colorado 80301 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
11 November 2021 (11.11.2021)

WO 2021/195279 A3

(54) Title: SMALL MOLECULE INHIBITORS OF ONCOGENIC CHD1L WITH PRECLINICAL ACTIVITY AGAINST COLORECTAL CANCER

(57) Abstract: Treatment of CHD1L-driven cancers, including TCF transcription-driven cancers and EMT-driven cancers using CHD1L inhibitors. Small molecule inhibitors of CHDL1 which inhibit CHD1L ATPase and inhibit CHD1L-dependent TCF-transcription have been identified. CHD1L inhibitors prevent the TCF-complex from binding to Wnt response elements and promoter sites. More specifically, CHD1L inhibitors induce the reversion of EMT. CHD1L inhibitors are useful in the treatment of various cancers and particularly CRC and m-CRC. The CHD1L-driven cancer is among others, CRC, breast cancer, glioma, liver cancer, lung cancer or gastrointestinal (GI) cancers. CHD1L inhibitors of formulas I and XX and salts thereof as defined herein are provided as well as pharmaceutical compositions containing CHD1L inhibitors. Synergistic combinations of CHD1L inhibitors with other antineoplastic agents are also described.

SMALL MOLECULE INHIBITORS OF ONCOGENIC CHD1L WITH PRECLINICAL ACTIVITY AGAINST COLORECTAL CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional applications 62/994,259, filed March 24, 2020, and 63/139,394, filed January 20, 2021, both of which are incorporated by reference herein in their entirety.

STATEMENT REGARDING U.S. GOVERNMENT SUPPORT

This invention was made with Government support under grant number W81XWH1810142 awarded by the Department of Defense (DoD). The U.S. Government has certain rights in this invention.

BACKGROUND

The integrity of the genome is maintained by conformational changes to chromatin structure that regulate accessibility to DNA for gene expression and replication. Chromatin structure is maintained by post-translational modifications of histones and rearrangement of nucleosomes. [Lorch et al., **2010**; Kumar et al., **2016**; Swygert et al., **2014**] ATP-dependent chromatin remodelers are enzymes that alter chromatin by changing histone composition, and by evicting or translocating nucleosomes along DNA. Their activity plays a critical role in cellular function by regulating gene expression and the accessibility of DNA for replication, transcription, and DNA repair. [Erdel et al., **2011**; Brownlee et al., **2015**] Dysregulation of chromatin remodeling is associated with human disease, particularly cancer. [Zhang et al., **2016**; Valencia & Kadoch, **2019**]

In the last decade, the chromatin remodeler known as CHD1L (chromodomain helicase/ATPase DNA binding protein 1-like), also known as ALC1 (amplified in liver cancer 1), has emerged as an oncogene implicated in the pathology of prominent human cancers.(Ma et al., **2008**; Chenget a l., **2013**] CHD1L is also involved in multi-drug resistance, ranging from upregulation of drug resistance efflux pumps (e.g. ABCB1) [Li et al., **2019**] to PARP1 mediated DNA repair [Pines et al., **2012**; Tsuda et al., **2017**], and anti-apoptotic activity. [Li et al., **2013**; Chen et al., **2009**] Moreover, amplification or overexpression of CHD1L are correlated with poor prognoses for patients, including low overall survival (OS) and metastatic disease. [He et al., **2015**; Hyeon et al., **2013**; Su et al., **2014**; Mu et al., **2015**; Su et al., **2015**; Li et al., **2013**; He et al., **2012**; Chen et al., **2010**] CHD1L overexpression has also been implicated in tumor progression and as a predictor of poor patient survival. [Ji et al., **2013**] The multifunctional oncogenic mechanisms of CHD1L make it an attractive therapeutic target in cancer. While the cancer driving mechanisms of

CHD1L have been studied in liver [Li et al., **2019**], breast [Wu et al., **2014**], and lung [Li et al., **2019**] cancer, little is known about the pathological mechanisms associated with CHD1L in colorectal cancer (CRC).

CRC is the third most prevalent cancer diagnosed each year and CRC patients have the second highest mortality rate worldwide. [Jemal et al., **2011**] Early detection of CRC combined with surgery and 5-fluorouracil (5FU) based combination chemotherapy has minimally improved the overall survival rate. [Jemal et al., **2011**; Fakih, **2015**] The current chemo and targeted therapies are largely ineffective against metastatic CRC (mCRC), evidenced by a low 11% 5-year overall survival rate. [Jemal et al., **2011**; Fakih, **2015**] There is an unmet need in the art to identify and characterize targets involved in the pathology of CRC tumor progression and metastasis.

A majority of CRC patients possess mutations in the Wnt signaling pathway, leading to aberrant T-Cell Factor/Lymphoid Enhancer Factor-transcription or TCF-complex. [Kinzler & Voelstein, **1996**; Cancer Genome Atlas, **2012**] Such mutations can lead to constitutive β-catenin translocation and transactivation of TCF-transcription. [Clevers, **2006**; Korinek et al., **1997**] The TCF-complex is orchestrated by TCF4 (a.k.a. TCFL2), which is activated through interactions with an array of coactivators such as β-catenin, PARP1, and CREB Binding protein (CBP). [Shitashige et al., **2008**] Recently, TCF4 was shown to be a specific driver of both early metastasis from adenomas (i.e. polyps) and from late stage mCRC. [Hyeo et al., **2013**; Su et al., **2014**]

It has been reported that TCF transcription functions as a master regulator of epithelial-mesenchymal transition (EMT) [Sánchez-Tillá et al., **2011**; Zhou et al., **2016**; Abraham et al., **2019**]. This process can transform relatively benign epithelial tumor cells into mesenchymal cells with increased cancer stem cell (CSC) stemness and other malignant properties that drive mCRC. [Chaffer et al., **2016**] It has recently been reported that alterations in certain CRC driver genes are common in both primary and metastatic tumor pairs. [Hu et al., **2019**] More specifically, aberrant TCF4 is reported to be a specific driver of mCRC. [Hu et al., **2019**] and CRC can metastasize in early adenomas (i.e., polyps [see also Magri & Bardelli, **2019**] which is likely caused by TCF-driven EMT [Chaffer et al. **2016**; Chaffer & Weinberg, **2011**] These reports indicate that TCF-transcription is a driving force at all stages of CRC progression and metastasis.

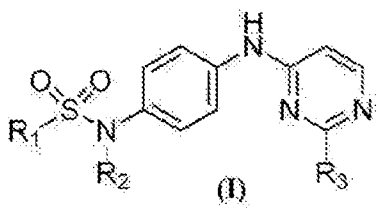
EMT is a major driving force in numerous human diseases, especially solid tumor progression, drug and radiation therapy resistance, evasion of the immune response and

immunotherapy, and promotion of metastasis. [Chaffer et al. **2016**; Chaffer & Weinberg, **2011**; Scheel & Weinberg, **2012**]

Due to the significance of the Wnt signaling pathway and TCF-transcription in cancer and other diseases [Clevers, **2006**], small molecule drugs that inhibit the Wnt signaling pathway and TCF-transcription have been examined. [Lee et al., **2011**; Polakis, **2012**] Therapeutic strategies considered include receptor targets (e.g. Frizzled); preventing Wnt ligand secretion (e.g. porcupine); inhibiting β-catenin destruction complex (e.g. tankyrases); and protein-protein inhibition (PPI) with β-catenin and co-activators (e.g. CBP). While clinical trials may be underway, no drug has as yet been clinically approved that targets the Wnt/TCF pathway. [Lu et al., **2016**] In contrast, the present invention describes a new therapeutic strategy, particularly for identifying small molecule drugs, for treatment of Wnt/TCF driven CRC in which CHD1L is identified as a DNA binding factor required for TCF-transcription regulating the malignant phenotype in CRC.

For example, U.S. Patent 9,616,047 reports small molecule inhibitors of β-catenin or disruptors of a β-catenin/TCF-4 complex which are said to attenuate colon carcinogenesis. Inhibitors of β-catenin reported therein include esculetin, as well as, compounds designated HI-B1–HI-B20, HI-B22–-HI-B-24, HI-B26, HI-B32 and HI-B34, the structures of each of which is provided in the patent. The patent further describes, in a number of generic chemical formula therein, compounds said to be useful as β-catenin inhibitors and for the treatment of colon carcinogenesis. This patent is incorporated by reference herein in its entirety for the structures of specific compounds, generic formulae and variable definitions of compounds said therein to be useful in the invention therein. The compounds identified herein are structurally distinct from those described in this patent.

CN109761909 published May 17, 2019 reports (as described in the Espacenet Eng. Abstract thereof) certain N-(4-(pyrimidine-4-amino)phenyl)sulfonamide compounds or salts of a certain formula inhibit Hsp90-Cdc37 (heat shock protein Hsp90 and its auxillary chaperone Cdc37) interactive client protein expression, and are reported useful for treating or preventing various diseases mediated by an Hsp90 signal channel. The formula given in the published application is:



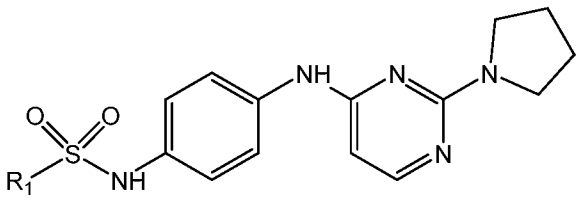
(I)

where variables are defined according to the Espacenet English machine translation as follows:

R_1 is mes-trimethylphenyl, 4-methylphenyl, 4-trifluoromethylphenyl, naphthyl, 2,3,4,5,-tetramethylphenyl, 4-methoxyphenyl, 4-tert-butylphenyl, 2,4-dimethoxyphenyl, 2,5-dimethoxyphenyl or 4-phenoxyphenyl;

R_2 is hydrogen, methyl acetate, acetate, aminoacetyl, 4-formic acid benzyl, 4-isopropylbenzyl, 4-chlorobenzyl or 4-methoxybenzyl; and

R_3 is chlorine, -ORa or –NRbRc, where, Ra is a chain C1-3 alkyl, C5-6 cycloalkyl, C1-2 alkoxy, mono- or di-C1-2 alkylamino, or C5-6 nitrogen-containing or oxygen-containing heterocyclic group; and Rb and Rc are C1-5 alkyl groups, respectively. More specifically, R_3 is chlorine, 2-hydroxytetrahydropyrrolyl, ethanolamino, 2,3-dihydroxy-1-methylpropylamino, 2,3-dihydroxypropylamino, piperazinyl, N-methylpiperazinyl, azepyl, piperidinyl, 2-methylpropylamino, propoxy, methylamino, ethylamino, cyclopropylamino, 1-ethylpropylamino, tetrahydropyran-4-ylmethoxy or 2-methoxyethoxy. The reference also refers to a compound of formula I-5:



This published application is incorporated by reference herein in its entirety for the structures of specific compounds, generic formulae and variable definitions of compounds said therein to be useful in the invention therein. Structures disclosed in this published application can be excluded from any chemical formula of the present application.

The present invention examines the clinicopathological characteristics of CHD1L in CRC, and the results herein indicate that CHD1L is a druggable target involved in TCF-transcription. A mechanism for CHD1L-mediated TCF-transcription is also proposed herein. Small molecule inhibitors of CHD1L are identified herein which are able to prevent TCF transcription, reverse EMT, and other malignant properties in a variety of cell models including tumor organoids and patient derived tumor organoids (PDTOs) Certain CHD1L inhibitors identified herein display drug-like pharmacological properties, including *in vivo* pharmacokinetic (PK) and pharmacodynamic (PD) profiles, important for translational development towards the treatment of CRC and other cancers.

SUMMARY

This invention relates to the treatment of CHD1L-driven cancers, more specifically TCF transcription-driven cancers and yet more specifically EMT-driven cancers. CHD1L is found to be an essential component of the TCF transcription complex. Small molecule inhibitors of CHDL1 which inhibit CHD1L ATPase and inhibit CHD1L-dependent TCF-transcription have been identified. CHD1L inhibitors are believed to prevent the TCF-complex from binding to Wnt response elements and promoter sites. More specifically, CHD1L inhibitors induce the reversion of EMT. CHD1L inhibitors are useful in the treatment of various cancers and particularly CRC and m-CRC. Particularly with respect to CRC, CHD1L inhibitors are shown in embodiments to inhibit cancer stem cell (CSC) stemness and invasive potential. IN embodiments, CHD1L inhibitors induce cytotoxicity in CRC PDTOs. In specific embodiments, the CHD1L-driven cancer is CRC, breast cancer, glioma, liver cancer, lung cancer or gastrointestinal (GI) cancers. In specific embodiments, the TCF transcription-driven cancer is CRC, including mCRC. In specific embodiments, the EMT-driven cancer is CRC, including mCRC.

The invention provides a method for treatment of CHD1L-driven cancers, more specifically TCF transcription-driven cancers and yet more specifically EMT-driven cancers, including GI cancer, particularly CRC and mCRC, which comprises administration to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for CHD1L inhibition, effective inhibition of aberrant TCF transcription or effective for induction of EMT reversion. In embodiments, the CHD1L inhibitor is a compound of any one of formulas I- XX or XXX-XVII. More specifically, the invention provides a method of inhibiting aberrant TCF-transcription, particularly in CRC, by administration of an effective amount of a CHD1L inhibitor. Yet more specifically, the invention provides a method of inducing reversion of EMT, particularly in CRC or mCRC, by administration of an effective amount of a CHD1L inhibitor. The invention provides a method of inhibiting Cancer Stem Cell (CSC) stemness and/or invasive potential, particularly in CRC, by administration of an effective amount of a CHD1L inhibitor. The invention provides a method for treatment of cancerous tumors of CHD1L-driven cancers, or TCF transcription-driven cancers or EMT-driven cancers, particularly in CRC, by administration of an effective amount of a CHD1L inhibitor.

In embodiments, CHD1L inhibitors are selective for inhibition of CHD1L. In embodiments, CHD1L inhibitors herein are not PARP inhibitors. In embodiments, CHD1L inhibitors herein are not inhibitors of topoisomerases. In particular, CHD1L inhibitors herein are not inhibitors of DNA topoisomerase. In particular, CHD1L inhibitors herein are not inhibitors of topoisomerase type IIα. In embodiments, CHD1L inhibitors herein are not inhibitors of β-

catenin, particularly inhibitors such as described in U.S. Patent 9,616,047. In embodiments, CHD1L inhibitors herein are not inhibitors of Hsp90-Cdc37 interactive client protein expression, particularly inhibitors as described in CN109761909.

The invention also provides a method to prevent tumor growth, invasion and/or metastasis in CHD1L-driven, TCF-transcription, or EMT-driven cancers by administering to a patient in need thereof of an amount of a CHD1L inhibitor of this invention which is effective for CHD1L inhibition, inhibition of aberrant TCF transcription, or effective for reversion of EMT. In specific embodiment, tumors are solid tumors. In embodiments, tumors are those associated with GI cancer. In embodiments, tumors are those associated with CRC. In embodiments, tumors are those associated with mCRC.

In specific embodiments, the invention provides a method for treatment of CRC, including mCRC, which comprises administration to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for inhibition of CHD1L. In specific embodiments, the invention provides a method for treatment of CRC, including mCRC, which comprises administration to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for inhibition of aberrant TCF transcription. In specific embodiments, the invention provides a method for treatment of CRC, including mCRC, which comprises administration to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for induction of reversion of EMT.

In specific embodiments, the invention provides a method for inducing apoptosis in cancer cells which comprises contacting a cancer cell with an effective amount of a CHD1L inhibitor. In an embodiment, the CHD1L inhibitor is provided in an amount effective for inhibition of aberrant TCF transcription. In an embodiment, the CHD1L inhibitor is provided in an amount effective for induction of reversion of EMT. In an embodiment, the cancer cells are CRC cancer cells. In an embodiment, the cancer cells are mCRC cancer cells. In an embodiment, the method is applied *in vivo*. In an embodiment, the method is applied *in vivo* in a patient. In an embodiment, the method is applied *in vitro*.

In embodiments of the methods herein comprising administration of the CHD1L inhibitor, the CHD1L inhibitor is administered by any known method and dosing schedule to achieve desired benefits. In an embodiment, administration is oral administration. In an embodiment, administration is by intravenous injection.

The invention also provides a method of treatment of drug-resistant cancer which comprises administering to a patient in need thereof of an amount of a CHD1L inhibitor, which is effective for CHD1L inhibition, inhibition of aberrant TCF transcription or induction of reversion of EMT, in combination with a known treatment to which the cancer has become resistant. In specific embodiments, the treatment to which the cancer has become resistant is conventional chemotherapy and other targeted therapies. In specific embodiments, the invention provides a method of increasing the efficacy of a DNA-damaging drug in cancer which comprises combined treatment of the cancer with the DNA damaging drug and a CHD1L inhibitor where the CHD1L is administered in an amount effective for decreasing resistance to the DNA-damaging drug. In an embodiment, the DNA-damaging drug is a topoisomerase inhibitor. In particular, the DNA-damaging drug is a DNA topoisomerase inhibitor. In particular, the DNA-damaging drug is a topoisomerase type IIα inhibitor. In particular, the DNA-damaging drug is etoposide or teniposide. In particular, the DNA-damaging drug is SN38 or a prodrug thereof. In an embodiment, the DNA-damaging drug is a thymidylate synthase inhibitor. In an embodiment, the thymidylate synthase inhibitor is a folate analogue. In an embodiment, the thymidylate synthase inhibitor is a nucleotide analogue. In specific embodiments, the thymidylate synthase inhibitor is raltitrexed, pemetrexed, nolatrexed or ZD9331. In a particular embodiment, the DNA-damaging drug is 5-fluorouracil or capecitabine.

In an embodiment, the cancer is a CDH1L-driven cancer. In an embodiment, the cancer is a TCF transcription-driven cancer. In an embodiment, the cancer is an EMT-driven cancer. In an embodiment, the treatment is for CRC. In an embodiment, the treatment is for mCRC. In embodiments, the DNA-damaging drug and the CHD1l inhibitor are administered by any known method on a dosing schedule appropriate for realizing the combined therapeutic benefit. In embodiments, the CHD1L inhibitor is administered orally and the DNA-damaging drug is administered by any known administration method and dosing schedule. In embodiments, the CHD1L inhibitor is administered prior to administration of the DNA-damaging drug. In embodiments, the CHD1L inhibitor is administered prior to and optionally after administration of the DNA-damaging drug. In embodiments, the CHD1L inhibitor is administered orally prior to and optionally after administration of the DNA-damaging drug by intravenous injection.

The invention provides methods for treatment of CHD1L-driven cancer, TCF-transcription-driven cancer, or EMT-driven cancer which comprises administration to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for CHD1L inhibition or inhibition of aberrant TCF transcription or induction of reversion of EMT in combination with an

alternative method of treatment for the cancer. In an embodiment, the cancer is GI cancer or more specifically CRC cancer and yet more specifically is mCRC. In an embodiment, the alternative method for treatment is administration of one or more of 5-fluorouracil, 5-fluorouracil in combination with folinic acid (also known as leucovorin), a topoisomerase inhibitor, or a cytotoxic or antineoplastic agent. In embodiments, the CHD1L inhibitor is administered in combination with 5-fluorouracil or in combination with 5-fluorouracil and folinic acid. In embodiments, the CHD1L inhibitor is administered in combination with a topoisomerase inhibitor and in particular with irinotecan (a prodrug of SN38 also known as camptothecin) or any other known prodrug of SN38. In embodiments, the combined treatment using a CHD1L inhibitor and a topoisomerase inhibitor exhibits at least additive activity against the cancer. In embodiments, the combined treatment of a CHD1L inhibitor with a topoisomerase inhibitor exhibits synergistic activity (greater than additive activity) against the cancer.

In embodiments, the CHD1L inhibitor is administered in combination with a cytotoxic or antineoplastic agent, in particular a platinum-based antineoplastic agent and more particularly cisplatin, carboplatin or oxaliplatin. In embodiments, the combined treatment using a CHD1L inhibitor and a platinum-based antineoplastic agent exhibits at least additive activity against the cancer. In embodiments, the combined treatment of a CHD1L inhibitor with a platinum-based antineoplastic agent exhibits synergistic activity (greater than additive activity) against the cancer. In embodiments, the platinum-based antineoplastic agent and the CHD1l inhibitor are administered by any known method on a dosing schedule appropriate for realizing the combined therapeutic benefit. In embodiments, the CHD1L inhibitor is administered orally and the platinum-based neoplastic agent is administered by any known administration method and dosing schedule. In embodiments, the CHD1L inhibitor is administered prior to administration of the platinum-based neoplastic agent. In embodiments, the CHD1L inhibitor is administered prior to and optionally after administration of the platinum-based antineoplastic agent. In embodiments, the CHD1L inhibitor is administered orally prior to and optionally after administration of the platinum-based neoplastic agent by intravenous injection.

In embodiments, the CHD1L inhibitor is administered in combination with a chemotherapy regimen for treatment of GI cancer, particularly CRC, and mCRC. In embodiments, the CHD1L inhibitor is administered in combination with the chemotherapy regimen designated FOLFOX. In embodiments, the CHD1L inhibitor is administered in combination with the chemotherapy regimen designated FOLFIRI. In embodiments, the chemotherapy regime and the CHD1l inhibitor are administered by any known method on a dosing schedule

appropriate for realizing the combined therapeutic benefit. In embodiments, the CHD1L inhibitor is administered orally and the chemotherapy regime is administered by any known administration method and dosing schedule. In embodiments, the CHD1L inhibitor is administered prior to administration of the chemotherapy regime. In embodiments, the CHD1L inhibitor is administered prior to and optionally after administration of the chemotherapy regime. In embodiments, the CHD1L inhibitor is administered orally prior to and optionally after administration of the PARP inhibitor by intravenous injection.

The invention provides a method for treatment of cancers that are sensitive to Poly(ADP)-ribose) polymerase I (PARPI) in which a CHD1L inhibitor is used in combination with a PARP inhibitor. In embodiments, an amount of a CHD1L inhibitor effective for CHD1L inhibition, inhibition of aberrant TCF transcription or induction of reversion of EMT is used in combination with an amount of a PARP inhibitor effective for treating cancer to at least enhance the effectiveness of the cancer treatment. In embodiments, the combined treatment using a CHD1L inhibitor and a PARP inhibitor exhibits at least additive activity against the cancer. In embodiments, the combined treatment of a CHD1L inhibitor with a PARP inhibitor exhibits synergistic activity (greater than additive activity) against the cancer. In embodiments, the cancer is a cancer sensitive to treatment by a PARP inhibitor. In embodiments, the cancer is a cancer that is or has become resistant to treatment by a PARP inhibitor. In embodiments, the cancer is a cancer sensitive to treatment by a PARP inhibitor. or which has become resistant to treatment by a PARP inhibitor and which is a CHD1L-driven, a TCF-driven or an EMT-driven cancer. In embodiments, the cancer is a homologous recombination deficient cancer (see, for example, Zhou et al. BioRxiv 2020). In embodiments, the cancer treated is a cancer sensitive to a PARP inhibitor and more particularly is breast or ovarian cancer. In specific embodiments, the cancer is BRCA-deficient breast or ovarian cancer. In embodiments, the cancer treated is GI cancer, CRC or mCRC. In embodiments, combined treatment of the CHD1L inhibitor with the PARP inhibitor reverses resistance of the cancer to treatment by the PARP inhibitor. In embodiments, the PARP inhibitor is olaparib, veliparib or talozoparib. In embodiments, the PARP inhibitor is rucaparib or niraparib. The invention also provides a method for treating a cancer which comprises administration of an amount of a PARP inhibitor effective for treatment of the cancer combined with administration of an amount of a CHD1L inhibitor effective for inhibiting CHD1L. In embodiments, the PARP inhibitor and the CHD1l inhibitor are administered by any known method on a dosing schedule appropriate for realizing the combined therapeutic benefit. In embodiments, the CHD1L inhibitor is administered orally and the PARP inhibitor is administered by intravenous injection. In embodiments, the CHD1L inhibitor and the PARP inhibitor are both administered by intravenous injection. In

embodiments, the CHD1L inhibitor is administered prior to administration of the PARP inhibitor. In embodiments, the CHD1L inhibitor is administered prior to and optionally after administration of the PARP inhibitor. In embodiments, the CHD1L inhibitor is administered after administration of the PARP inhibitor. In embodiments, the CHD1L inhibitor is administered orally prior to and optionally after administration of the PARP inhibitor by intravenous injection.

The invention also provides a method for identifying a CHD1L inhibitor, which inhibits CHD1L- dependent TCF transcription which comprises determining if a selected compound inhibits a CHD1L ATPase, as described in examples herein. In specific embodiments, inhibition of cat-CHD1L ATPase is determined. In embodiments, compounds exhibiting % inhibition of 30% or greater are selected as inhibiting a CHD1L ATPase. In embodiments, compounds exhibiting % inhibition of 80% or greater are selected as inhibiting a CHD1L ATPase. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 10 μM in dose response assays against CHD1L ATPase, particularly cat-CHD1L ATPase. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 5 μM in dose response assays against CHD1L ATPase, particularly cat-CHD1L ATPase. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 3 μM in dose response assays against CHD1L ATPase, particularly cat-CHD1L ATPase. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 5 μM. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 3 μM. In specific embodiments, CHD1L inhibitors exhibit IC_{50} less than 1 μM.

In specific embodiments, CHD1L inhibitors are assessed for inhibition of TCF-transcription in a 2D cancer cell model, particularly using one or more CRC cell lines, such as described in examples herein. In specific embodiments, inhibition of TCF-transcription is determined using a TOPflash reporter construct and more specifically a TOPflash luciferase reporter construct as described herein. In specific embodiments, inhibition of TCF-transcription by CHD1L inhibitors in the cell model is dose-dependent. In specific embodiments, inhibition of TCF-transcription by CHD1L inhibitors in the cell model is dose-dependent in the range of 1 to 50 μM. In specific embodiments, a CHD1L inhibitor exhibits % TCF-transcription normalized to cell viability of 75% or less at 20 μM. In specific embodiments, a CHD1L inhibitor exhibits % TCF-transcription normalized to cell viability of 50% or less at 40 μM. In specific embodiments, CHD1L inhibitors exhibit dose dependent inhibition of TCF-transcription with IC_{50} less than 10 μM assayed with TOPflash reporter in a cancer cell line. In specific embodiments, CHD1L inhibitors exhibit dose dependent inhibition of TCF-transcription with IC_{50} less than 5 μM assayed with TOPflash reporter in a cancer cell line. In specific embodiments, CHD1L inhibitors exhibit dose dependent inhibition of TCF-

transcription with IC_{50} less than 3 μM assayed with TOPflash reporter in a cancer cell line. In embodiments, the cancer cell line is a CRC cancer cell, a breast cancer cell, a glioma cell, a liver cancer cell, a lung cancer cell or a GI cancer cell. In an embodiment, the cancer cell line is a CRC cancer cell line. In a specific embodiment, the CRC cancer cell line is SW620.

In specific embodiments, CHD1L inhibitors are assessed for their ability to reverse or inhibit EMT. In specific embodiments, CHD1L inhibitors are assessed for their ability to reverse EMT in tumor organoids. In embodiments, reversion or inhibition of EMT is assessed in tumor organoids expressing vimentin where dose-dependent decrease in vimentin expression indicates reversion or inhibition of EMT. In embodiments, reversion of EMT is assessed in tumor organoids expressing E-cadherin where dose-dependent increase in E-cadherin expression indicates reversion or inhibition of EMT. In embodiments, reversion of EMT is assessed in tumor organoids expressing E-cadherin, vimentin or both, where dose-dependent decrease in vimentin and dose-dependent increase in E-cadherin expression indicates reversion or inhibition of EMT. In specific embodiments, dose-dependent reversion or inhibition of EMT is measured over a compound concentration of 0.1 to 100 μM. In specific embodiments, dose-dependent reversion of EMT is measured over a compound concentration of 0.3 to 50 μM.

In specific embodiments, CHD1L inhibitors are assessed for their ability to inhibit clonogenic colony formation which is a well-established assay to measure cancer stem cell stemness. In embodiments, cells are pretreated with a selected concentration of CHD1L inhibitors prior to plating. In embodiments, cells are cultured at low density such that only CSC will form colonies over 10 days in culture. In embodiments, cells are pretreated for 12-36 h. In embodiments, cells are pretreated for 24 h. In embodiments, cells are pretreated with CHD1L inhibitors at concentration in the range of 0.5-50 μM with appropriate controls. In embodiments, CHD1L inhibitors exhibit 40% or more inhibition of clonogenic colony counts, compared to no compound control, for CHD1L concentration of 40 μM. In embodiments, CHD1L inhibitors exhibit 40% or more inhibition of clonogenic colony counts, compared to no compound control, for CHD1L concentration of 20 μM. In embodiments, CHD1L inhibitors exhibit 40% or more inhibition of clonogenic colony counts, compared to no compound control, for CHD1L concentration of 2 μM. In embodiments, inhibition of clonogenic colony formation lasts over 10 days in culture.

In specific embodiments, CHD1L inhibitors are further assessed for loss of invasive potential employing any known method and particularly employing a method as described in the examples herein.

In specific embodiments, CHD1L inhibitors are further assessed for antitumor activity as measured by induction of cytotoxicity in tumor organoids. In embodiments, cells are treated for a selected time (e.g., 24-96 h, preferably 72 h) with selected concentration of CHD1L inhibitor (1-100 μM). In embodiments, cytotoxicity is measured using any of a variety of cytotoxicity reagents known in the art, such as small molecules which, enter damaged cells and exhibit a measurable change on entry (e.g., fluorescence, such as, CellTox™ Green reagent (Promega, Madison, WI) or IncuCyteCytotox reagents (Sartorius, France). In embodiments, cytotoxicity is measured by measurement of LDH (lactate dehydrogenase) released from dead cells.

In embodiments, the CHD1L inhibitors useful in methods of treatment herein are those of formulas I- XX, and XXX-XLII or pharmaceutically acceptable salts or solvates thereof. In embodiments, the invention provides novel compounds of any formula herein and in particular of of formulas I-XX, XXXV-XLII or salts or solvates thereof. In embodiments, the CHD1L inhibitors are those of formula I. In embodiments, the CHD1L inhibitors are those of formula XX. In embodiments, the CHD1L inhibitors are those of formulas I-IX, Xi-XiX, XX, or XXXV-XLII.

In specific embodiments, the methods of the invention employ CHD1L inhibitors that are selected from one or more of compounds **1-73** or pharmaceutically acceptable salts or solvates thereof. Two or more CHD1L inhibitors can be employed in combination in the methods herein. In specific embodiments, the CHD1L inhibitors employed in the invention are selected from one or more of compounds **6-39** or pharmaceutically acceptable salts thereof. In specific embodiments, the CHD1L inhibitors employed in the methods of the invention are selected from one or more of compounds **40-51** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the CHD1L inhibitors employed in the methods of the invention are selected from one or more of compounds **52-68** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the CHD1L inhibitors employed in the methods of the invention are selected from one or more of compounds **70-73** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the CHD1L inhibitors employed in methods of this invention are compounds **6**, **8, 52, 54, 56, 61, 62, 65 or 66** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the CHD1L inhibitors employed in methods of this invention are compounds **6**, **8** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the CHD1L inhibitors employed in methods of this invention are compounds **52**, **54** or pharmaceutically acceptable salts or solvates thereof. In specific embodiments, the

CHD1L inhibitors employed in methods of this invention are compounds **22**, **23**, **26** or **27** or pharmaceutically acceptable salts thereof.

In specific embodiments, the methods of the invention employ CHD1L inhibitors of formula XX and include all embodiments described herein for formula XX. The invention also provides novel compounds of formula XX, salts thereof and pharmaceutical compositions contains such compounds and salts.

The invention is also directed to CHD1L inhibitors of this invention and pharmaceutically acceptable compositions comprising any such inhibitors. In embodiments, the invention is directed to any compound or pharmaceutically acceptable salt or solvate thereof as described in chemical formulas herein which is novel. In particular, the invention is directed to CHD1L inhibitors and pharmaceutically acceptable salts thereof as described in formulas herein with the exception that the CHD1L inhibitor is not compounds **1-8** or salts or solvates thereof. In particular, the invention is directed to CHD1L inhibitors and pharmaceutically acceptable salts thereof as described in formulas herein with the exception that the CHD1L inhibitor is not compounds **1-9** or salts thereof. In embodiments, the invention is directed to any of compounds **9-39**, **40-68, 69-73** or pharmaceutically acceptable salts or solvates hereof or pharmaceutically acceptable compositions that contains such compounds or salts or solvates. In embodiments, the invention is directed to any of compounds **10-39** or **40-73** or pharmaceutically acceptable salts or solvates hereof or pharmaceutically acceptable compositions that contains such compounds or salts or solvates. In embodiments, the invention is directed to any of compounds **52-73** or pharmaceutically acceptable salts or solvates hereof or pharmaceutically acceptable compositions that contains such compounds or salts or solvates. In embodiments, a CHD1L inhibitor of the invention has Clog P of 5 or less. In embodiments, a CHD1L inhibitor of the invention has Clog P of 3-4.

In specific embodiments the invention is directed to the following compounds and to methods herein employing these compounds for the treatment of cancer, particularly CRC and mCRC: compounds **52-73**; compound **52** or **53**; compounds **54**, **55** or **67**; or compounds **57, 58** or **59**; or pharmaceutically acceptable salts or solvates thereof; any one of compound **8**, compound **52,** compound **53,** compound **54,** compound **55,** compound **56,** compound **57,** compound **58,** compound **59,** compound **61,** compound **62,** compound **65,** compound **66,** or compound **67.**

The invention also relates to the use of a CHD1L inhibitor in the manufacture of a medicament for the treatment of cancer, particularly for the treatment of cancer, CHD1L-

driven cancer, TCF-driven cancer, or EMT-driven cancer, particularly GI cancer, and more particularly CRC or mCRC. The invention further relates to a CHD1L inhibitor herein for use in the treatment of cancer, CHD1L-driven cancer, TCF-driven cancer, or EMT-driven cancer, particularly GI cancer, and more particularly CRC or mCRC.

Other embodiments and aspects of the invention will be readily apparent to one of ordinary skill in the art on review of the drawings, detailed description and examples herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B: Validation of CHD1L inhibitors identified from HTS. (**FIG. 1A**) cat-CHD1L ATPase C50 dose responses with hits **1-7**. Mean IC_{50} values are calculated from three independent experiments and representative graphs are shown. (**FIG. 1B**) SW620, HCT-16, and DLD1CHD1L-OE cells with TOPflash reporter were used to measure inhibition of TCF transcription using 3 doses over 24h.

Figures 2A-2D: CHD1L inhibitors reverse EMT and the malignant phenotype in CRC. Dose responses for CHD1L inhibitors that modulate EMT measured by high-content imaging of (**FIG. 2A**) downregulation of VimPro-GFP reporter and (**FIG. 2B**) Upregulation of EcadPro-RFP reporter. Mean EC_{50} values ± SEM are calculated from three independent experiments (**FIG. 2C**) CSC stemness measured by clonogenic colony formation after pretreatment with CHD1L inhibitors in DLD1CHD1L-OE and HCT-116 cells. (**FIG. 2D**) Inhibition of invasive potential of HCT-116 cells after treatment of CHD1L inhibitors. Welch's t-test statistical analysis was used to determine significance, where * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$.

Figures 3A-C: Compound **6** induces apoptosis in CRC cell lines and PDTOs. (**FIG. 3A**) Time course evaluation of the induction E-cadherin expression using Ecad-ProRFP reporter assay and cytotoxicity using Cell-Tox™ Green cytotoxicity assay (Promega, Madison, Wi). (**FIG. 3B**) Annexin V-FITC staining analysis of apoptosis after treatment of SN-38 and **6** for 12 hours. (**FIG. 3C**) Cytotoxicity of **6** in PDTO CRC102 using CellTiter-Blue® cell viability assay (Promega, Madison, WI). Mean EC_{50} values ± s.d. are calculated from six independent experiments and representative graph is shown with inset of a representative PDTO. Welch's t-test statistical analysis was used to determine significance, where * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$.

Figure 4: Accumulation of Compound **6** in SW620 xenograft tumors. Compound **6** was administered by i.p. injection to athymic nude mice QD for 5 days to measure accumulation in SW620 xenograft tumors.

Figure 5: Proposed mechanism of action of CHD1L mediated TCF-transcription. CHD1L is activated through binding TCF-complex members PARP1 and TCF4 [Abbott et al., **2020**] (1) Once activated, CHD1L is directed to hindered WREs localized on chromatin. (2) Chromatin remodeling and DNA translocation occurs exposing WRE sites. (3) TCF-complex binds to exposed WREs facilitated by CHD1L, promoting EMT genes and other genes associated with mCRC. CHD1L ATPase inhibitors effectively prevent step 1, leading to the reversion of EMT and other malignant properties of CRC.

Figures 6A-E Evaluation of Compound **8**. (**FIG. 6A**) Compound **8** displays potent low μM dose-dependent inhibition of TCF-transcription based on TOPFlash reported in SW260 cells cultures in 2D and over a 24 h time course. Compound **8** effectively reverses EMT in dual reporter SW620 tumor organoids over 72 h evidenced by downregulation of vimentin (**FIG. 6B**) and (**FIG. 6C**) upregulation of E-cadherin promoter activity in a dose-dependent manner. Compound **8** significantly inhibits (**FIG. 6D**) clonogenic colony formation over 10 days after pre-treatment for 24 h and (**FIG. 6E**) HCT116 invasive potential over 48 h. The students t-test indicates* $P \leq 0.05$

Figures 7A-B: Viability of Colorectal Cancer Tumor Organoids after Treatment with Exemplary CHDIL Inhibitors. The figures illustrate representative graphs of % viability as a function of log concentration of the indicated compound. (**FIG. 7A**) Treatment with Compound **6.9**; (**FIG. 7B**) Treatment with Compound **6.11**; Alternative compound numbers as used in Scheme 1 are given in parenthesis. IC_{50}, in some cases average IC_{50}, are provided in each figure. Viability data for a number of exemplary compounds are provided in Table 3.

Figures 8A-B: Assessment of CHD1L-mediated DNA repair and "on target" effects of CHD1L inhibitor **6** alone and in combination with irinotecan (prodrug of SN38). CHD1L is known to be essential for PARP-1-mediated DNA repair, causing resistance to DNA damaging chemotherapy [Ahel et al., 2009; Tsuda et al., 2017]. DLD1 CRC cells that have low level expression of CHD1L (DLD1 Empty Vector, EV) compared to DLD1 cells that were engineered to overexpress CHD1L (DLD1 Overexpressing, OE) were used. **FIG. 8A** is a Western blot comparing expression of CHD1L in DLD1(EV) to DLD1(OE) in view of control

expression of α-tubulin in these cells. **FIG. 8B** presents a graph of γ-H2AX intensity (relative to DMSO) for compound alone, SN38 alone, and a combination of the two in DLD1 empty vector cells and DLD1 overexpressing cells. Compound **6** alone does not induce significant DNA damage, nor does it synergize with SN38 in DLD1 cells with low expression of CHD1L. This graph demonstrates CHD1L inhibitor "on target" effects that synergize with SN38 inducing DNA damage in DLD1 cells overexpressing CHD1L.

Figures 9A- 9C: Synergy studies with exemplary CHD1L inhibitors and irinotecan (Prodrug of SN38). (**FIG. 9A**) Synergy studies with compounds **6** and **6.3** in SW620 Colorectal Cancer (CRC) Tumor Organoids. (**FIG. 9B**) Synergy studies with compound **6.9** in SW620 Colorectal Cancer (CRC) Tumor Organoids. (**FIG. 9C**) Synergy studies with compound **6.11** in SW620 Colorectal Cancer (CRC) Tumor Organoids. SN38 combinations of **6**, and **6.3** are 50-fold, and 150-fold more potent, respectively, than SN38 alone in killing colon SW620 tumor organoids. SN38 combination of **6.9** and **6.11** are both over 100-fold more potent than SN38 alone. Each of compounds **6**, **6.3**, **6.9** and **6.11** exhibit synergism with irinotecan (and SN38) for killing SW620 tumor organoids.

Figure 10: *In vivo* synergy studies of compound **6** in combination with irinotecan in mice. Figure 10 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 28 days) of treatment with compound **6** alone (2), irinotecan alone (3) or a combination thereof (4), compared to control (1). A data Table is also provided showing data statistical significance. The combination of irinotecan and compound **6** significantly inhibit colon SW620 tumor xenografts to almost no tumor volume within 28 days of treatment compared to the single agent treatment groups.

Figure 11: *In vivo* synergy of CHD1L inhibitor compound **6** and irinotecan continues post treatment. Figure 11 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 41 days) of treatment with irinotecan alone (1) or a combination of compound **6** and irinotecan (2). A data Table is also provided showing data statistical significance. The combination of irinotecan and compound **6** significantly inhibits colon SW620 tumors to almost no tumor volume beyond the last treatment (day 28) compared to irinotecan alone. Within 2-weeks of the last treatment of irinotecan alone tumor volume rose to above the volume of the last treatment, signifying tumor recurrence. In contrast the combination maintained a lower tumor volume.

Figure 12: Compound **6** alone and in combination with irinotecan significantly increases the survival of CRC tumor-bearing mice compared to vehicle and irinotecan alone. Figure 12

includes a graph of survival (%) as a function of time up to 52 days after last treatment on day 28 with compound **6** alone (2), irinotecan alone (3) or a combination thereof (4), compared to control (1). A data Table is also provided showing data statistical significance. Survival rate was significantly higher with the combination treatment compared to single dosage compounds or control.

Figure 13: *In vivo* synergy studies of compound **6.11** incombination with irinotecan in mice. Figure 13 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 20 days) of treatment with compound **6.11** alone (2), irinotecan alone (3) or a combination thereof (4), compared to control (1). A data Table is also provided showing data statistical significance. The combination of irinotecan and compound **6.11** significantly inhibit colon SW620 tumor xenografts to almost no tumor volume within 20 days of treatment compared to the irinotecan alone.

Figure 14: *In vivo* synergy of CHD1L inhibitor compound **6.11** and irinotecan continues post treatment. Figure 14 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 41 days) of treatment with irinotecan alone (1) or a combination of compound **6** and irinotecan (2). Treatment was stopped at day 33 (Tx released). The combination of irinotecan and compound **6.11** significantly inhibits colorectal SW620 tumors beyond the last treatment (day 33) compared to irinotecan alone.

Figure 15: *In vivo* synergy of CHD1L Inhibitor **6.11** and irinotecan significantly increases survival benefit. Compound **6.11** in combination with irinotecan significantly Increases the survival of CRC tumor-bearing mice compared to vehicle and irinotecan alone. Figure 15 includes a graph of survival (%) as a function of time up to 50 days after last treatment on day 33 with compound **6** alone (2), irinotecan alone (3) or a combination thereof (4), compared to control (1). A data Table is also provided showing data statistical significance. Survival rate was significantly higher with the combination treatment compared to irinotecan alone or control.

DETAILED DESCRIPTION

The invention relates generally to the characterization of a relatively new oncogene, CHD1L, as a tumorigenic factor associated with poor prognosis and survival in CRC. A new biological function for CHD1L as a DNA binding factor for the TCF transcription complex required for promoting TCF-driven EMT and other malignant properties has been demonstrated. Abbott et al. **2020** and the supplementary information for this article, which is available from the

journal web site (mct.aacrjournals.org), provide description of a portion of the experiments and data presented herein and are each incorporated by reference herein in its entirety.

CHD1L is amplified (Chr1q21) and overexpressed in many types of cancer. [Ma et al., **2008**; Cheng et al., **2013**] CHD1L overexpression has been characterized as a marker for poor prognosis and metastasis in numerous cancers. [Ma et al., **2008**; Cheng et al., **2008**; Hyeon et al., **2013**; Su et al., **2014**] While the collective literature demonstrating CHD1L as an oncogene and driver of malignant cancer is compelling, the rigor of the prior research and the hypothesis that CHD1L is an oncogene with potential as a molecular target in CRC is tested herein. *In silico* analyses of transcriptome data from a large cohort of 585 CRC patients obtained over 15 years was reported. [Marisa et al., **2013**] Strikingly, CHD1L expression was correlated with poor survival, with low- CHD1L patients living significantly longer than high-CHD1L patients. Using the same cohort, Marisa et al., **2013** identified six distinct subtypes for improved clinical stratification of CRC and CHD1L is universally expressed in all six subtypes, indicating its potential as a therapeutic target for CRC. CHD1L also correlated with tumor node metastasis, with increased expression moving from N0 (no regional spread) to N3 (distant regional spread). Transcriptome data from a UCCC patient cohort (n=25) was analyzed and it was found that CHD1L expression significantly correlated with stage IV and mCRC. Literature reports and the work herein demonstrate that CHD1L is an oncogene promoting malignant CRC and its high expression correlates with poor prognosis and survival of CRC patients.

A new biological function for CHD1L as a DNA binding factor for the TCF-transcription complex required for promoting TCF-driven EMT and other malignant properties is demonstrated. Using HTS drug discovery the first known inhibitors of CHD1L have been identified and characterized which display good pharmacological efficacy in cell-based models of CRC, including PDTOs. CHD1L inhibitors effectively prevent CHD1L-mediated TCF-transcription, leading to the reversion of EMT and other malignant properties, including CSC stemness and invasive potential. Notably, CHD1L inhibitor **6** displays the ability to induce cell death that is consistent with the reversion of EMT and induction of cleaved E-cadherin mediated extrinsic apoptosis through death receptors. Furthermore, compound **6** synergizes with SN38 (i.e., irinotecan) displaying potent DNA damage induction compared to SN38 alone, which is consistent with the inhibition of PARP1/CHD1L mediated DNA repair. CHD1L inhibitors having drug-like physicochemical properties and favorable in vivo PK/PD disposition with no acute liver toxicity have been identified.

Based on the data presented herein, a mechanism of action for CHD1L-mediated TCF-driven EMT involved in CRC tumor progression and metastasis is presented (FIG. 5). In this

mechanism, TCF-complex specifically recruits CHD1L to dynamically regulate metastatic gene expression. Central to this mechanism, CHD1L binds to nucleosome hindered WREs when directed by the TCF-complex via protein interactions with PARP1 and TCF4. Importantly, PARP1 is characterized as the major cellular activator of CHD1L through macro domain binding that releases auto inhibition. [Lehmann et al., **2017**; Gottschalk et al., **2009**] Moreover, PARP1 is a required component of the TCF-complex forming interactions with β-catenin and TCF4. [Idogawa et al., **2005**] Therefore, the mechanism indicates that CHD1L is recruited by the TCF-complex and activated by PARP1 and TCF4. Once activated, CHD1L exposes WREs by nucleosome translocation, facilitating TCF-complex binding to WREs and transcription of malignant genes promoting EMT. CHD1L inhibitors have a unique mechanism of action by inhibiting CHD1L ATPase activity, which prevents exposure of WREs to the TCF-complex, inhibiting transcription of TCF-target genes associated with EMT and particularly with mCRC.

Small molecule inhibitors of CHD1L, as described herein, have been identified in screens based on inhibition of CHD1L ATPase activity. Certain inhibitors identified exhibit drug-like physicochemical properties and favorable *in vivo* PK/PD disposition with no acute liver toxicity. Such inhibitors are effective as a treatment for CRC and mCRC (metastatic CRC) among other CHD1L-driven cancers.

Well-known methods for assessment of drugability can be used to further assess active compounds of the invention for application to given therapeutic application. The term “drugability” relates to pharmaceutical properties of a prospective drug for administration, distribution, metabolism and excretion. Drugability is assessed in various ways in the art. For example, the “Lipinski Rule of 5" for determining drug-like characteristics in a molecule related to in vivo absorption and permeability can be applied [Lipinski et al., **2001**; Ghose, et al., **1999**]

The invention provides methods for combination therapy in which administration of CDH1L inhibitor is combined with administration of one or more anticancer agent which is not a CDH1L inhibitor. In embodiments, the other anticancer agents is a topoisomerase inhibitor, a platinum-based anti-neoplastic agent, a PARP inhibitor or combinations of two or more of such inhibitors and agents. In embodiments, the combination therapy combines administration of a CDH1L inhibitor with a topoisomerase inhibitor. In embodiments, the combination therapy combines administration of a CDH1L inhibitor with a platinum-based anti-neoplastic agent. In embodiments, the combination therapy combines administration of a CDH1L inhibitor with a PARP inhibitor. In embodiments, the combination therapy combines administration of a CDH1L inhibitor with a topoisomerase inhibitor and

administration of a PARP inhibitor. In embodiments, the combination therapy combines administration of a CDH1L inhibitor with chemotherapy for the specific cancer being treated. In embodiments herein, the combination of a CDH1L inhibitor and the other anti-neoplastic agent exhibits synergistic activity in combination.

In embodiments herein, therapy employing CDH1L can be combined with radiation therapy suitable for a given cancer.

Various PARP inhibitors are known in the art. [See, for example Rouleau et al., **2010**; Yi et al., **2019**; Zhou et al., **2020**; Wahlberg et al., **2012**; D'Andrea, **2018**] Each of these references is incorporated by reference herein it is entirety for descriptions of PARP inhibitors, mechanism of PARP inhibitor action, cancers treated using PARP inhibitors, and resistance to PARP inhibitors. In a specific embodiment herein, PARP-resistance cancer is treated with a combination of a CDH1L inhibitor and the PARP inhibitor.

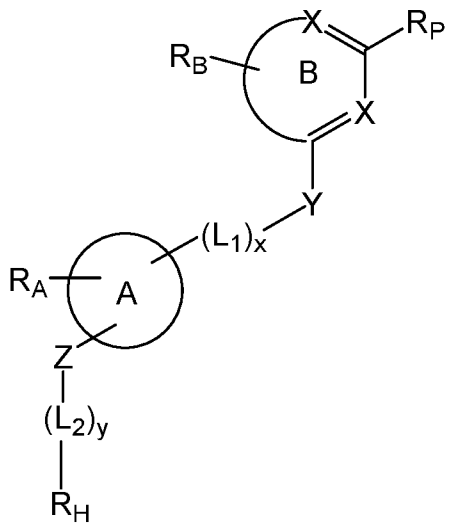
Various topoisomerase inhibitors are known in the art and have been employed clinically. (See, for example, Hevener, **2018**). This reference is incorporated by reference herein in its entirety for descriptions of types of topoisomerase inhibitors, specific topoisomerase inhibitors, mechanisms of topoisomerase inhibition, cancers treated using topoisomerase inhibitors and combination therapies using topoisomerase inhibitors. In embodiments, topoisomerase inhibitors useful in methods herein include camptothecin and prodrugs thereof, irinotecan, topotecan, belotecan, indotecan, or indimitecan. In embodiments, topoisomerase inhibitors useful in methods herein include etoposide or teniposide. In embodiments, topoisomerase inhibitors useful in methods herein include namitecan, silatecan, vosaroxin, aldoxorubicin, becatecarin, or edotecarin.

Various platinum-based anti-neoplastic agents (also called platins) are known in the art and have been employed clinically or are in clinical trials. [See, for example, Wheate et al., **2010**] his reference is incorporated by reference herein in its entirety for descriptions of types of platinum-based anti-neoplastic agents, specific platinum-based anti-neoplastic agents, mechanisms of action of such agents, cancers treated using such agents and combination therapies using platinum-based anti-neoplastic agents. In embodiments, platinum-based anti-neoplastic agents useful in methods herein include cisplatin, carbon platin,oxaliplatin, nedaplatin, lobaplatin, or heptaplatin. In embodiments, platinum-based anti-neoplastic agents include satraplatin, or picoplatin. Platinum-based anti-neoplastic agents may be liposomally encapsulated (e.g., Lypoplatin™) or bound in nanopolymers (e.g., ProLindac™).

Various thymidylate synthase inhibitors are known in the art and have been employed clinically particularly in the treatment of CRC [Papamichael, **2009;** Lehman, **2002**].

Thymidylate synthase inhibitors include folate analogues and nucleotide analogues. In specific embodiments, the thymidylate synthase inhibitor is raltitrexed, pemetrexed, nolatrexed or ZD9331. In more specific embodiments, the thymidylate synthase inhibitor is 5-fluorouracil or capecitabine.

The invention provides CHD1L inhibitors of the following formulas:
Compounds useful in the methods of this invention include those of formula I:



or salts, or solvates thereof,

where:

the B ring is a heteroaryl ring or ring system having one, two or three 5- or 6-member rings, any two or three of which can be fused rings, where the rings are carbocyclic, heterocyclic, aryl or heteroaryl rings and at least one of the rings is heteroaryl;

in the B ring, each X is independently selected from N or CH and at least one X is N;

R_P is a primary or secondary amine group [$–N(R_2)(R_3)$] or is a $–(M)_x$-P group, where P is $–N(R_2)(R_3)$ or an aryl or heteroaryl group, where x is 0 or 1 to indicate the absence or present of M and M is an optionally substituted linker $–(CH_2)_n–$ or $–N(R)(CH_2)_n–$, where each n is independently an integer from 1-6 (inclusive);

Y is a divalent atom or group selected from the group consisting of –O–, –S–, $–N(R_1)–$,

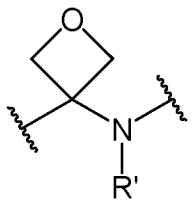
$–CON(R_1)–$, $–N(R_1)CO–$, $—SO_2N(R_1)–$, or $–N(R_1)SO_2–$;

L_1 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where x is 0 or 1 to indicate the absence or presence of L_1;

the A ring is a carbocyclic or heterocyclic ring or ring system having one, two or three rings, two or three of which can be fused, each ring having 3-10 carbon atoms and optionally 1-6 heteroatoms and wherein each ring is optionally saturated, unsaturated or aromatic;

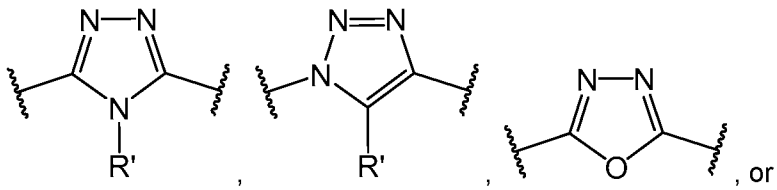
Z is a divalent group containing at least one nitrogen substituted with a R' group,

where in embodiments, Z is a divalent group selected from –N(R')–, –CON(R')–, –N(R')CO–, –CSN(R')–, –N(R')CS–, –N(R')CON(R')–, $–SO_2N(R')–$, $–N(R')SO_2–$, $–CH(CF_3)N(R')–$, $–N(R')CH(CF_3)–$, $–N(R')CH_2CON(R')CH_2–$, $–N(R')COCH_2N(R')CH_2–$,

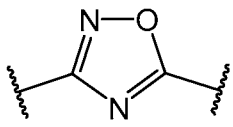


,

or the divalent Z group comprises a 5- or 6-member heterocyclic ring having at least one nitrogen ring member, for example,



, or



;

L_2 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where z is 0 or 1 to indicate the absence or presence of L_1;

R is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

each R' is independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_1 is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_2 and R_3 are independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted or

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_A and R_B represent hydrogens or 1-10 non-hydrogen substituents on the indicated A and B ring or ring systems, respectively, wherein R_A and R_B substituents are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or disubstituted amino (-NR_CR_D), alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, acyl, haloalkyl, –$COOR_C$, –$OCOR_C$, –$CONR_CR_D$, -$OCONR_CR_D$, -NR_CCOR_D, -SR_C, -SOR_C, -SO_2R_C,and –$SO_2NR_CR_D$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted;

each R_C and R_D is selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted with one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, heterocyclyl, aryl-substituted alkyl, or heterocyclyl-substituted alkyl; and

R_H is an optionally substituted aryl or heteroaryl group;

wherein optional substitution includes, substitution with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, –$COOR_E$, –$OCOR_E$, –$CONR_ER_F$, -$OCONR_ER_D$, -NR_ECOR_F, -SR_E, -SOR_E, -SO_2R_E, and –$SO_2NR_ER_F$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted and

each R_E and R_F is selected from hydrogen, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, each of which groups is optionally substituted with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4

alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, and C1-C6 acyl.

In embodiments of formula I:

R is selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted;

each R' is independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted;

R_1-R_3 are independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted;

One or more of R_1-R_3 is cycloalkyl substituted alkyl, for example, a cyclopropylmethyl, a cyclopentylmethyl, or a cyclohexylmethyl;

R is hydrogen or a C1-C3 alkyl;

each R' is independently hydrogen or C1-C3 alkyl;

R_1 is hydrogen or C1-C3 alkyl;

R_2 and R_3 are independently selected from hydrogen, or a C1-C3 alkyl; or

R_2 and R_3 together with the N to which they are attached form a 5-7member heterocycliuc ring which is saturated.

In specific embodiments of formula I:

Ring A is an optionally substituted phenyl;

Ring A is an optionally substituted naphthyl;

Ring B is an optionally substituted pyridyl,

Ring B is an optionally substituted pyrimidyl;

Ring B is an optionally substituted pyrazinyl;

Ring B is an optionally substituted triazinyl;

Ring B is an optionally substituted quinazolinyl;

Ring B is an optionally substituted pteridinyl;

Ring B is an optionally substituted quinolinyl;

Ring B is an optionally substituted isoquinolinyl;

Ring B is an optionally substituted naphthyridinyl;

Ring B is an optionally substituted pyridopyrimidyl;

Ring B is an optionally substituted pyrimidopyridyl;

Ring B is an optionally substituted pryanopyridyl;

Ring B is an optionally substituted pyranopyrimidyl;

Ring B is an optionally substituted purine;

Ring A is an optionally substituted phenyl and Ring B is an optionally substituted pyrimidinyl; or

Ring A is an optionally substituted phenyl and Ring B is an optionally substituted pteridinyl.

Preferred A and B ring substitution includes one or more C1-C3 alkyl, C3-C7 cycloalkyl, C4-C10 cycloalkyl substituted alkyl, C2-C4 alkenyl, C1-C3 alkoxy, C1-C3 acyl, halogen, hydroxyl, C1-C3 haloalkyl, mono- or disubstituted phenyl or mono- or disubstituted benzyl. More specific A and B ring substitution includes methyl, ethyl, isopropyl, cyclopropyl, cyclopropylmethyl, methoxy, ethoxy, phenyl, benzyl, halophneyl, halobenzyl, Cl, Br, F, CF_3-, HO-,CF_3O-, CH_3CO- and CHCO.

In more specific embodiments, the B ring has structure as shown in Scheme 4, formula RBI, where X^1 and X^2 are selected from CH and N and at least one of X^1 and X^2 is N and X^3-X^6 are selected from CH, CH_2, O, S, N and NH where the B ring is saturated, unsaturated or aromatic, dependent upon choice of X^1-X^6 and R_B represents optional substitution as defined for formula I. In embodiments, R_B represents hydrogens and the B ring is unsubstituted. In embodiments, R_B represents one or more halogen, C1-C3 alkyl, C1-C3 acyl, C1-C3 alkoxy. In embodiments, R_B represents one or more F, Cl or Br, methyl, ethyl, acetyl or methoxy or combinations thereof. In embodiments, of formula I the B ring is selected from any of RB2-RB5, as shown in Scheme 4.

In embodiments of formula I:

x is 1 and L_1 is $–(CH_2)_n–$, where n is 1 or 2;

x is 0 and L1 is absent;

y is 1 and L_2 is $–(CH_2)_n–$, where n is 1 or 2;

y is 1, and L_2 is -CH=CH-;

y is 1, and L_2 is trans -CH=CH-;

both of x and y are 0;

x is 1 and y is 0 and L_1 is $–(CH_2)_n–$, where n is 1 or 2;

y is 1 and x is 0 and L_2 is $–(CH_2)_n–$, where n is 1 or 2; or

both of x and y are 1 and both of L_2 and L_1 are $–(CH_2)_n–$, where n is 1 or 2.

In embodiments of formula I:

Y is –O–, –S–, $–N(R_1)–$, $–CON(R_1)–$, or $–N(R_1)CO–$;

Y is $–N(R_1)–$, $–CON(R_1)–$, or $–N(R_1)CO–$;

R_1 is hydrogen, a C1-C3 alkyl or a C1-C3 haloalkyl, particularly C1-C3 fluoroalkyl;

R_1 is hydrogen, a methyl group or CF_3-;

R_1 is hydrogen;

Y is $–N(R_1)–$, $–CON(R_1)–$, or $–N(R_1)CO–$ and R_1 is hydrogen, methyl or CF_3-;

Y is –NH–, –CONH–, or –NHCO–;

Y is $–N(R_1)–$ and R_1 is hydrogen, C1-C3 alkyl or C1-C3 haloalkyl, particularly C1-C3 fluoroalkyl; or

Y is $–N(R_1)–$ and R_1 is hydrogen, methyl or CF_3-.

In embodiments of formula I, both x and y are 0 and Y is $–N(R_1)–$. In embodiment of formula I, both x and y are 0 and Y is –NH–.

In embodiments of formula I:

Z is $–N(R')–$, $–CON(R')–$, or $–N(R')CO–$;

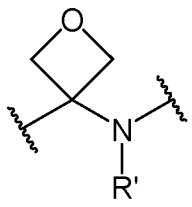
Z is $–CH(CF_3)N(R')–$;

Z is $–SO_2N(R')–$;

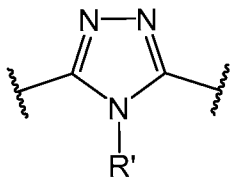
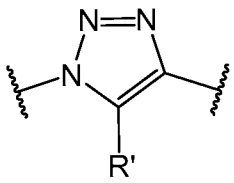
Z is $–N(R')CON(R')–$;

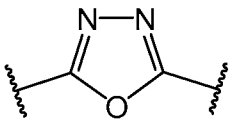
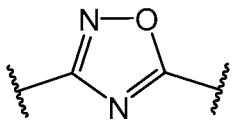
Z is $–N(R')CH_2CON(R')CH_2–$;

Z is



;

Z is  or  ;

Z is  or  ;

R' is hydrogen, a C1-C6 alkyl or a C1-C3 haloalkyl, particularly a C1-C3 fluoroalkyl;

R' is hydrogen or a C1-C3 alkyl;

R' is hydrogen, methyl or CF_3-;

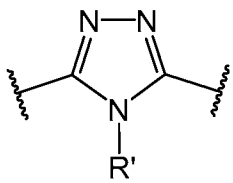
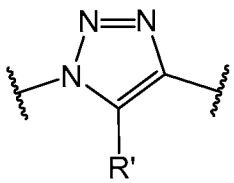
R' is hydrogen or methyl;

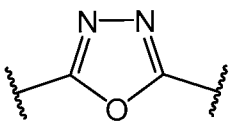
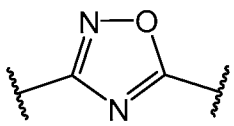
R' is hydrogen;

Z is –N(R')–, –CON(R')–, or –N(R')CO– and R' is hydrogen or methyl;

Z is –CON(R')– or –N(R')CO– and R' is hydrogen or methyl;

Z is –N(R')CON(R')– and both R' are hydrogen;

Z is  or  and R' is hydrogen; or

Z is  or  and R' is hydrogen.

In embodiments of formula I,

x is 0;

x is 1 and L_2 is $–(CH_2)_n–$, where n is 1-3;

y is 0, x is 1 and L_2 is $–(CH_2)_n–$, where n is 1-3;

x is 0 and Z is –N(R')–, –CON(R')–, or –N(R')CO–;

x is 0, Z is –N(R')–, –CON(R')–, or –N(R')CO– and R' is hydrogen or methyl;

x is 0 and Z is–CON(R')–;

x is 0, Z is–CON(R')– and R' is hydrogen or methyl;

x is 0, Z is–CON(R')– and R' is hydrogen

x is 1, L_2 is $–(CH_2)_n–$, where n is 1-3, and Z is $–N(R_1)–$, –CON(R')–, or –N(R')CO–;

x is 1, L_2 is $–(CH_2)_n–$, where n is 1-3, and Z is –CON(R')–;

x is 1, L_2 is $–CH_2–$, and Z is –CON(R')–;

x is 1, L_2 is $–CH_2-CH_2–$, and Z is –CON(R')–;

x is 0 or 1, L_2, if present, is $–CH_2–$ or $–CH_2-CH_2–$ and Z is –CON(R')–;

x is 0 or 1, L_2, if present, is $–CH_2–$ or $–CH_2-CH_2–$, Z is –CON(R')– and R' is hydrogen;

y is 0, x is 0 or 1, L_2, if present, is $–CH_2–$ or $–CH_2-CH_2–$, Z is –CON(R')– and R' is hydrogen;

y is 0, Y is $–N(R_1)–$, x is 0 or 1, L_2, if present, is $–CH_2–$ or $–CH_2-CH_2–$, and Z is –CON(R')–; or

y is 0, Y is $–N(R_1)–$, R_1 is hydrogen, x is 0 or 1, L_2, if present, is $–CH_2–$ or $–CH_2-CH_2–$, Z is –CON(R') and R' is hydrogen.

In embodiments of formula I,

R_P contains at least one nitrogen; or

when R_P is –(M)x-P, and x = 0, then P is $–N(R_2)(R_3)$ or P is a heterocyclic or heteroaryl group having at least one ring N; or

when R_P is –(M)x-P, x = 1, and M = $-(CH_2)_n$, then P is $–N(R_2)(R_3)$ or P is a heterocyclic or heteroaryl group having at least one ring N.

In embodiments of formula I, R_P is:

$–N(R_2)(R_3)$;

–(M)-N(R_2)(R_3), where M is an optionally substituted linker –(CH_2)$_n$– or –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is hydrogen or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-N(R_2)(R_3), M is an optionally substituted linker –(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is hydrogen or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-N(R_2)(R_3), M is an optionally substituted linker –(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is hydrogen or an optionally substituted alkyl group having 1-3 carbon atoms, where optional substitution is substitution with one or more halogen or one or more C1-C3 alkyl groups;

–(M)-N(R_2)(R_3), M is optionally substituted –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive and R is hydrogen) and R is hydrogen or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-N(R_2)(R_3), M is optionally substituted –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is H or an optionally substituted alkyl group having 1-3 carbon atoms, where optional substitution is substitution with one or more halogen or one or more C1-C3 alkyl groups;

–(M)-N(R_2)(R_3), where M is an optionally substituted linker –(CH_2)^n– and n is 1, 2 or 3;

–(M)-N(R_2)(R_3), where M is an optionally substituted linker –N(R)(CH_2)$_n$– and n is 1, 2 or 3;

–(M)-N(R_2)(R_3), where M is –(CH_2)$_n$– and n is 1, 2 or 3;

–(M)-N(R_2)(R_3), where M is –N(R)(CH_2)$_n$– and n is 1, 2 or 3;

–(M)$_x$-P group, where P is a aryl or heteroaryl group, where x is 0 or 1 to indicate the absence or presence of M and M is an optionally substituted linker –(CH_2)$_n$– or –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is H or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-P group, where P is a aryl or heteroaryl group, and M is an optionally substituted linker –(CH_2)$_n$– or –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is H or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-P group, where P is a aryl or heteroaryl group, and M is an optionally substituted linker –N(R)(CH_2)$_n$–, where each n is independently an integer from 1-6 (inclusive) and R is H or an optionally substituted alkyl group having 1-3 carbon atoms;

–(M)-P group, where P is a aryl or heteroaryl group, and M is an optionally substituted linker $-(CH_2)_n-$, where each n is independently an integer from 1-3 (inclusive) and R is H or an optionally substituted alkyl group having 1-3 carbon atoms;

P is an optionally substituted phenyl or naphthyl;

P is an optionally substituted phenyl or naphthyl and optional substitution is with one or more halogen, C1-C3 alkyl, C1-C3 alkoxy or C1-C3 haloalkyl;

P is an optionally substituted heteroaryl group having a 5- or 6-member ring or two fused 5- or 6-member rings;

P is an optionally substituted heteroaryl group having a 5- or 6-member ring or two fused 5- or 6-member rings and having 1 to 3 nitrogen ring members;

R_2 in R_P is hydrogen (i.e., $-N(R_2)(R_3)$ is a primary amine group);

both R_2 and R_3 in R_P are groups other than hydrogen (i.e., $-N(R_2)R_3$) is a secondary amine group);

R_2 is hydrogen and R_3 is an optionally substituted 3-8-member cycloalkyl group;

R_2 is hydrogen and R3 is a C1-C3 alkyl group substituted with a 3-8-member cycloalkyl group;

R_2 is hydrogen and R_3 is an optionally substituted aryl group having 6-12 carbon atoms;

R_2 is hydrogen and R_3 is an optionally substituted heteroaryl group having 6-12 carbon atoms and 1-3 heteroatoms (N, O, or S);

R_2 is hydrogen and R_3 is an optionally substituted heteroaryl group having 6-12 carbon atoms and 1-3 ring nitrogens;

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_P is $-(CH_2)_n-N(R_2)(R_3)$, where n is 1 or 2 and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_P is $-N(R)(CH_2)_n-N(R_2)(R_3)$, where n is 1 or 2, R is hydrogen or methyl and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_P is –M-N(R_2)(R_3) and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which contains no double bonds;

R_P is -N(R_2)(R_3) and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which contains no double bonds;

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which contains one, two or three double bonds;

R_P is -N(R_2)(R_3) and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which contains one, two or three double bonds;

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heteroaryl ring; or

R_P is -N(R_2)(R_3) and R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heteroaryl ring.

In specific embodiments of formula I, R_P or –N(R_2)(R_3) is:

any one of R_N1-R_N31 of Scheme 2;

R_N1;

R_N3;

R_N2 or R_N4;

R_N5 or R_N6;

R_N7 or R_N8;

R_N9;

R_N10;

R_N11;

R_N12;

R_N13;

R_N14;

R_N15;

R_N16;

R_N17 or R_N18;

R_N19 or R_N20;

R_N21;

R_N22;

R_N23 or R_N24

R_N25;

R_N26-R_N29;

R_N30;

R_N31;

R_N1, R_N2, R_N3, R_N4, R_N11, R_N13, or R_N14; or

R_N1-R_N31 which is unsubstituted.

In embodiments of formula I, R_H is:

optionally substituted phenyl;

unsubstituted phenyl;

optionally substituted naphthyl;

unsubstituted naphthyl;

optionally substituted naphthy-2-yl;

optionally substituted naphthy-1-yl;

naphthy-2-yl;

naphthy-1-yl;

optionally substituted thiophenyl;

halogen substituted thiophenyl;

bromine substituted thiophenyl

optionally substituted thiophen-2-yl;

halogen substituted thiophen-2-yl;

bromine substituted thiophen-2-yl

4-halothiophen-2-yl;

4-bromothiophen-2-yl;

optionally substituted furyl;

optionally substituted fur-2-yl

optionally substituted indolyl;

unsubstituted indolyl;

indol-3-yl;

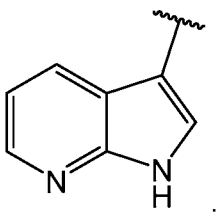
indol-2-yl;

indol-1-yl;

optionally substituted pyridinopyrrolyl;

optionally substituted pyridinopyrrol-2-yl;

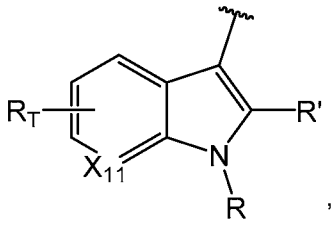
optionally substituted benzoimidazolyl;



.

In specific embodiments, optional substitution of R_H is substitution with one or more halogen, C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 haloalkyl, C1-C3 fluoroalkyl, C4-C7 cycloalkylalkyl, OH, amino, C1-C6 acyl, $-COOR_E$, $-OCOR_E$, $-CONR_ER_F$, $-OCONR_ER_D$, $-NR_ECOR_F$, $-SR_E$, $-SOR_E$, $-SO_2R_E$,and $-SO_2NR_ER_F$, where R_E and R_e are as defined above and in particular are hydrogen, C1-C3 alkyl, phenyl or benzyl. More specifically, optional substitution of R_H is substitution with one or more halogen (particularly Br or Cl), C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 fluoroalkyl (particularly CF_3-).

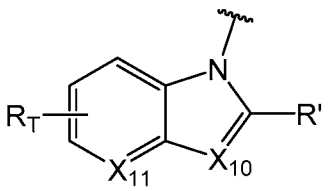
In embodiments, R_H has formula:



where:

X_{11} is CH, CR_T or N; R_T is optional R_H ring substitution as described above and R and R' are independently hydrogen, C1-C6 alkyl group, C4-C10 cycloalkylalkyl group, aryl group, heterocyclyl group, or heteroaryl group each of which groups are optionally substituted. In specific embodiments, R_T is hydrogen or substitution with one or more of halogen, OH, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; R' is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; and R is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl.

In embodiments, R_H has formula:



where:

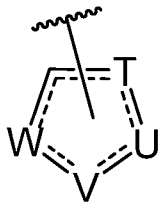
X_{11} is CH, CR_T or N; X_{10} is CH, CR_T or N; R_T is R_H ring optional substitution as described above and R and R' are independently hydrogen, C1-C6 alkyl group, C4-C10 cycloalkylalkyl group, aryl group, heterocyclyl group, or heteroaryl group each of which groups are optionally substituted. In specific embodiments, R_T is hydrogen or substitution with one or more of halogen, OH, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; R' is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; and R is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl.

In embodiments, R_H is selected from the following formulas in Scheme 3:
R12-3, R12-4, R12-5, R12-7, R12-8, R12-10, R12-23, R12-25, R12-27, R12-29, or R12-31; or
R12-12, R12-13, R2-145, R12-15, R12-16, R12-17, R12-18, R12-19, R12-20, R12-21, R12-21 or R12-22, where p is 0; or

R12-33, R12-34, R12-35, R12-36, R12-37, R12-38, R12-39 R12-40, R12-41, R12-42, where p is 0; or

R12-70 or R12-71, where p is 0.

In embodiments, R_H is selected from 5-membered heterocyclic groups of general formula:



YY1

where:

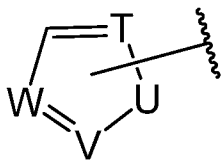
T, U, V, and W are selected from O, S, C(R")(R"), C(R")–/, C(R"), C–/, N(R"), or N–/;

where the group contains one or two double bonds dependent upon choice of T, U, V, and W;

where the R_H group is bonded to the –(L_2)y-Z–moiety in the compound of formula I through C-/, C(R")–/, or N–/; and

where R" indicates optional substitution on N or C.

More specifically, R_H is selected from 5-membered heterocyclic groups of formula:



YY2

where:

T is C(R"), C–/, or N; or

U is O, S, C(R")(R"), C(R")–/, N(R"), or N–/;

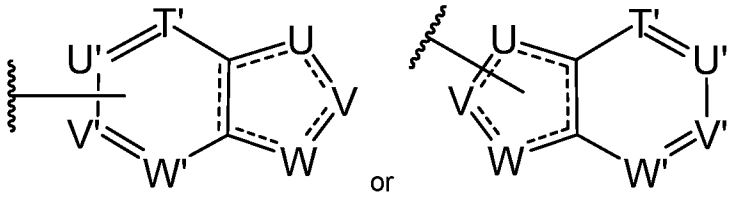
V is CR", C–/, or N and

W is CR", C–/, N, where the R_H group is bonded to the –(L_2)y-Z–moiety in the compound of formula I through C-/, C(R")–/, or N–/,

where the R_H group is bonded to the –(L_2)y-Z–moiety in the compound of formula I through C–/, C(R")–/, or N–/; and

where R" indicates optional substitution on N or C. The symbol "–/" indicates a monovalent bond through which the heterocyclic group is bonded in the compounds herein e.g., C–/ indicates a monovalent bond from a ring carbon through which the heterocyclic group is bonded into compounds herein.

In embodiments, R_H is a fused ring heterocyclic group of formula:



YY3 YY4

where:

U, V and W are selected from O, S, N, C(R'')(R''), C(R'')–/, C(R''), C–/, N(R''), or N–/;

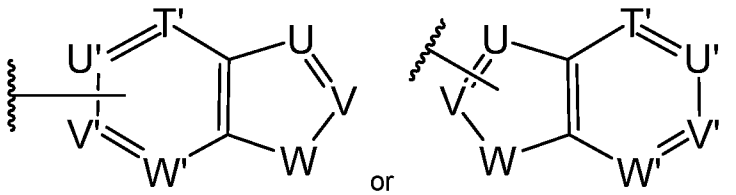
T', U', V' and W are selected from C(R''), C–/, N(R''), or N–/;

where the R_H group is bonded to the –(L_2)y-Z–moiety in the compound of formula I through C-/, C(R'')–/, or N–/ in the indicated ring;

where the group contains bonds dependent upon choice of, U, V, and W; and

where R'' indicates optional substitution on N or C.

More specifically, R_H is a fused heterocyclic group of formula:



YY5 YY6

where:

U, and V are selected from N, C(R''), or C–/,/;

W is selected from O, S, C(R'')(R''), C(R'')–/, N(R''), or N–/;

T', U', V' and W' are selected from C(R''), C–/, N(R''), or N–/;

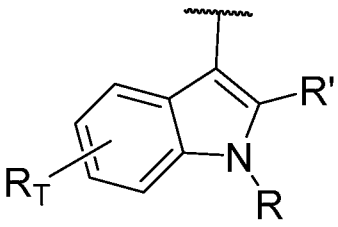
where the R_H group is bonded to the –(L_2)y-Z–moiety in the compound of formula I through C-/, C(R'')–/, or N–/ in the indicated ring; and

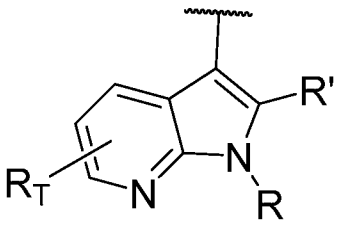
where R'' indicates optional substitution on N or C.

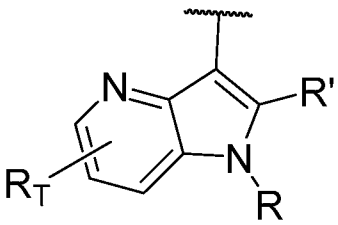
Each R'', independently, is selected from hydrogen, halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, –$COOR_E$, –$OCOR_E$, –$CONR_ER_F$, -$OCONR_ER_D$, -NR_ECOR_F, -SR_E, -SOR_E, -SO_2R_E, and –$SO_2NR_ER_F$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted;

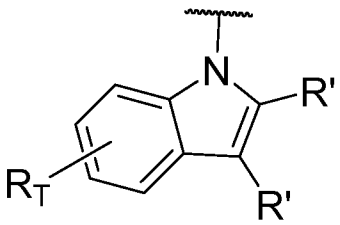
where each R_E and R_F is selected from hydrogen, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, each of which groups is optionally substituted with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, and C1-C6 acyl.

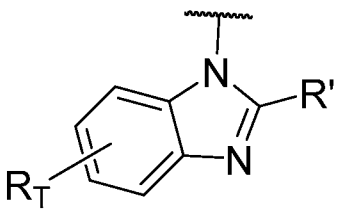
In more specific embodiments, R_H is selected from any one of:

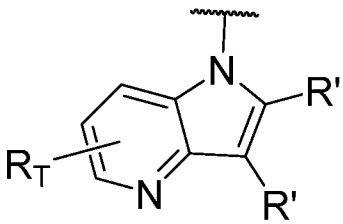

RH1

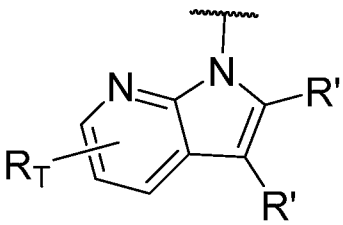

RH2

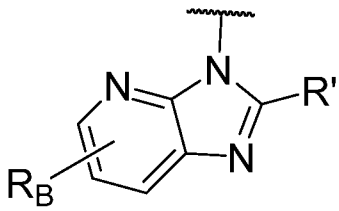

RH3

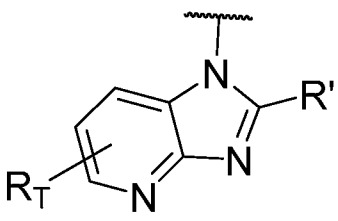

RH4

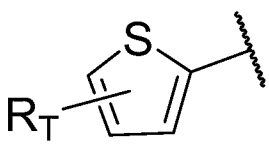

RH5

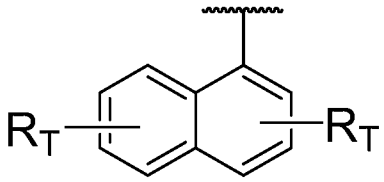

RH6


RH7

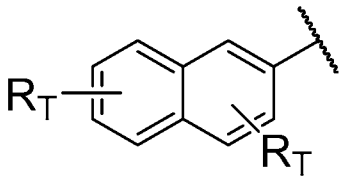

RH8


RH9


RH10



RH11



RH12,

where:

R_T is R_H ring optional substitution as described above and R and R' are independently hydrogen, C1-C6 alkyl group, C4-C10 cycloalkylalkyl group, aryl group, heterocyclyl group, or heteroaryl group each of which groups are optionally substituted. In specific embodiments, R_T is hydrogen or substitution with one or more of halogen, OH, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; R' is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl; and R is hydrogen, C1-C3 alkyl, C1-C3 alkoxy, or C1-C3 alkyl substituted with a C3-C6 cycloalkyl. In specific embodiments, R and R' are independently hydrogen, C1-C3 alkyl or C4-C7 cycloalkylalkyl. In specific embodiments, R_T represents hydrogens or substitution with one halogen, particularly Br.

In embodiments, R_H is a 6-member optionally substituted heterocyclic or heteroaryl group having 1-3 nitrogen in the ring, 1 or 2 oxygens, sulfurs or both in the ring, or 1 or 2 nitrogens and one oxygen or sulfur in the ring, where optional substitution is defined as in formula I. The heterocyclic group can be unsaturated, partially unsaturated or a heteroaryl group.

In embodiments, R_H is an optionally substituted fused heterocyclic or heteroaryl group having two fused 6-member rings having 1-5 nitrogens in the fused rings, 1-3 oxygens, sulfurs or both in the fused rings or 1-4 nitrogens and 1 or 2 oxygens, sulfurs or both in the fused rings, where optional substitution is defined as in formula I. In more specific embodiments, the fused rings have 1, 2, 3 or 4 nitrogens in the fused rings. In more specific embodiments, the fused rings have 1 or 2 oxygens or sulfurs in the fused rings. In more specific embodiments the fused rings have 1 or 2 nitrogens and one oxygen or sulfur in the fused rings. The fused ring heterocyclic group can be unsaturated, partially unsaturated or a heteroaryl group.

In specific embodiments, the R_H group is selected from phenyl, oxazinyl, pyridinyl, pyrimidinyl, thinly, pyranyl, thiazinyl, 4H-pyranyl, naphthyl, quinolinyl, isoquinolinyl, quinoxalinyl, quinazolinyl, pteridinyl, purinyl and chromanyl, where the R_H group is attached to the $-(L_2)y-Z-$moiety in the compound of formula I at any available ring position. In specific embodiments, the R_H group is attached to the $-(L_2)y-Z-$moiety in the compound of formula I at a carbon in the ring.

In specific embodiments of formula I, -Z-(L_2)y-R_H is a group other than –NH-SO_2-R_W, where R_W is R_1 is mes-trimethylphenyl, 4-methylphenyl, 4-trifluoromethylphenyl, naphthyl, 2,3,4,5,-tetramethylphenyl, 4-methoxyphenyl, 4-tert-butylphenyl, 2,4-dimethoxyphenyl, 2,5-dimethoxyphenyl or 4-phenoxypheny. In specific embodiments of formula I, -Z-(L_2)y- is a moiety other than –NR_X-SO_2-, where R_X is H, hydrogen, methyl acetate, acetate, aminoacetyl, 4-formic acid benzyl, 4-isopropylbenzyl, 4-chlorobenzyl or 4-methoxybenzyl. In embodiments of formula I, -Z- is other than –NR_X-SO_2-, where R_X is H, hydrogen, methyl acetate, acetate, aminoacetyl, 4-formic acid benzyl, 4-isopropylbenzyl, 4-chlorobenzyl or 4-methoxybenzyl.

In embodiments of formula I, R_H is other than a phenyl group or an optionally substituted phenyl group. I_N embodiments of formula I, R_H is a heterocyclic group that is substituted with a single halogen, particularly a Br.

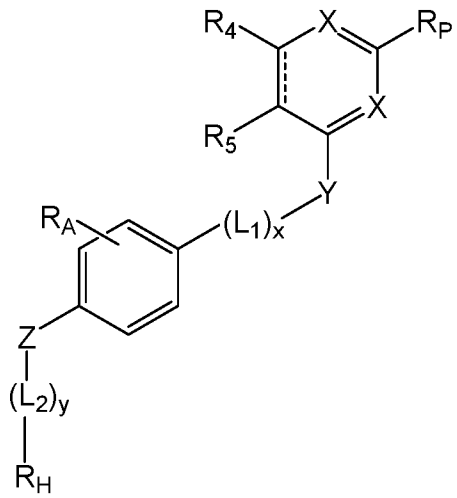
In embodiments of formula I, R_P or –N(R_2)(R_3) are optionally substituted amine groups illustrated in Scheme 2, R_N1-R_N31. Exemplary optional substitution of groups is illustrated in Scheme 2. The illustrated R substituent groups can be positioned on any available ring position. In the moieties of Scheme 2, preferred alkyl are C1-C3 alkyl, acyl includes formyl, preferred acyl are C1-C6 acyl and more preferably acetyl, hydroxyalkyl are C1-C6 hydroxyalkyl and preferably are –CH_2-CH_2-OH, for amine groups, preferred alkyl are C1-C3 alkyl, preferred alkyl for –SO_2alkyl are C1-C3 alkyl and more preferred is methyl.

In specific embodiments of formula I, –N(R_2)(R_3) is R_N1. In specific embodiments, –N(R_2)(R_3) is R_N3. In specific embodiments, –N(R_2)(R_3) is R_N2 or R_N4. In specific embodiments, –N(R_2)(R_3) is R_N5 or R_N6. In specific embodiments, –N(R_2)(R_3) is R_N7 or R_N8. In specific embodiments, –N(R_2)(R_3) is R_N9. In specific embodiments, –N(R_2)(R_3) is R_N10. In specific embodiments, –N(R_2)(R_3) is R_N11. In specific embodiments, –N(R_2)(R_3) is R_N12. In specific embodiments, –N(R_2)(R_3) is R_N13. In specific embodiments, –N(R_2)(R_3) is R_N14. In specific embodiments, –N(R_2)(R_3) is R_N15. In specific embodiments, –N(R_2)(R_3) is R_N16. In specific embodiments, –N(R_2)(R_3) is R_N17 or R_N18. In specific embodiments, –N(R_2)(R_3) is R_N19 or R_N20. In specific embodiments, –N(R_2)(R_3) is R_N21. In specific embodiments, –N(R_2)(R_3) is R_N22. In specific embodiments, –N(R_2)(R_3) is R_N23 or R_N24. In specific embodiments, –N(R_2)(R_3) is R_N25. In an embodiment, –N(R_2)(R_3) is R_N1, R_N2, R_N3, R_N4, R_N11, R_N13, or R_N14. In an embodiment, –N(R_2)(R_3) is R_N26-R_N29. In an embodiment, –N(R_2)(R_3) is R_N30. In an embodiment, –N(R_2)(R_3) is R_N31.

In embodiments of formula I, R_H is a moiety illustrated in Scheme 3 R12-1 to R12-69. In an embodiment, R_H is R12-35-R12-42. In embodiments, R_H is any of R12-43-R12-69. In

embodiments, R_H is any of R12-43-R12-45. In embodiments, R_H is any of R12-46-R12-48. In embodiments, R_H is any of R12-49-R12-51. In embodiments, R_H is any of R12-52-R12-54. In embodiments, R_H is any of R12-55-R12-58. In embodiments, R_H is any of R12-59-R12-62 In embodiments, R_H is any of R12-63-R12-66. In embodiments, R_H is any of R12-67-R12-69. In moieties of Scheme 3, preferred alkyl groups are C-C6 alkyl groups or more preferred C1-C3 alkyl groups, preferred halogen are F, Cl and Br, acyl includes formyl and preferred acyl are –CO-C1-C6 alky and more preferred is acetyl, phenyl is optionally substituted with one or more halogen, alkyl or acyl.

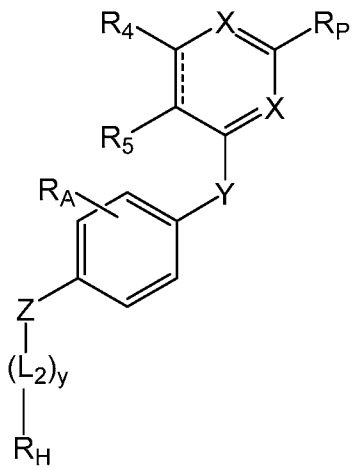
In specific embodiments, compounds useful in the methods herein include those of formula II:



or salts, or solvates thereof,

where variables are as defined in formula I and the dotted line represents a single or double bond. In embodiments, x is 1, and y is 1. In embodiments, both X are nitrogens. In embodiments, R_P is $–N(R_2)(R_3)$. In embodiments, L_1 and L_2 are $–(CH_2)n–$, where n are independently is 1, 2 or 3. In embodiments, R_H is a heterocyclic or heteroaryl group. In embodiments, Y is $–N(R_1)–$, $–CON(R_1)–$, or $–N(R_1)CO–$. In embodiments, Z is $–CON(R')–$ or $–N(R')CO–$. In embodiments, R' is hydrogen, a C1-C3 alkyl or a Ci-C3 haloalky. In embodiments, R' is hydrogen, methyl or trifluoromethyl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula III:

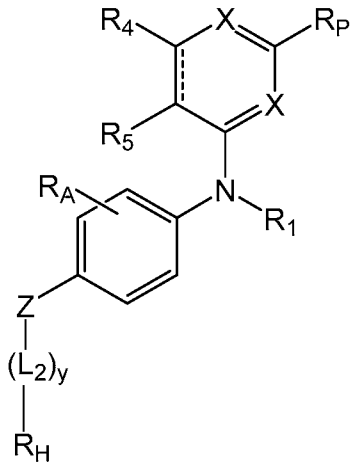


or salts, or solvates thereof,

where variables are as defined in formula I and the dotted line represent a single or double bond.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, R_P is $–N(R_2)(R_3)$. In embodiments, L_2 is $–(CH_2)n–$, where n is 1, 2 or 3. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments, Y is $–N(R_1)–$, $–CON(R_1)–$, or $–N(R_1)CO–$. In embodiments, Z is –CON(R')– or –N(R')CO–. In embodiments, R' is hydrogen, a C1-C3 alkyl or a Ci-C3 haloalky. In embodiments, R' is hydrogen, methyl or trifluoromethyl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula IV:

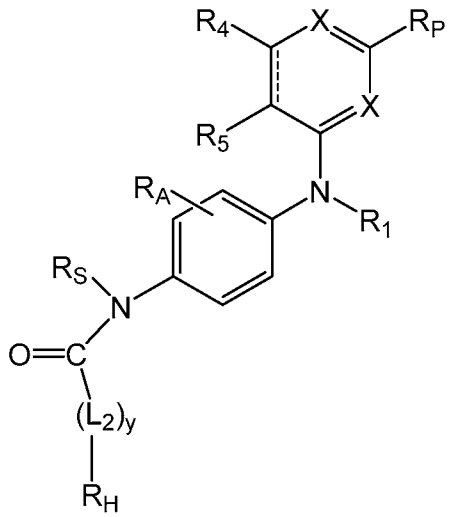


or salts or solvates thereof;

where variables are as defined in formula I and the dotted line represents a single or double bond.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, R_P is $–N(R_2)(R_3)$. In embodiments, L_2 is $–(CH_2)n–$, where n is 1, 2 or 3. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments R_1 is hydrogen In embodiments, R1 is hydrogen, methyl or trifluoromethyl. In embodiments, Z is –CON(R')– or –N(R')CO–. In embodiments, R' is hydrogen, a C1-C3 alkyl or a Ci-C3 haloalky. In embodiments, R' is hydrogen, methyl or trifluoromethyl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula V:

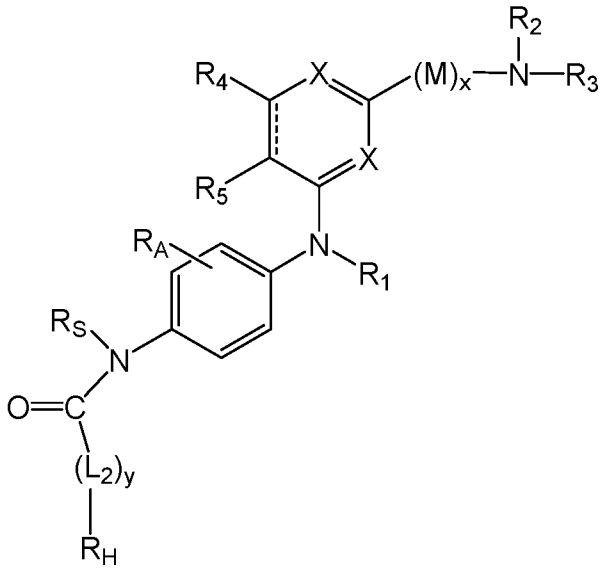


or salts or solvates thereof;

where variables are as defined in formula I and the dotted line represents a single or double bond.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, R_P is $–N(R_2)(R_3)$. In embodiments, L_2 is $–(CH_2)n–$, where n is 1, 2 or 3. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, Rs is hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula VI:

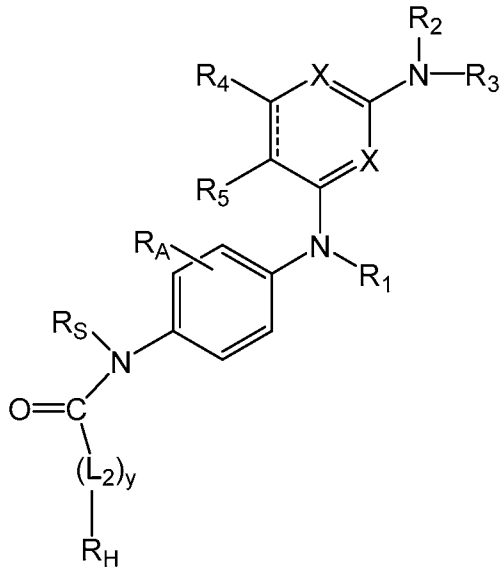


or salts or solvates thereof;

where variables are as defined in formula I and the dotted line represents a single or double bond.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, y is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, Rs is hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula VII:

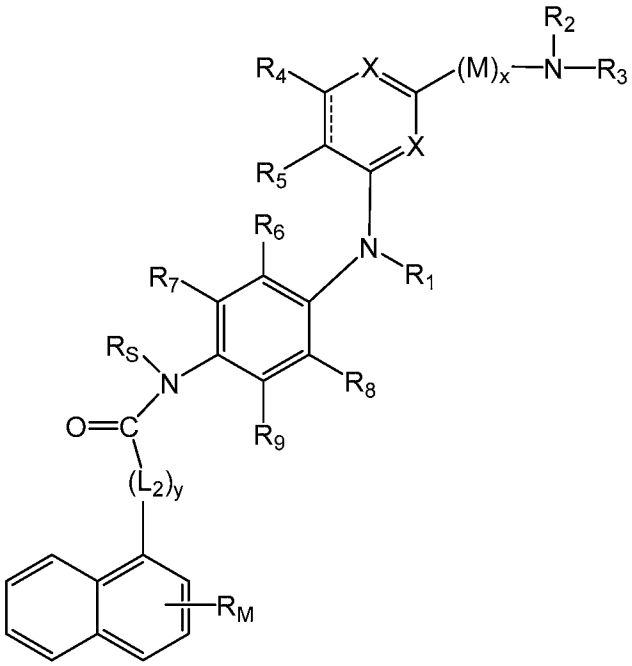


or salts or solvates thereof;

where variables are as defined in formula I and the dotted line represents a single or a double bond.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, y is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, Rs is hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_A is hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R' is hydrogen, methyl, methoxy or trifluoromethyl. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_H is any one of RH1-RH12.

In specific embodiments, compounds useful in the methods herein include those of formula VIII:



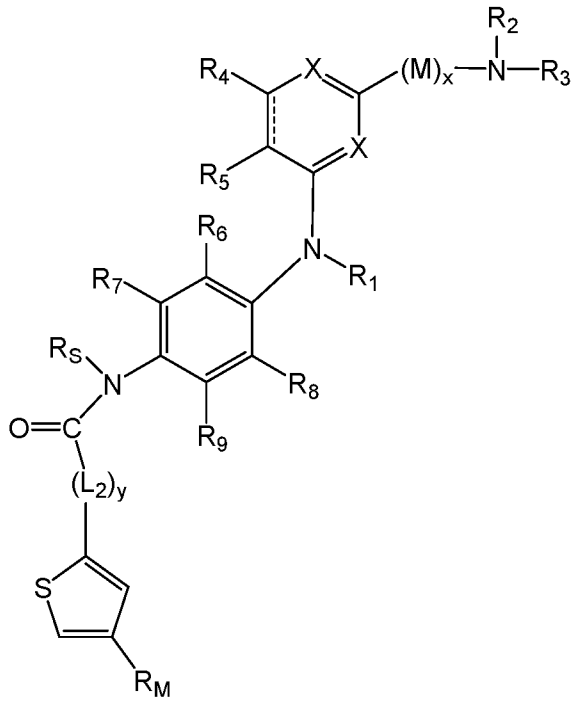
or salts or solvates thereof;

where variables are as defined in formula I, the dotted line represents a single or a double bond, R_6-R_9 are independently selected from hydrogen and R_A groups defined in formula I. R_M represents optional substitution on the fused ring and R_M takes the values of R_A in formula I.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, R_7-R_9 are independently selected from hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_7-R_9 are independently selected from hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R_7-R_9 are all hydrogens. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_M is one or more hydrogen, halogen, C1-C3 alkyl group, C4-C7 cycloalkylalkyl group or C1-C3 haloalkyl group. In embodiments, R_M is one or more

hydrogen, halogen, particularly Br, methyl or trifluoromethyl. In embodiments, R_M is hydrogen.

In specific embodiments, compounds useful in the methods herein include those of formula IX:

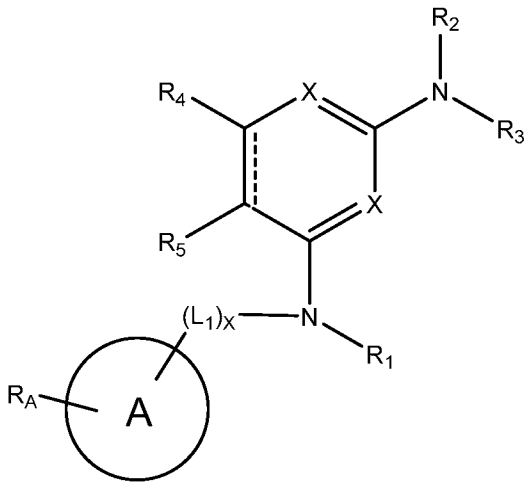


or salts or solvates thereof; where variables are as defined in formula I, the dotted line represents a single or a double bond. R_6-R_9 are independently selected from hydrogen and R_A groups defined in formula I and R_M represents optional substitution as defined in formula I.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and M is –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 1 and M is -(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, R_7-R_9 are independently selected from hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_7-R_9 are independently selected from hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R_7-R_9 are all hydrogens. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_M is

hydrogen, halogen, C1-C3 alkyl group or C1-C3 haloalkyl group. In embodiments, R_M is hydrogen, halogen, particularly Br, methyl or trifluoromethyl.

In other embodiments, the invention provides a compound of formula XI:



or salts, or solvates thereof,

where:

each X is independently selected from N or CH and at least one X is N;

the A ring is a carbocyclic or heterocyclic ring having 3-10 carbon atoms and optionally 1-6 heteroatoms and which optionally is saturated, unsaturated or aromatic;

L_1 is an optional 1-3 carbon linker that is optionally substituted, where x is 0 or 1 to indicate the absence of presence of L_1;

R_1 is selected from the group consisting of hydrogen, alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted;

R_2 and R_3 are independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, each of which groups is optionally substituted or

R_2 and R_3 together form an optionally substituted 5- to 8-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_4 and R_5 are independently selected from hydrogen, halogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, each of which groups is optionally substituted or

R_4 and R_5 together form an optionally substituted 5- or 6-member ring which optionally contains one or two double bonds or is aromatic and optionally contains 1-3 heteroatoms;

where the dotted line is a single or double bond dependent upon selection of R_4 and R_5;

and

R_A represents hydrogens or 1-10 substituents on the indicated ring, wherein R_A substituents are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or disubstituted amino, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, $-OR_{15}$, $-COR_{15}$, $-COOR_{15}$, $-OCOR_{15}$, $-CO-NR_{16}R_{17}$, $-OCON\ R_{16}R_{17}$, $-NR_{16}-CO-R_{15}$, $-SR_{15}$, $-SOR_{15}$, $-SO_2R_{15}$, $-SO_2-NR_{16}R_{17}$, R10, –NH-CO-$(L_2)_y$-R_{12}, or –NH-CO-$(L_2)_y$-R_{12}, where L_2 is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two carbons of the liker are optionally replaced with O or S, where y is 0 or 1 to show the absence or presence of L_2;

R_{10} is selected from alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, or aryl, each of which groups is optionally substituted with one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, heterocyclyl, aryl-substituted alkyl, or heterocyclyl-substituted alkyl;

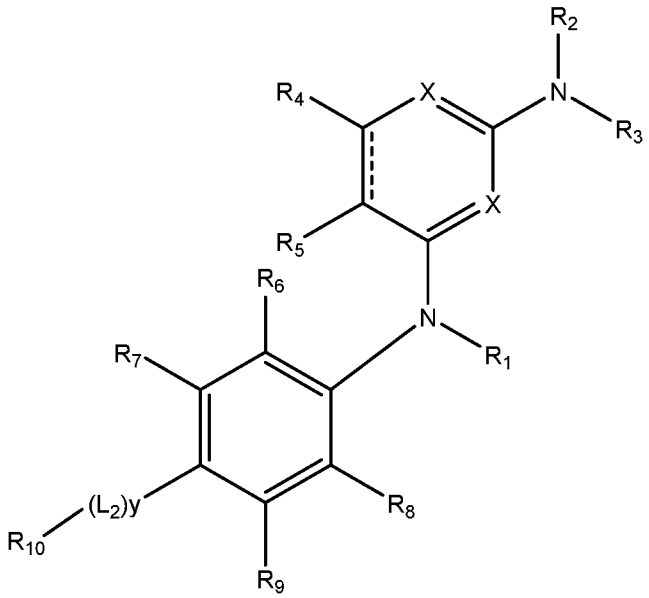
R_{12} is selected from cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, or aryl, each of which groups is optionally substituted, or R_{12} is a C1-C3 alky substituted with cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, or aryl each of which is optionally substituted and where optional substitution is one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, or heterocyclyl;

each R_{15} is independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, arylalkyl (an alkyl group substituted with an aryl) and heterocyclylalkyl, cycloalkylalkyl, cycloalkenylalkyl, each of which groups is optionally substituted; and

each R_{16} and R_{17} is independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, arylalkyl (an alkyl group substituted with an aryl) and heterocyclylalkyl, cycloalkylalkyl, cycloalkenylalkyl, each of which groups is optionally substituted;

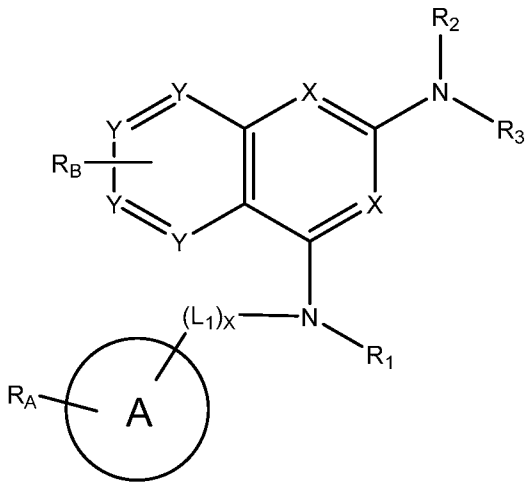
wherein optional substitution includes substitution with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C6-C12 aryl, and C6-C12 heterocyclyl.

In an embodiment, the compound has formula XII:



or a salt or solvate thereof where variables are as defined for formula XI.

In an embodiment, the compound has formula XIII:



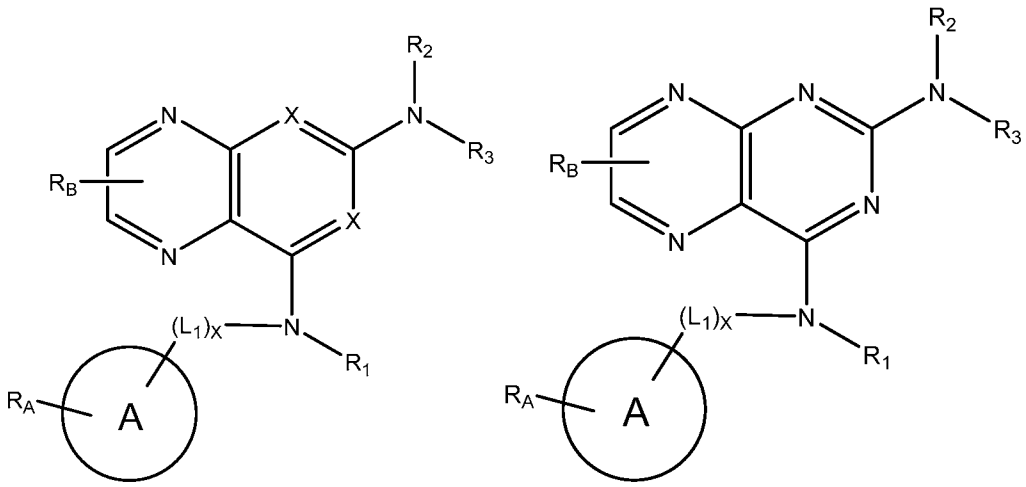
or a salt, or a solvate thereof, wherein variables are as defined in formula XI and where;

each Y is independently selected from N or CH;

R_B represents hydrogens or 1-10 substituents on the indicated ring, wherein R_A substituents are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or disubstituted amino, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, -OR_{15}, -COR_{15},

-$COOR_{15}$, -$OCOR_{15}$, -CO-$NR_{16}R_{17}$, -OCON $R_{16}R_{17}$, -NR_{16}-CO-R_{15}, -SR_{15}, -SOR_{15}, -SO_2R_{15}, -SO_2-$NR_{16}R_{17}$, or -$(L_2)_y$-R_{10}, where L_2 is an optional 1-6 carbon atom linker group which linker is optionally substituted, and where y is 0 or 1 to show the absence or presence of L_2.

In embodiments, the compound has formula XIV or XV:

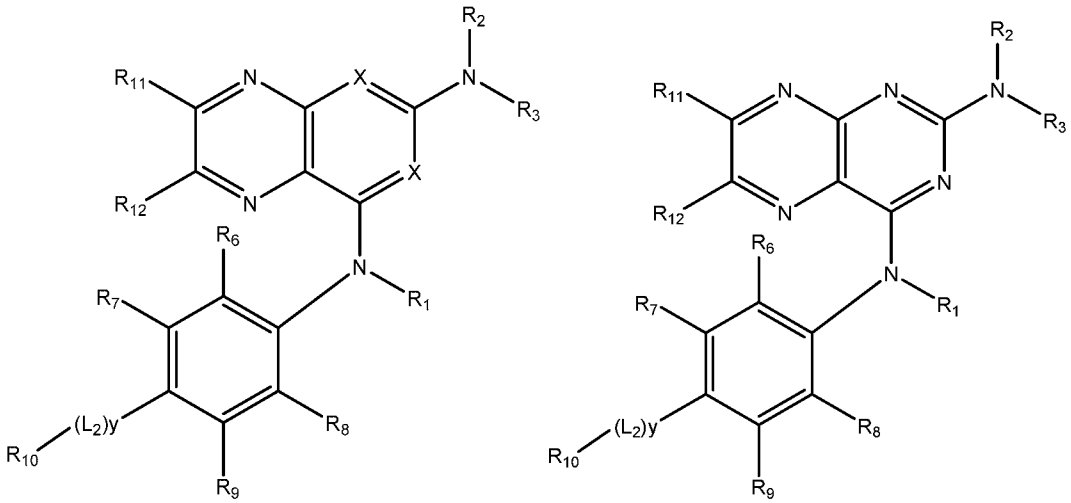


XIV XV

or a salt or solvate thereof,

where variables are as defined in formula XI, XII or XIII.

In embodiments, the compound has formula XVI or XVII:



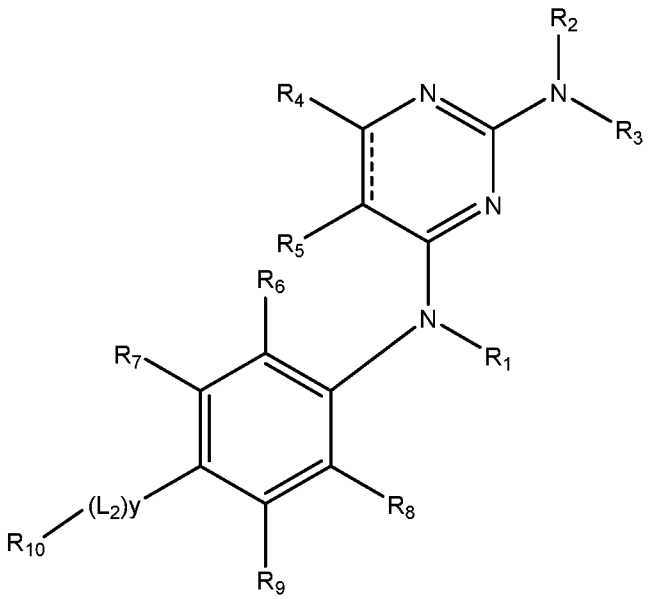
XVI XVII

or a salt or solvate thereof,

where variables are as defined in formula XI or XV, and

R_{11} and R_{12} are independently selected from hydrogen, halogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, or heterocyclyl group, each of which groups is optionally substituted.

In embodiments, the compound has formula XVIII:



or salts (or solvates) thereof,

wherein:

R_1 is selected from the group consisting of hydrogen, alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted (need to define substitution);

R_2 and R_3 together form an optionally substituted 5- or 6-member heterocyclic ring which can contain one or two double bonds or be aromatic;

R_4 and R_5 are independently selected from hydrogen, halogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, or heterocyclyl group, each of which groups is optionally substituted or

R_4 and R_5 together form an optionally substituted 5- or 6-member heterocyclic ring which can contain one or two double bonds or be aromatic;

the dotted line is a single or double bond dependent upon choice of R_4 and R_5;

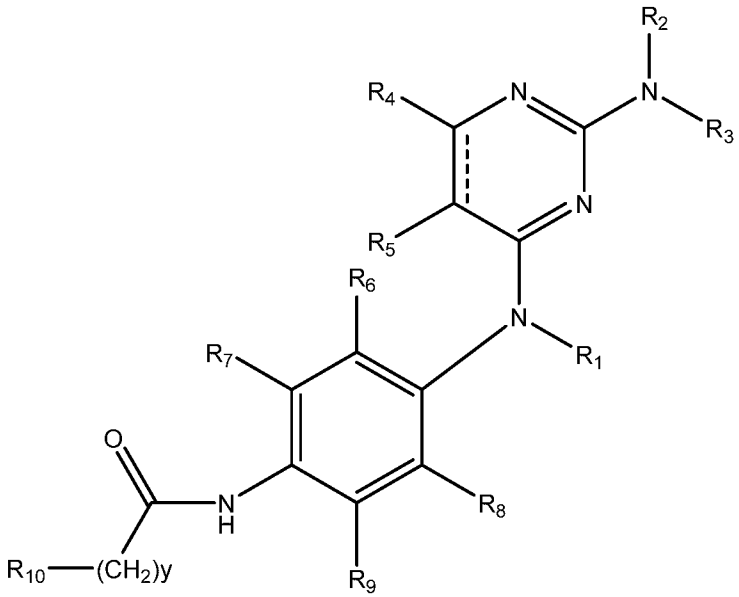
R_6-R_9 are independently selected from hydrogen, halogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, or heterocyclyl group, each of which groups is optionally substituted;

L is an optional 1-6 atom linker group, where x is 1 or 0 to show the presence or absence of the L group; and

R_{10} is selected from alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted.

For example, L is a 2-6 atom linker group; (e.g., --CH_2-O-, -CH_2-CH_2-O-, -O-CH_2-, -O-CH_2-CH_2-, -CO-NH-, --NH-CO-, -CH_2-CO-NH-, -CH_2-CH_2-CO-NH-)

In an embodiment, the compound is of formula XIX:



or salts (or solvates) thereof,

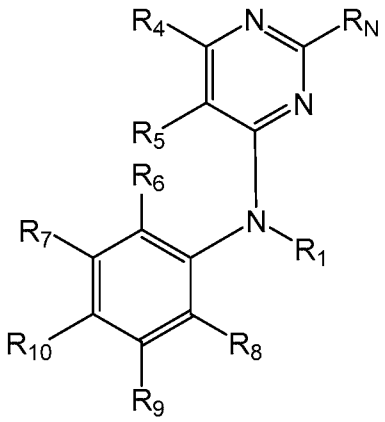
where:

R_1-R_9 are as defined above; the dotted line represents a single or double bond dependent on choice of R_4 and R_5;

y is 0 or an integer ranging from 1-3 inclusive; and

R_{10} is selected from alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted.

In embodiments, the CDH1L inhibitor is a compound of formula XX:



and salts or solvates thereof, where R_1-R_9 represent hydrogen or optional substituents, R_{10} is a moiety believed to be associated with potency; and R_N is a moiety believed to be associated with physicochemical properties such as solubility. In embodiments, R_5 is a substituent other than hydrogen which is believed to be associated with metabolic stability. In specific embodiments, R_5 is a halogen, particularly F or Cl, a C1-C3 alkyl group, particularly a methyl group. In embodiments, R_4 is a substituent other than hydrogen and in particular is a C1-C3 alkyl group, and more particularly is a methyl group. In a specific embodiment, R_5 is F and R_4 is methyl. In embodiments, R_6-R_9 are selected from hydrogen, C1-C3-alkyl, halogen, hydroxyl, C1-C3 alkoxy, formyl, or C_1-C_3 acyl. In embodiments, one or two of R_6-R_9 are moieties other than hydrogen. In an embodiment, one of R_6-R_9 is a halogen, particularly fluorine. In specific embodiments, all of R_6-R_9 are hydrogen. In embodiments, R_N is an amino moiety –N(R_2)(R_3). In specific embodiments, R_N is an optionally substituted heterocyclic group having a 5- to 7- member ring optionally containing a second heteroatoms (N, S or O). In embodiments, R_N is optionally substituted pyrrolidin-1-yl, piperidin-1-yl, azepan-1-yl, piperazin-1-yl, or morpholino. In R_N is substituted with one substituent selected from C1-C3 alkyl, formyl, C1-C3 acyl (particularly acetyl), hydroxyl, halogen (particularly F or Cl), hydroxyC1-C3 alkyl (particularly –CH_2-CH_2-OH). In embodiments, R_N is unsubstituted pyrrolidin-1-yl, piperidin-1-yl, azepan-1-yl, piperazin-1-yl, or morpholino.

In embodiments, R_{10} is –NRy-CO-(L_2)y-R_{12} or –CO-NRy--(L_2)y-R_{12}, where y is 0 or 1 to indicate the absence of presence of L_2 which is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two, carbons of the linker are optionally replaced with O, NH, NRy or S, where Ry is hydrogen or a 1-3 carbon alkyl, and R_{12} is an aryl group, cycloalkyl group, heterocyclic group, or heteroaryl group, each of which

is optionally substituted. I_N embodiments, y is 1. L_2 is $–(CH_2)p-$, where p is 0-3. In embodiments, R_{12} is thiophen-2-yl, thiophen-3-yl, furany-2-yl, furan-3-yl, pyrrol-2-yl, pyrrol-3-yl, oxazol-4-yl, oxazol-5-yl, oxazol-2-yl, indol-2-yl, indol-3-yl, benzofuran-2-yl, benzofuran-3-yl, benzo[b]thiophen-2-yl, benzo[b]thiophen-3-yl, isobenzofuran-1-yl, isoindol-1-yl, or benzo[c]thiophen-1-yl. In embodiments, R_1 is hydrogen or methyl. In embodiments, R_{12} is thiophen-2-yl, furany-2-yl, pyrrol-2-yl, oxazol-4-yl, indol-2-yl, benzofuran-2-yl, or benzo[b]thiophen-2-yl. In embodiments, R_{12} is thiophen-2-yl or indol-2-yl. In embodiments, R_1 is hydrogen or methyl.

In more general embodiments of formula XX:

R_1 is selected from the group consisting of hydrogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted;

R_N is $–NR_2R_3$, R_2 and R_3 are independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, each of which groups is optionally substituted or R_2 and R_3 together form an optionally substituted 5- to 8- member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_4 $–R_9$ are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or dialkyl substituted amino, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted cycloalkyl, optionally substituted cycloalkenyl, optionally substituted aryl, optionally substituted heterocyclyl, $-OR_{15}$, $-COR_{15}$, $-COOR_{15}$, $-OCOR_{15}$, $-CO-NR_{15}R_{16}$, $-OCONR_{15}R_{16}$, $-NR_{15}-CO-R_{16}$, $-SR_{15}$, $-SOR_{15}$, $-SO_2R_{15}$, and $-SO_2-NR_{15}R_{16}$;

R_{10} is $–NRy-CO-(L_2)y-R_{12}$, $-CO-NRy-(L_2)y-R_{12}$, where L_2 is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two, carbons of the liker are optionally replaced with O or S, where y is 0 or 1 to show the absence or presence of L_2;

R_{12} is selected from cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, or aryl, each of which groups is optionally substituted, or R_{12} is a C1-C3 alky substituted with cycloalkyl, cycloalkenyl, heterocyclyl, heteroaryl, or aryl each of which is optionally substituted and where optional substitution is one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, or heterocyclyl;

each R_{15} and R_{16} is independently selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl, arylalkyl and heterocyclylalkyl, cycloalkylalkyl, cycloalkenylalkyl, each of which groups is optionally substituted; and

wherein optional substitution includes, substitution with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C6-C12 aryl, C6-C12 heterocyclyl, -OR_{17}, -COR_{17}, -$COOR_{17}$, -$OCOR_{17}$, -CO-$NR_{17}R_{18}$, -$OCONR_{17}R_{18}$, -NR_{17}-CO-R_{18}, -SR_{17}, -SOR_{17}, -SO_2R_{17}, and -SO_2-$NR_{17}R_{18}$, where R_{17} and R_{18} are independently hydrogen or a C_1-C_6 alkyl.

In embodiments of formula XX, R_N is an optionally substituted cyclic amine group selected from any of R_N1-R_N31 (Scheme 2). Exemplary optional substitution of groups is illustrated in Scheme 2. The illustrated R substituent groups can be positioned on any available ring position. In the moieties of Scheme 2, preferred alkyl are C1-C3 alkyl, acyl includes formyl, preferred acyl are C1-C6 acyl and more preferably acetyl, hydroxyalkyl are C1-C6 hydroxyalkyl and preferably are –CH_2-CH_2-OH, for amine groups, preferred alkyl are C1-C3 alkyl, preferred alkyl for –SO_2alkyl are C1-C3 alkyl and more preferred is methyl.

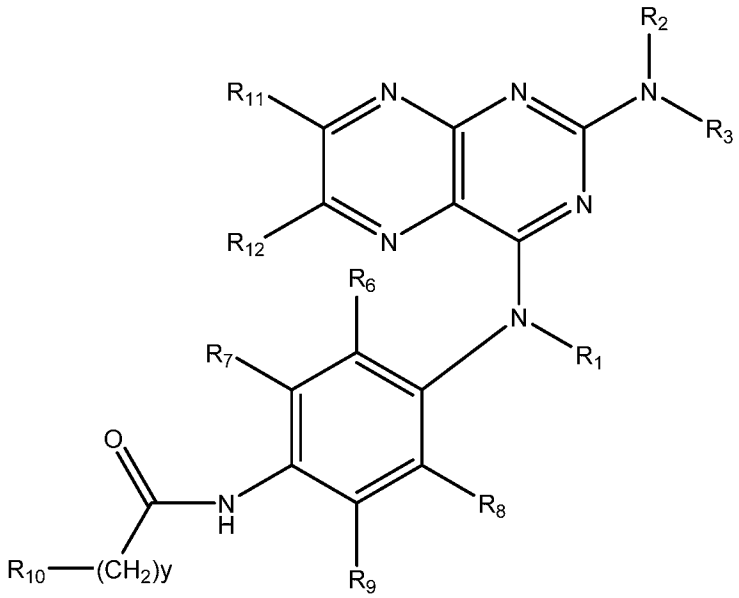
In specific embodiments of formula XX, R_N is R_N1. In specific embodiments, R_N is R_N3. In specific embodiments, R_N is R_N2 or R_N4. In specific embodiments, R_N is R_N5 or R_N6. In specific embodiments, R_N is R_N7 or R_N8. In specific embodiments, R_N is R_N9. In specific embodiments, R_N is R_N10. In specific embodiments, R_N is R_N11. In specific embodiments, R_N is R_N12. In specific embodiments, R_N is R_N13. In specific embodiments, R_N is R_N14. In specific embodiments, R_N is R_N15. In specific embodiments, R_N is R_N16. In specific embodiments, R_N is R_N17 or R_N18. In specific embodiments, R_N is R_N19 or R_N20. In specific embodiments, R_N is R_N21. In specific embodiments, R_N is R_N22. In specific embodiments, R_N is R_N23 or R_N24. In specific embodiments, R_N is R_N25. In an embodiment, R_N is R_N1, R_N2, R_N3, R_N4, R_N11, R_N13, or R_N14. In an embodiment, R_N is R_N26-R_N29. In an embodiment, R_N is R_N30. In an embodiment, R_N is R_N31.

In embodiments of formula XX, R_{12} is an optionally-substituted thienyl, thienylmethyl, furyl, furylmethyl, indolyl or methylindolyl. In embodiments, R_{12} is a moiety illustrated in Scheme 3 R12-1 to R12-69. In moieties of Scheme 3, preferred alkyl groups are C-C6 alkyl groups or more preferred C1-C3 alkyl groups, preferred halogen are F, Cl and Br, acyl includes formyl and preferred acyl are –CO-C1-C6 alky and more preferred is acetyl, phenyl is optionally substituted with one or more halogen, alkyl or acyl. In embodiments, R_{12} is a methyl, ethyl group or propyl substituted with a moiety as illustrated in Scheme 3 R12-1 to R12-22. In an embodiment, R_{12} is R12-1. In an embodiment, R_{12} is R12-2. In an embodiment, R_{12} is R12-3. In an embodiment, R_{12} is R12-4. In an embodiment, R_{12} is R12-5. In an embodiment, R_{12} is R12-6. In an embodiment, R_{12} is R12-7. In an embodiment, R_{12} is R12-8. In an embodiment, R_{12} is R12-9. In an embodiment, R_{12} is R12-10. In an embodiment, R_{12} is R12-11. In an embodiment, R_{12} is R12-12. In an embodiment, R_{12} is R12-13. In an embodiment,

R_{12} is R12-14. In an embodiment, R_{12} is R12-15. In an embodiment, R_{12} is R12-16. In an embodiment, R_{12} is R12-17. In an embodiment, R_{12} is R12-18 In an embodiment, R_{12} is R12-19. In an embodiment, R_{12} is R12-20. In an embodiment, R_{12} is R12-21. In an embodiment, R_{12} is R12-22. In an embodiment, R_{12} is R12-23-R12-26. In an embodiment, R_{12} is R12-27-R12-30. In an embodiment, R_{12} is R12-31-R12-34. In an embodiment, R_{12} is R12-35-R12-42. In embodiments, R12 is any of R12-43-R12-69. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-43-R12-69. In embodiments, R12 is any of R12-43-R12-45. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-43-R12-45. In embodiments, R12 is any of R12-46-R12-48. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-46-R12-48. In embodiments, R12 is any of R12-49-R12-51. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-49-R12-51. In embodiments, R12 is any of R12-52-R12-54. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-52-R12-54. In embodiments, R12 is any of R12-55-R12-58. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-55-R12-58. In embodiments, R12 is any of R12-59-R12-62 In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-59-R12-62. In embodiments, R12 is any of R12-63-R12-66. In embodiments, R_{12} is a methyl, ethyl group or propyl group substituted with a moiety as illustrated in Scheme 3 R12-63-R12-66. In embodiments, R12 is any of R12-67-R12-69. In embodiments, R_{12} is a methyl, ethyl or propyl group substituted with a moiety as illustrated in Scheme 3 R12-67-R12-69. In embodiments, R12 is a moiety as illustrated in Scheme 3 R12-70 or R12-71.

In embodiments herein of formula XX, R_N is an optionally substituted cyclic amine group selected from any of R_N1-R_N25 (Scheme 2) and R_{12} is a thienyl, thienylmethyl, furyl, furylmethyl, indolyl or methylindoyl. In embodiments of formula XX, R_N is R_N1, R_N2, R_N3, R_N4, R_N11, R_N13, R_N14 or R_N25 and R_{12} is a thienyl, thienylmethyl, furyl, furylmethyl, indolyl or methylindoyl. In embodiments, R_{10} is –NHCOR_{12}. In embodiments, R_{10} is –CONHR_{12}. In embodiments herein of formula XX, R_{10} is –CO-NH-R_{12} and R_N is any one of R_N1-R_N25 and R_{12} is any one of R12-1-R12-22. In embodiments herein of formula XX, R_{10} is –CO-NH-R_{12} and R_N is any one of R_N1-R_N25 and R_{12} is any one of R12-1-R12-69.

In embodiments, the compound is of formula XXX:



or salts (or solvates) thereof,

wherein:

R_1 is selected from the group consisting of hydrogen, alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted (need to define substitution);

R_2 and R_3 together form an optionally substituted 5- or 6-member heterocyclic ring which can contain one or two double bonds or be aromatic;

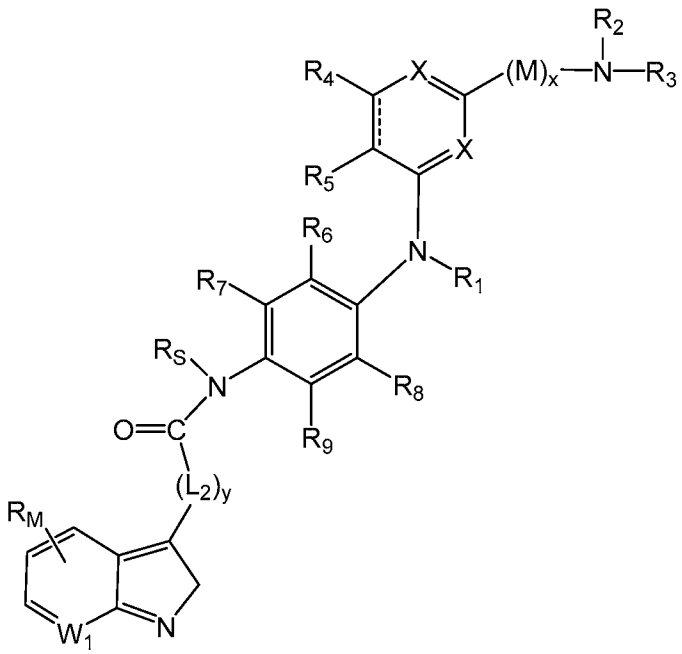
R_6-R_9 are independently selected from hydrogen, halogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, or heterocyclyl group, each of which groups is optionally substituted;

Y is 0 or an integer ranging from 1-3 inclusive;

R_{10} is selected from alkyl group. alkenyl group, cycloalkyl group, cycloalkenyl group, heterocyclyl group, or aryl group, each of which groups is optionally substituted; and

R_{11} and R_{12} are independently selected from hydrogen, halogen, alkyl group, alkenyl group, cycloalkyl group, cycloalkenyl group, or heterocyclyl group, each of which groups is optionally substituted. In embodiments, R_{10} is any one of RH1-RH12.

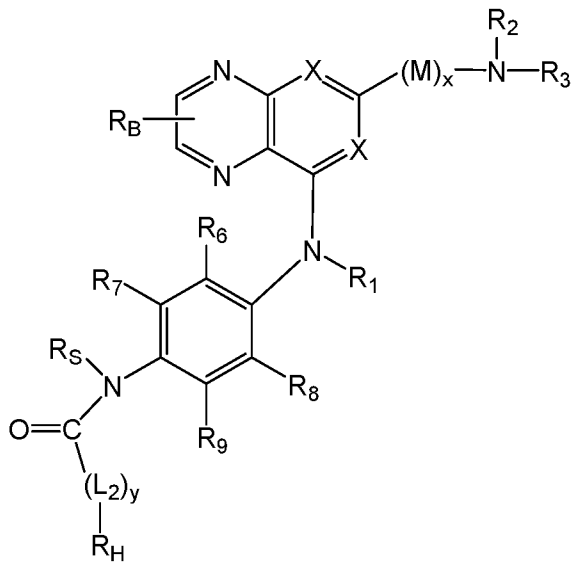
In specific embodiments, compounds useful in the methods herein include those of formula XXXI:



or salts or solvates thereof; where variables are as defined in formula I, R_6-R_9 are independently selected from hydrogen and R_A groups defined in formula I, R_M represents optional substitution on the fused ring and R_M takes the values of RA in formula I and W_1 is N or CH.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and M is –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 1 and M is -(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, R_7-R_9 are independently selected from hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_7-R_9 are independently selected from hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R_7-R_9 are all hydrogens. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_4 and R_5 together form a 5- or 6-member carbocyclic or heterocyclic ring which is saturated, partially unsaturated or is heteroaromatic. In embodiments, R_M is one or more hydrogen, halogen, C1-C3 alkyl group or C1-C3 haloalkyl group. In embodiments, R_M is one or more hydrogen, halogen, particularly Br, methyl or trifluoromethyl. In embodiments, R_M is hydrogen.

In embodiments, compounds useful in the methods of this invention include compounds of formula XXXII:

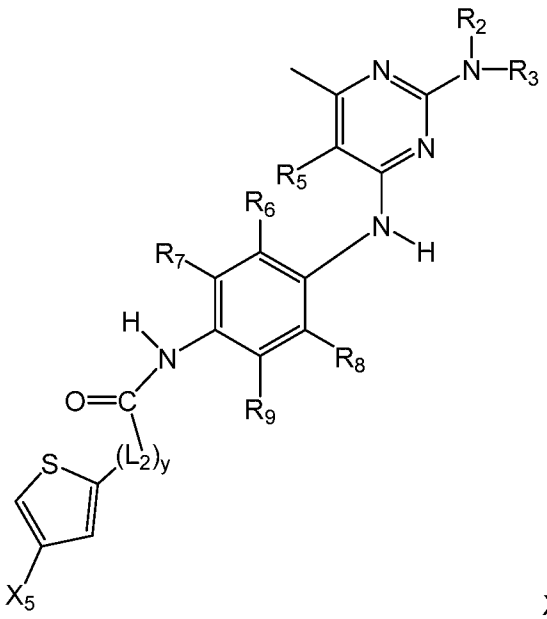


or salts or solvates thereof,

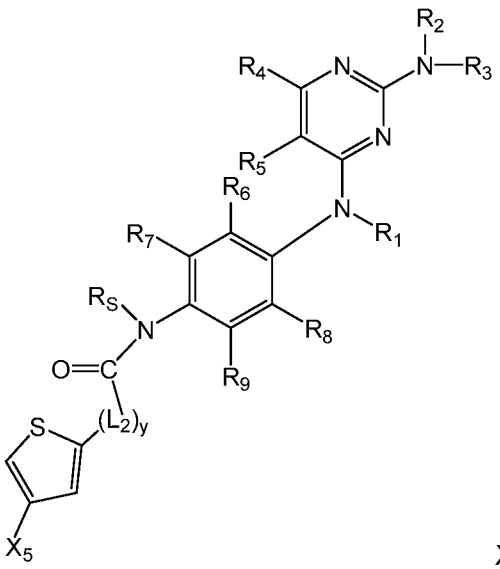
where variables are as defined in formula I, R_B represents optional substitution as defined in formula I and R_6-R_9 are hydrogen or take values of R_A from formula I.

In embodiments, y is 1. In embodiments, y is 0. In embodiments, both X are nitrogens. In embodiments, x is 1 and M is –N-(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 1 and M is -(CH_2)n–, where n is 1, 2 or 3. In embodiments, x is 0. In embodiments, L_2 is –(CH_2)n–, where n is 1, 2 or 3. In embodiments R_1 is hydrogen In embodiments, R_1 is hydrogen, methyl or trifluoromethyl. In embodiments, R_6-R_9 are independently selected from hydrogen, C1-C3 alkyl, optionally substituted C1-C3 alkyl, or aryl. In embodiments, R_6-R_9 are independently selected from hydrogen, halogen C1-C3 alkyl, C1-C3 alkoxyl, C1-C3 acyl, or C1-C3 haloalkyl. In embodiments, R_7-R_9 are all hydrogens. In embodiments, R_4 and R_5 are selected from hydrogen, halogen, C1-C3 alkyl, C1-C3 alkoxyl, or C1-C3 haloalkyl. In embodiments, R_B is one or more hydrogen, halogen, C1-C3 alkyl group or C1-C3 haloalkyl group. In embodiments, R_B is one or more hydrogen, halogen, particularly Br, methyl or trifluoromethyl. In embodiments, R_B is hydrogen. In embodiments, R_H is a heterocyclyl or heteroaryl group. In embodiments, R_H is optionally substituted naphthyl, thiophene, indoyl, or pyridinopyrroyl.

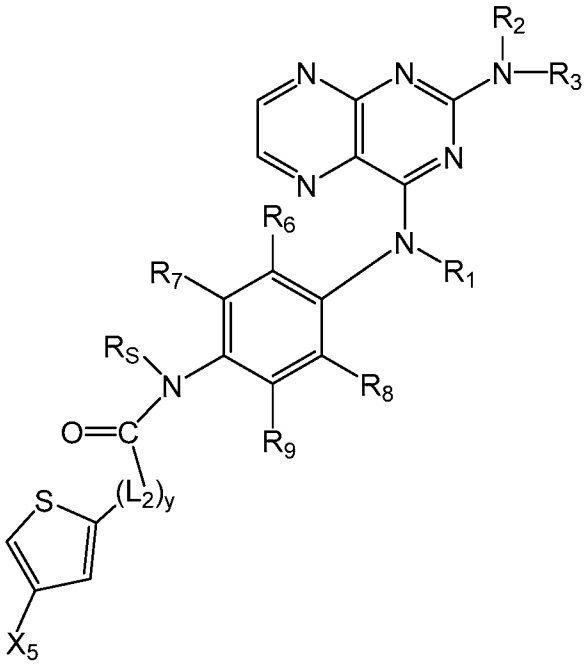
Compounds of formulas XXXV-XLII are useful in the methods herein:



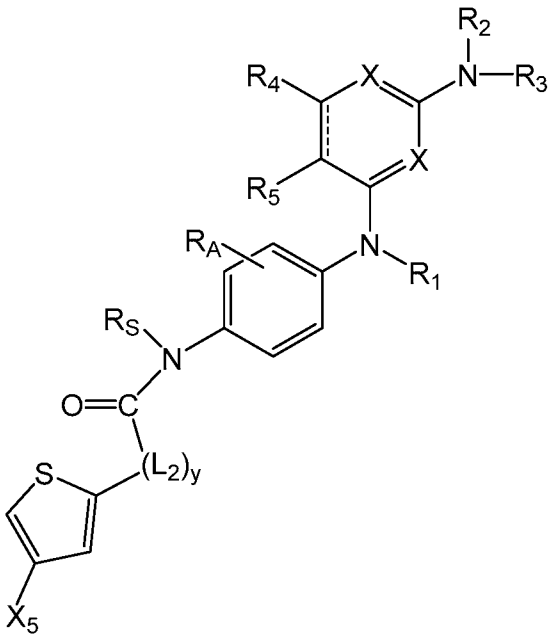
XXXV,



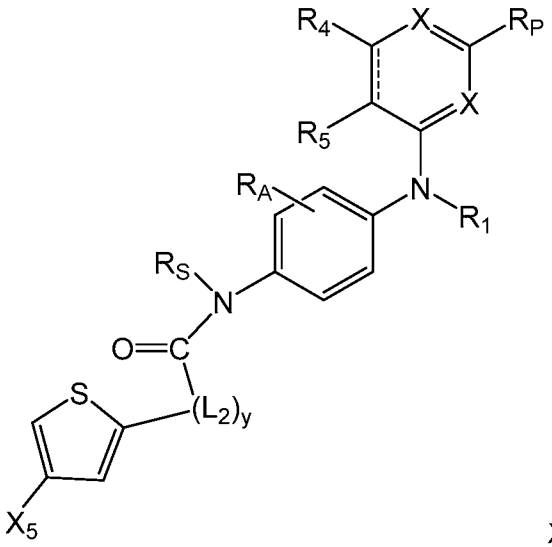
XXXVI,



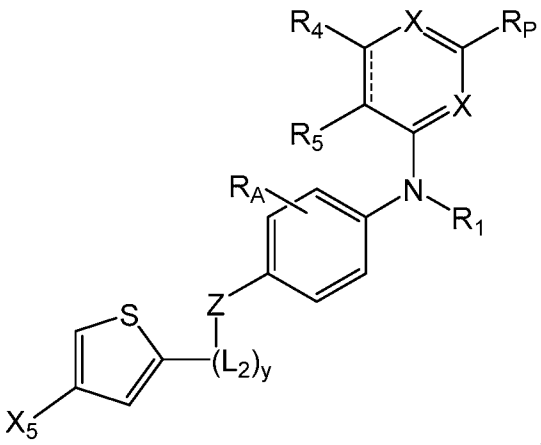
XXXVII,



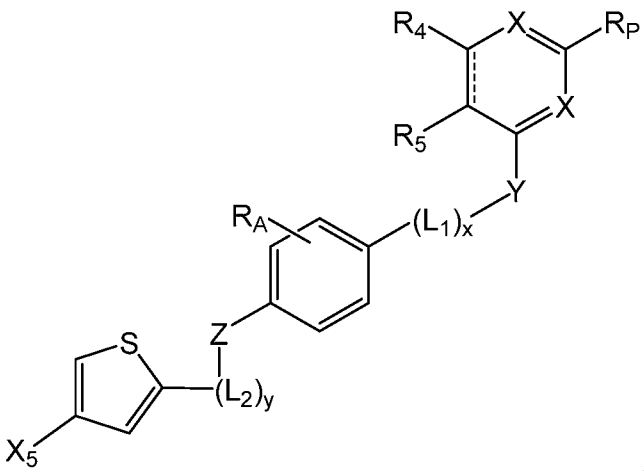
XXXVIII,



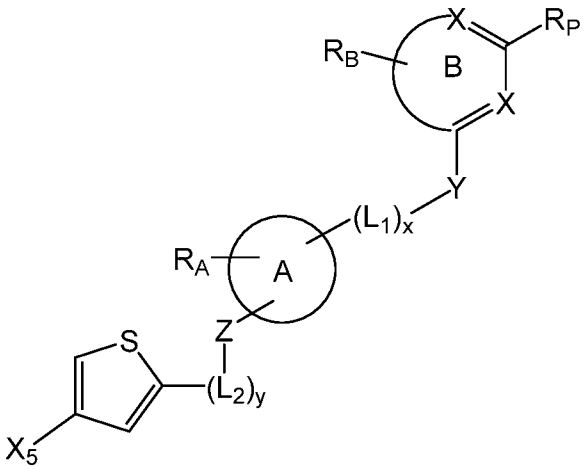
XXXIX,



XL.



XLI, or



XLII,

where variables are as defined in formulas I-XIX above and X_5 is a halogen, including F, Cl and Br and in a specific embodiment is Br. In specific embodiments of formulas XXXV-XLII, y is 0. In specific embodiments of formulas XXXV-XLII, y is 1 and L_2 is $-(CH_2)n-$ and n is 1, 2 or 3. In specific embodiments of formulas XXXV-XLII, the A ring is a phenyl ring where R_A is hydrogen. In specific embodiments R_P is a group selected from any one of R_N-1 to R_N-31. In specific embodiments, the B ring of formula XLII is that of formula RBI as shown in Scheme 4. In more specific embodiments, the B ring of formula XLII is that of RB2-RB5 of Scheme 4.

The invention provides salts, particularly pharmaceutically acceptable salts of each of the compounds of any of formulas I-IX, XI-XIX, XXX-XXXII, XXXV-XLII and formula XX below. The invention provides solvates and salts thereof, particularly pharmaceutically acceptable solvates and salts of each of the compounds of any of formulas I-XIX, XXX-XXXII, XXXV, XXXV-XLII and formula XX below. A preferred solvate is a hydrate. The invention provides pharmaceutical compositions comprising any compound of any one of the formulas herein.

An aliphatic compound is an organic compound containing carbon and hydrogen joined together in straight chains, branched chains, or non-aromatic rings and which may contain single, double, or triple bonds. Aliphatic compounds are distinguished from aromatic compounds. The term aliphatic group herein refers to a monovalent group containing carbon and hydrogen that is not aromatic. Aliphatic groups include alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl, as well as aliphatic groups substituted with other aliphatic groups, e.g., alkenyl groups substituted with alkyl groups, alkyl groups substituted with cycloalkyl groups.

The terms alkyl or alkyl group refer to a monoradical of a straight-chain or branched saturated hydrocarbon. Alkyl groups include straight-chain and branched alkyl groups. Unless otherwise indicated alkyl groups have 1-8 carbon atoms (C1-C8 alkyl groups) and preferred are those that contain 1-6 carbon atoms (C1-C6 alkyl groups) and more preferred are those that contain 1-3 carbon atoms (C1-C3 alkyl groups). Alkyl groups are optionally

substituted with one or more non-hydrogen substituents as described herein. Exemplary alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, various branched-pentyl, n-hexyl, various branched hexyl, all of which are optionally substituted, where substitution is defined elsewhere herein. Substituted alkyl groups include fully halogenated or semihalogenated alkyl groups, such as alkyl groups having one or more hydrogens replaced with one or more fluorine atoms, chlorine atoms, bromine atoms and/or iodine atoms. Substituted alkyl groups include fully fluorinated or semifluorinated alkyl.

Cycloalkyl groups are alkyl groups having at least one 3- or higher member carbon ring. Cycloalkyl groups include those having 3-12-member carbon rings. Cycloalkyl groups include those having 3-20 carbon atoms and those having 3-12 carbon atoms. More specifically, cycloalkyl groups can have at least one 3-10-member carbon ring. Cycloalkyl groups can have a single carbon ring having 3-10 carbons in the ring. Cycloalkyl groups are optionally substituted. Cycloalkyl groups can be bicyclic having 6-12 carbons. Exemplary cycloalkyl groups include among others, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl and cyclodecyl groups. Bicyclic alkyl groups include fused bicyclci grouos and bridged bicyclic groups. Exemplary bicycloalkyl groups include, among others, bicyclo[2.2.2]octyl, bicyclo[4.4.0] decyl (decalinyl), and bicyclo[2.2.2]heptyl (norbornyl).

Cycloalkylalkyl groups are alkyl groups as described herein which are substituted with a cycloalkyl group as dcribed herein. More specifically, the alkyl group is a methyl or an ethyl group and the cycloalkyl group is a cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl group. Cycloalkyl groups are optionally substituted. In specific embodiments, optional substitution iincludes substitution with one or more halogens, alkyl groups having 1-3 carbon atoms, alkoxy groups having 1-3 carbo atoms, hydroxyl and nitro groups

The term alkylene refers to a divalent radical of a straight-chain or branched saturated hydrocarbon. Alkylene groups can have 1-12 carbon atoms unless otherwise indicated. Alkylene groups include those having 2-12, 2-8, 2-6 or 2-4 carbon atoms. Linker groups (L1) herein include alkylene groups, particularly straight chain, unsubstituted alkylene groups, -(CH2)n-, where n is 1-12, n is 1-10, n is 1-9, n is 1-8, n is 1-7, n is 1-6, n is 1-5, n is 1-4, n is 1-3, n is 2-10, n is 2-9, n is 2-8, n is 2-7, n is 2-6, n is 2-5 or n is 2-4.

An alkoxy group is an alkyl group, as broadly discussed above, linked to oxygen (Ralkyl-O-). An alkoxy grou is monovalent.

An alkenylene group is a divalent radical of a straight-chain or branched alkylene group which has one or more carbon-carbon double bonds. In specific embodiments, the same

carbon atom is not part of two double bonds. In an alkenylene group one or more CH2-CH2 moieties of the alkylene group are replaced with a carbon-carbon double bond. In specific embodiments, an alkenylene group contains 2-12 carbon atoms or more preferably 3-12 carbon atoms. In specific embodiments, an alkenylene group contains one or two double bonds. In specific embodiments, the alkenylene group contains one or two trans-double bonds. In specific embodiments, the alkenylene group contains one or two cis-double bonds. Exemplary alkenylene groups include:

$-(CH_2)n-CH=CH-(CH_2)n-$, where n is 1-4 and more preferably is 2; and

$-(CH_2)n-CH=CH-CH=CH-(CH_2)n-$, where n is 1-4 and more preferably is 1 or 2.

An alkoxyalkyl group is an alkyl group in which one or more of the non-adjacent internal $–CH_2-$ groups are replaced with –O-, such a group may also be termed an ether group. The alkoxyalkyl group is monovalent. These groups may be straight-chain or branched, but straight-chain groups are preferred. Alkoxyalkyl groups include those having 2-12 carbon atoms and 1, 2, 3 or 4 oxygen atoms. More specifically, alkoxyalkyl groups include those having 3 or 4 carbons and 1 oxygen, or those having 4, 5 or 6 carbons and 2 oxygens. Each oxygen in the group is bonded to a carbon in the group. The group is bonded into a molecule via a bond to a carbon in the group.

An alkoxyalkylene group is a divalent alkoxyalkyl group. This group can be described as an alkylene group in which one or more of the internal $–CH_2-$ groups are replaced with an oxygen. These groups may be straight-chain or branched, but straight-chain groups are preferred. Alkoxyalkylene groups include those having 2-12 carbon atoms and 1, 2, 3 or 4 oxygen atoms. More specifically, alkoxyalkylene groups include those having 3 or 4 carbons and 1 oxygen, or those having 4, 5 or 6 carbons and 2 oxygens. Each oxygen in the group is bonded to a carbon in the group. The group is bonded into a molecule via bonds to a carbon in the group. Linker groups (L1) herein include alkoxyalkylene groups, particularly straight chain, unsubstituted alkoxyalkylene groups. Specific alkoxyalkylene groups include, among others, $-CH_2-O-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-CH_2-CH_2-CH_2-$,$-CH_2-CH_2-O-CH_2-$, $-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-$, $-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-$, $-CH_2-CH_2-CH_2-O-CH_2-CH_2-CH_2-O-CH_2-$, and $-CH_2-CH_2-CH_2-O-CH_2-CH_2-CH_2-O-CH_2-CH_2-$.

The term acyl group refers to the group –CO-R where R is hydrogen, an alkyl or aryl group as described herein.

Aryl groups include monovalent groups having one or more 5- or 6-member aromatic rings. Aryl groups can contain one, two or three, 6-member aromatic rings. Aryl groups can contain

two or more fused aromatic rings. Aryl groups can contain two or three fused aromatic rings. Aryl groups are optionally substituted with one or more non-hydrogen substituents. Substituted aryl groups include among others those which are substituted with alkyl or alkenyl groups, which groups in turn can be optionally substituted. Specific aryl groups include phenyl groups, biphenyl groups, and naphthyl groups, all of which are optionally substituted as described herein. Substituted aryl groups include fully halogenated or semihalogenated aryl groups, such as aryl groups having one or more hydrogens replaced with one or more fluorine atoms, chlorine atoms, bromine atoms and/or iodine atoms. Substituted aryl groups include fully fluorinated or semifluorinated aryl groups, such as aryl groups having one or more hydrogens replaced with one or more fluorine atoms.

Alkyl groups include arylalkyl groups in which an alkyl group is substituted with an aryl group. Arylalkyl groups include benzyl and phenethyl groups among others. Arylalkyl groups are optionally substituted as described herein. Substituted arylalkyl groups include those in which the aryl group is substituted with 1-5 non-hydrogen substituents and particularly those substituted with 1, 2 or 3 non-hydrogen substituents. Useful substituents include among others, methyl, methoxy, hydroxy, halogen, and nitro. Particularly useful substituents are one or more halogens. Specific substituents include F. Cl, and nitro.

A heterocyclic group is a monovalent group having one or more saturated or unsaturated carbon rings and which contains one to three heteroatoms (e.g., N, O or S) per ring. These groups optionally contain one, two or three double bonds. To satisfy valence requirements, a ring atom may be bonded to one or more hydrogens or be substituted as described herein. One or more carbons in the heterocyclic ring can be –CO- groups. Heterocyclic groups include those having 3-12 carbon atoms, and 1-6, heteroatoms, wherein 1 or 2 carbon atoms are replaced with a –CO- group. Heterocyclic groups include those having 3-12 or 3-10 ring atoms of which up to three can be heteroatoms other than carbon. Heterocyclic groups can contain one or more rings each of which is saturated or unsaturated. Heterocyclic groups include bicyclic and tricyclic groups. Preferred heterocyclic groups have 5- or 6-member rings. Heterocyclic groups are optionally substituted as described herein. Specifically, heterocyclic groups can be substituted with one or more alkyl groups. Heterocyclic groups include those having 5- and 6- member rings with one or two nitrogens and one or two double bonds. Heterocyclic groups include those having 5- and 6-member rings with an oxygen or a sulfur and one or two double bonds. Heterocyclic group include those having 5- or 6-member rings and two different heteroatoms, e.g., N and O, O and S or N and S. Specific heterocyclic groups include among others among others, pyrrolidinyl, piperidyl, piperazinyl, pyrrolyl, pyrrolinyl, furyl, thienyl, morpholinyl, oxazolyl, oxazolinyl, oxazolidinyl, indolyl, triazoly, and triazinyl groups.

Heterocycylalky groups are alkyl groups substituted with one or more heterocycyl groups wherein the alkyl groups optionally carry additional substituents and the heterocycyl groups are optionally substituted. Specific groups are heterocycyl-substituted methyl or ethyl groups.

Heteroaryl groups are monovalent groups having one or more aromatic rings in which at least one ring contains a heteroatom (a non-carbon ring atom). Heteroaryl groups include those having one or two heteroaromatic rings carrying 1, 2 or 3 heteroatoms and optionally have one 6-member aromatic ring. Heteroaryl groups can contain 5-20, 5-12 or 5-10 ring atoms. Heteroaryl groups include those having one aromatic ring contains a heteroatom and one aromatic ring containing carbon ring atoms. Heteroaryl groups include those having one or more 5- or 6-member aromatic heteroaromatic rings and one or more 6-member carbon aromatic rings. Heteroaromatic rings can include one or more N, O, or S atoms in the ring. Heteroaromatic rings can include those with one, two or three N, those with one or two O, and those with one or two S, or combinations of one or two or three N, O or S. Specific heteroaryl groups include furyl, pyridinyl, pyrazinyl, pyrimidinyl, quinolinyl, purinyl, indolyl groups. In a specific embodiment, the heteroaryl group is an indolyl group and more specifically is an indol-3-yl group:

Heteroatoms include O, N, S, P or B. More specifically heteroatoms are N, O or S. In specific embodiments, one or more heteroatoms are substituted for carbons in aromatic or carbocyclic rings. To satisfy valence any heteroatoms in such aromatic or carbocyclic rings may be bonded to H or a substituent group, e.g., an alkyl group or other substituent.

Heteroarylalkyl groups are alkyl groups substituted with one or more heteroaryl groups wherein the alkyl groups optionally carry additional substituents and the aryl groups are optionally substituted. Specific alkyl groups are methyl and ethyl groups.

The term amino group refers to the species $–N(H)_2$. The term alkylamino refers to the species -NHR″ where R″ is an alkyl group, particularly an alkyl group having 1-3 carbon atoms. The term dialkylamino refers to the species $–N(R″)^2$ where each R″ is independently an alkyl group, particularly an alkyl group having 1-3 carbon atoms.

Groups herein are optionally substituted. Most generally any alky, cycloalkyl, aryl, heteroaryl and heterocyclic groups can be substituted with one or more halogen, hydroxyl group, nitro group, cyano group, isocyano group, oxo group, thioxo group, azide group, cyanate group, isocyanate group, acyl group, haloakyl group, alkyl group, alkenyl group or alkynyl group (particularly those having 1-4 carbons), a phenyl or benzyl group (including those that are halogen or alkyl substituted), alkoxy, alkylthio, or mercapto (HS-). In specific embodiments,

optional substitution is substitution with 1-12 non-hydrogen substituents. In specific embodiments, optional substitution is substitution with 1-6 non-hydrogen substituents. In specific embodiments, optional substitution is substitution with 1-3 non-hydrogen substituents. In specific embodiments, optional substituents contain 6 or fewer carbon atoms. In specific embodiments, optional substitution is substitution by one or more halogen, hydroxy group, cyano group, oxo group, thioxo group, unsubstituted C1-C6 alkyl group or unsubstituted aryl group. The term oxo group and thioxo group refer to substitution of a carbon atom with a =O or a =S to form respectively –CO-- (carbonyl) or –CS- (thiocarbonyl) groups.

Specific substituted alkyl groups include haloalkyl groups, particularly trihalomethyl groups and specifically trifluoromethyl groups. Specific substituted aryl groups include mono-, di-, tri, tetra- and pentahalo-substituted phenyl groups; mono-, di , tri-, tetra-, penta-, hexa-, and hepta-halo-substituted naphthalene groups; 3- or 4-halo-substituted phenyl groups, 3- or 4-alkyl-substituted phenyl groups, 3- or 4-alkoxy-substituted phenyl groups, 3- or 4-RCO-substituted phenyl, 5- or 6-halo-substituted naphthalene groups. More specifically, substituted aryl groups include acetylphenyl groups, particularly 4-acetylphenyl groups; fluorophenyl groups, particularly 3-fluorophenyl and 4-fluorophenyl groups; chlorophenyl groups, particularly 3-chlorophenyl and 4-chlorophenyl groups; methylphenyl groups, particularly 4-methylphenyl groups, and methoxyphenyl groups, particularly 4-methoxyphenyl groups.

The term aromatic as applied to cyclic groups refers to ring structures which contain double bonds that are conjugated around the entire ring structure, possibly through one or more heteroatoms such as an oxygen atom, sulfur atom or a nitrogen atom. Aryl groups, and heteroaryl groups are examples of aromatic groups. The conjugated system of an aromatic group contains a characteristic number of electrons, for example, 6 or 10 electrons that occupy the electronic orbitals making up the conjugated system, which are typically un-hybridized p-orbitals.

The term carbocyclic refers to a monovalent group having a carbon ring or ring system which comprises 3 to 12 carbon atoms and may be monocyclic, bicyclic or tricyclic. The ring does not contain any heteroatoms. The ring may be unsaturated, partially unsaturated or saturated.

Compounds and substituent groups of formulas herein are optionally substituted. A substituent refers to a single atom (for example, a halogen atom) or a group of two or more atoms that are covalently bonded to each other, which are covalently bonded to an atom or atoms in a molecule to satisfy the valency requirements of the atom or atoms of the

molecule, typically in place of a hydrogen atom. Examples of substituents include among others alkyl groups, hydroxyl groups, alkoxy groups, acyloxy groups, mercapto groups, and aryl groups. Substituent groups may themselves be substituted.

Substituted or substitution refer to replacement of a hydrogen atom of a molecule or of an chemical group or moiety with one or more additional substituents such as, but not limited to, halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, nitro, sulfato, or other R-groups.

Carbocyclic or heterocyclic rings are optionally substituted as described generally for other groups, such as alkyl and aryl groups herein. Substitution if present is typically on ring C, ring N or both. In addition, carbocyclic and heterocyclic ring can optionally contain a -CO-, -CO-O-, -CS- or –CS-O- moiety in the ring.

As to any of the chemical groups herein that are substituted, i.e., contain one or more non-hydrogen substituents, it is understood, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

Protected derivatives of the disclosed compounds also are contemplated. A variety of suitable protecting groups for use with the disclosed compounds are disclosed in Greene and Wuts, Protective Groups in Organic Synthesis; 3rd Ed.; John Wiley & Sons, New York, 1999. In general, protecting groups are removed under conditions which will not affect the remaining portion of the molecule. These methods are well known in the art and include acid hydrolysis, hydrogenolysis, and the like. One preferred method involves the removal of an ester, such as cleavage of a phosphonate ester using Lewis acidic conditions, such as in TMS-Br mediated ester cleavage to yield the free phosphonate. A second preferred method involves removal of a protecting group, such as removal of a benzyl group by hydrogenolysis utilizing palladium on carbon in a suitable solvent system such as an alcohol, acetic acid, and the like or mixtures thereof. A t-butoxy-based group, including t-butoxy carbonyl protecting groups can be removed utilizing an inorganic or organic acid, such as HCl or trifluoroacetic acid, in a suitable solvent system, such as water, dioxane and/or methylene chloride. Another exemplary protecting group, suitable for protecting amino and hydroxy functions amino is trityl. Other conventional protecting groups are known, and suitable protecting groups can be selected by those of skill in the art in consultation with Greene and Wuts, Protective Groups in Organic Synthesis; 3rd Ed.; John Wiley & Sons, New York, 1999. When an amine is deprotected, the resulting salt can readily be neutralized to yield the free

amine. Similarly, when an acid moiety, such as a phosphonic acid moiety is unveiled, the compound may be isolated as the acid compound or as a salt thereof. Protected derivatives of compounds herein can, for example, be employed in the synthesis of structurally related compounds herein.

The present invention provides novel therapeutic strategies for targeting TCF-driven EMT, a process that promotes tumor cell heterogeneity, MDR, and metastasis. The inventors' structure-based drug design has produced novel potent CHD1L inhibitors which in an embodiment target TCF-driven EMT. Reversion of EMT by CHD1L inhibitors may be an effective treatment when used in combination with cytotoxic chemotherapy and targeted antitumor drugs as well as radiation therapy. These EMT-targeting agents may also sensitize both primary tumors and metastatic lesions to clinically relevant therapies, and potentially inhibit tumor cell metastasis.

Thus, one aspect of this invention are CHD1L inhibitors which can be used to treat or prevent metastasis of a wide variety of advanced solid tumors and blood cancers. Pharmaceutically acceptable salts, prodrugs, stereoisomers, and metabolites of all the CHD1L inhibitor compounds of this invention also are contemplated.

The invention expressly includes pharmaceutically usable solvates of compounds according to formulas herein. Specifically, useful solvates are hydrates. The compounds of formula I or salts thereof can be solvated (e.g., hydrated). The solvation can occur in the course of the manufacturing process or can take place (e.g., as a consequence of hygroscopic properties of an initially anhydrous compound of formulas herein (hydration)).

Compounds of the invention can have prodrug forms. Prodrugs of the compounds of the invention are useful in the methods of this invention. Any compound that will be converted *in vivo* to provide a biologically, pharmaceutically or therapeutically active form of a compound of the invention is a prodrug. Various examples and forms of prodrugs are well known in the art. A prodrug is an active or inactive compound that is modified chemically through in vivo physiological action, such as hydrolysis, metabolism and the like, into an active compound following administration of the prodrug to a subject. The term prodrug as used throughout this text means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting in vivo biotransformation product of the derivative is the active drug as defined in the compounds described herein. Prodrugs preferably have excellent aqueous solubility, increased bioavailability, and are readily metabolized into the active TOP2A inhibitors in vivo. Prodrugs of compounds described herein may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either by routine manipulation or in vivo, to the parent compound. The suitability

and techniques involved in making and using prodrugs are well known by those skilled in the art. Examples of prodrugs are found, inter alia, in Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985), Methods in Enzymology, Vol. 42, at pp. 309-396, edited by K. Widder, et. al. (Academic Press, 1985); A Textbook of Drug Design and Development, edited by Krosgaard-Larsen and H. Bundgaard, Chapter 5, "Design and Application of Prodrugs," by H. Bundgaard, at pp. 113-191, 1991); H. Bundgaard, Advanced Drug Delivery Reviews, Vol. 8, p. 1-38 (1992); H. Bundgaard, et al., Journal of Pharmaceutical Sciences, Vol. 77, p. 285 (1988); and Nogrady (1985) Medicinal Chemistry A Biochemical Approach, Oxford University Press, New York, pages 388-392).

Administration of and administering a compound or composition should be understood to mean providing a compound or salt thereof, a prodrug of a compound, or a pharmaceutical composition comprising a compound. The compound or composition can be administered by another person to the patient (e.g., intravenously) or it can be self-administered by the subject (e.g., tablets or capsules). The term “patient” refers to mammals (for example, humans and veterinary animals such as dogs, cats, pigs, horses, sheep, and cattle). Administration of CHD1L inhibitors herein in combination with other agents, such as alternative anti-cancer, antineoplastic or cancer cytotoxic agents is contemplated. Such combined administration includes administration of two or more active ingredients at the same time or at times separated by minutes, hours or days as is found to be effective and consistent with the administration of any known alternative treatments with which the CHD1L inhibitor is to be combined. Combined administration further includes administration by the same method and/or location of the patient’s body or by different methods at different locations, again as is consistent with and consistent with the administration of known alternative treatments with which the CHD1L inhibitor is to be combined.

Pharmaceutical compositions herein comprise a named active ingredient in an amount effective for achieving the desired biological activity for a given form of administration to a given patient and optionally contain a pharmaceutically acceptable carrier. Pharmaceutical compositions can include an amount (for example, a unit dosage) of one or more of the disclosed compounds together with one or more non-toxic pharmaceutically acceptable additives, including carriers, diluents, and/or adjuvants, and optionally other biologically active ingredients. Such pharmaceutical compositions can be prepared by standard pharmaceutical formulation techniques such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (19th Edition).

Pharmaceutically acceptable carriers are those carriers that are compatible with the other ingredients in the formulation and are biologically acceptable. Carriers can be solid or liquid.

It is currently contemplated that preferred carrier are liquid carriers. Carriers can include one or more substances that can also act as solubilizers, suspending agents, fillers, glidants, compression aids, binders, tablet-disintegrating agents, or encapsulating materials. Liquid carriers can be used in preparing solutions, suspensions, emulsions, syrups and elixirs. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water (of appropriate purity, e.g., pyrogen-free, sterile, etc.), an organic solvent, a mixture of both, or a pharmaceutically acceptable oil or fat. The liquid carrier can contain other suitable pharmaceutical additives such as, for example, solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Compositions for oral administration can be in either liquid or solid form.

Suitable examples of liquid carriers for oral and parenteral administration include water of appropriate purity, aqueous solutions (particularly containing additives, e.g. cellulose derivatives, sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols e.g. glycols) and their derivatives, and oils. For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are used in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant. Liquid pharmaceutical compositions that are sterile solutions or suspensions can be administered by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. Compositions for oral administration can be in either liquid or solid form. The carrier can also be in the form of creams and ointments, pastes, and gels. The creams and ointments can be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type.

A “therapeutically effective amount” of the disclosed compounds is a dosage of the compound that is sufficient to achieve a desired therapeutic effect, such as an anti-tumor or anti-metastatic effect. In some examples, a therapeutically effective amount is an amount sufficient to achieve tissue concentrations at the site of action that are similar to those that are shown to modulate TCF-transcription and/or epithelial-mesenchymal transition (EMT) in tissue culture, in vitro, or in vivo. For example, a therapeutically effective amount of a compound may be such that the subject receives a dosage of about 0.1 μg/kg body weight/day to about 1000 mg/kg body weight/day, for example, a dosage of about 1 μg/kg body weight/day to about 1000 μg/kg body weight/day, such as a dosage of about 5 μg/kg body weight/day to about 500 μg/kg body weight/day.

The term modulate refers to the ability of a disclosed compound to alter the amount, degree, or rate of a biological function, the progression of a disease, or amelioration of a condition. For example, modulating can refer to the ability of a compound to elicit an increase or decrease in angiogenesis, to inhibit TCF-transcription and/or EMT, or to inhibit tumor metastasis or tumorigenesis.

Treatment refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. As used herein, the term ameliorating, with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. The phrase treating a disease is inclusive of inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease, or who has a disease, such as cancer or a disease associated with a compromised immune system. Preventing a disease or condition refers to prophylactically administering a composition to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing a pathology or condition, or diminishing the severity of a pathology or condition.

All references throughout this application, for example patent documents including issued or granted patents or equivalents; patent application publications; and non-patent literature documents or other source material; are hereby incorporated by reference herein in their entireties, as though individually incorporated by reference. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. References cited herein are incorporated by reference herein in their entirety to indicate the state of the art, in some cases as of their filing date, and it is intended that this information can be employed herein, if needed, to exclude (e.g., to disclaim) specific embodiments that are in the prior art. For example, when a compound is claimed, it should be understood that compounds known in the prior art, including certain compounds disclosed in the references disclosed herein (particularly in referenced patent documents), are not intended to be included in the claim.

Abbott et al., **2020** and the supplementary information for that journal article are each incorporated by reference herein in its entirety for descriptions of biological and chemical methods useful in making and assessing the activities and properties of the CHD1L inhibitors herein.

When a group of substituents is disclosed herein, it is understood that all individual members of the group and all subgroups, including any isomers and enantiomers of the group members, and classes of compounds that can be formed using the substituents are disclosed separately. When a compound is claimed, it should be understood that compounds known in the art including the compounds disclosed in the references disclosed herein are not intended to be included. When a Markush group or other grouping is used herein, all individual members of the group and all combinations and subcombinations possible of the group are intended to be individually included in the invention.

When a compound is described herein such that a particular isomer, enantiomer or diastereomer of the compound is not specified, for example, in a formula or in a chemical name, that description is intended to include each isomers and enantiomer (e.g., cis/trans isomers, R/S enantiomers) of the compound described individual or in any combination. Additionally, unless otherwise specified, all isotopic variants of compounds disclosed herein are intended to be encompassed by the invention. For example, it will be understood that any one or more hydrogens in a molecule disclosed can be replaced with deuterium or tritium. Isotopic variants of a molecule are generally useful as standards in assays for the molecule and in chemical and biological research related to the molecule or its use. Isotopic variants, including those carrying radioisotopes, may also be useful in diagnostic assays and in therapeutics. Methods for making such isotopic variants are known in the art.

Molecules disclosed herein may contain one or more ionizable groups [groups from which a proton can be removed (e.g., -COOH) or added (e.g., amines) or which can be quaternized (e.g., amines)]. All possible ionic forms of such molecules and salts thereof are intended to be included individually in the invention herein. With regard to salts of the compounds herein, one of ordinary skill in the art can select from among a wide variety of available counterions those that are appropriate for preparation of salts of this invention for a given application. In specific applications, the selection of a given anion or cation for preparation of a salt may result in increased or decreased solubility of that salt.

CHD1L inhibitors of this invention are commercially available or can be prepared without undue experimentation by the methods disclosed herein or by routine adaptation of such methods using starting materials and reagents which are commercially available or which can be made by known methods. It will be appreciated that it may be necessary, dependent upon the compound to be synthesized, to protect potentially reactive groups in starting materials from undesired conjugation. Useful protective groups, for various reactive groups are known in the art, for example as described in Wutts & Greene, **2007**.

Compounds herein can be in the form of salts, for example ammonium salts, with a selected anion or quaternized ammonium salts. The salts can be formed as is known in the art by addition of an acid to the free base. Salts can be formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, or organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, N-acetylcystein and the like.

In specific embodiments, compounds of the invention can contain one or more negatively charged groups (free acids) which may be in the form of salts. Exemplary salts of free acids are formed with inorganic base include, but are not limited to, alkali metal salts (e.g., Li^+, Na^+, K^+), alkaline earth metal salts (e.g., Ca^{2+}, Mg^{2+}), non-toxic heavy metal salts and ammonium (NH_4^+) and substituted ammonium ($N(R')_4^+$ salts, where R' is hydrogen, alkyl, or substituted alkyl, i.e., including, methyl, ethyl, or hydroxyethyl, specifically, trimethyl ammonium, triethyl ammonium, and triethanol ammonium salts), salts of cationic forms of lysine, arginine, N-ethylpiperidine, piperidine, and the like. Compounds of the invention can also be present in the form of zwitterions. Compound herein can be in the form of pharmaceutically acceptable salts, which refers to those salts which retain the biological effectiveness and properties of the free bases or free acids, and which are not biologically or otherwise undesirable.

The scope of the invention as described and claimed encompasses the racemic forms of the compounds as well as the individual enantiomers and non-racemic mixtures thereof. The compounds of the invention may contain one or more asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. The compounds can be, for example, racemates or optically active forms. The optically active forms can be obtained by resolution of the racemates or by asymmetric synthesis. In a preferred embodiment of the invention, enantiomers of the invention exhibit specific rotation that is + (positive). Preferably, the (+) enantiomers are substantially free of the corresponding (-) enantiomer. Thus, an enantiomer substantially free of the corresponding enantiomer refers to a compound which is isolated or separated via separation techniques or prepared free of the corresponding enantiomer. “Substantially free,” means that the compound is made up of a significantly greater proportion of one enantiomer. In preferred embodiments the compound is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments of the invention, the compound is made up of at least about 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including high performance liquid chromatography (HPLC)

and the formation and crystallization of chiral salts or prepared by methods described herein. [See, for example, Jacques et al., **1981**; Wilen et al., **1977**; Eliel, **1962**; Wilen, **1972**.]

Compounds of the invention, and salts thereof, may exist in their tautomeric form, in which hydrogen atoms are transposed to other parts of the molecules and the chemical bonds between the atoms of the molecules are consequently rearranged. It should be understood that all tautomeric forms, that may exist, are included within the invention.

Every formulation, compound or combination of components described or exemplified herein can be used to practice the invention, unless otherwise stated. Specific names of compounds are intended to be exemplary, as it is known that one of ordinary skill in the art can name the same compounds differently. When a compound is described herein such that a particular isomer or enantiomer of the compound is not specified, for example, in a formula or in a chemical name, that description is intended to include each isomers and enantiomer of the compound described individual or in any combination.

One of ordinary skill in the art will appreciate that methods, alternative therapies, starting materials, and synthetic methods other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents, of any such methods, device elements, starting materials, and synthetic methods are intended to be included in this invention. Whenever a range is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the invention.

The singular terms “a,” “an,” and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicates otherwise. The term “comprises” means “includes.” Also, “comprising A or B” means including A or B, or A and B, unless the context clearly indicates otherwise. It is to be further understood that all molecular weight or molecular mass values given for compounds are approximate and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this invention, suitable methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

As used herein, “comprising” is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude

materials or steps that do not materially affect the basic and novel characteristics of the claim. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of elements of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

Without wishing to be bound by any particular theory, there can be discussion herein of beliefs or understandings of underlying principles relating to the invention. It is recognized that regardless of the ultimate correctness of any mechanistic explanation or hypothesis, an embodiment of the invention can nonetheless be operative and useful.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

THE EXAMPLES

Example 1: Clinicopathological characterization of CHD1L in patients with CRC

CHD1L expression is correlated with poor prognosis in several cancers, but only limited information about the pathology of CHD1L in CRC is known. This example describes the pathogenic characterization and mechanisms of pathology for CHD1L in CRC patients. The clinicopathological characteristics of 585 patients with CRC were analyzed from the Cartes d'Identite des Tumeurs (CIT) program with respect to CHD1L expression (GEO: GSE39582). [Marisa et al., **2013**] These characteristics are summarized in Abbott et al., **2020**, supplementary information.

Additional data for this example are found in Abbott et al., **2020** and its supplementary information. Follow up information was available for all patients in the CIT cohort over a period of 15 years. For the entire patient cohort, high CHD1L expression is associated with lower OS ($P = 0.0167$) and median survival (MS) of 8.8 years for high CHD1L patients. Median survival was not reached in the low CHD1L cohort as 72% (115/159) of patients were censored and 26% (42/159) were deceased. Patient data were evaluated using the

TNM staging system. As Stage I and IV patients have a high likelihood of survival or death, respectively, survival of Stage II and III CRC patients was evaluated. High CHD1L expression was associated with a lower OS (P = 0.0191) and MS of 11 years for Stage II and III CRC, again median survival was not reach in the low CHD1L cohort. Survival was also analyzed with respect to CHD1L expression for each stage of CRC. Stage II patients showed a significant difference in survival (P = 00319) with a M.S. of 11 years, no significant difference was observed for Stage I, III or IV patients. Analysis of CHD1L expression indicated a significant difference in expression cancer stage. Patients with Stage I and II colorectal cancer versus patients with Stage III and IV were evaluated and showed a significant increase in CHD1L expression in the Stage III and IV versus early stage cohort (P = 0.0051). Analysis of CHD1L expression with respect to lymph node metastasis suggests that CHD1L is overexpressed in patients with increased regional lymph node metastasis (N1 P = 0.0128, N2 P = 0.05 compared to N0). Although the trend of CHD1L expression was the same for the N3 cohort, no significance was determined due to the limited number of patient samples available. No significant difference in CHD1L expression with respect to tumor size, metastasis or location was found.

Evaluation of CHD1L in CRC molecular subtypes.

The association of CHD1L expression with six molecular subtypes of CRC [Marisa et al., **2013**]: C1 (immune system down, n = 116), C2 (deficient mismatch repair, n = 104), C3 (KRAS mutant, n = 75), C4 (CSC, n = 59), C5 (activated WNT pathway, n = 152), and C6 (chromosomal instability normal, n = 60) was investigated. There is a significant difference of CHD1L expression among the six molecular subtypes (P < 0.001). CHD1L expression was high in C5, C4, and C3, and low in C2 and C6. The C2 subtype is associated with a decrease in the WNT signaling pathway and deficient for mismatch repair. The C4 and C6 subtypes are associated with poorer relapse-free survival compared to other subtypes. The C4 subtype is associated with increased CSC stemness and the C5 subtype is associated with activated WNT signaling and deregulated EMT pathways. The lower CHD1L expression in the C2 (deficient mismatch repair) subtype is consistent with its known function in DNA damage response. [Ahel et al., **2009**] Additionally, CHD1L expression was lower in patients with deficient mismatch repair than in patients without (P < 0.001). CHD1L expression was also higher in patients with KRAS mutations (P = 0.049). The expression of CHD1L in the C3, C4, and C5 molecular subtypes prompted a further investigation of the function of CHD1L expression in EMT, CSC stemness, and the WNT/TCF pathway.

CHD1L expression correlates with Wnt/TCF associated genes

Utilizing a smaller cohort of CRC patients (n = 26) from the UCCC GI tumor tissue bank, a similar trend was observed as with the larger CIT cohort CHD1L expression significantly correlated with late stage and metastatic CRC compared to early stage and primary CRC (Abbott et al., **2020**, Supplementary information). The expression is quantified as FPKM (fragments per kilobase exon per million fragments mapped). Metastatic tumor samples had significantly more CHD1L than primary tumors. Additionally, CHD1L levels were higher in Stage IV compared to Stage II/III patient cohorts. When analyzing CHD1L expression with genes involved in KEGG WNT pathway, using Spearman's correlation a significant positive correlation with 65 of 125 genes was observed. Among these were well-established genes involved in TCF-mediated transcription such as topoisomerase IIα (TOP2A) (r = 0.65, P = 0.004 [Zhou et al., **2016**; Abraham et al, **2019**], and TCF4 (r = 0.61, P = 0.0012) (Abbott et al., Supplementary Figure 2). Genes had $P < 0.05$ correlation value. Transcript expression was Log_2 normalized and quantified by FPKM (fragments per kilobase exon per million fragments mapped).

A significant positive correlation was observed between known CSC markers CD44 (r = 0.43, P = 0.038), LGR5 (r = 0.55, P = 0.0075) and CHD1L. When comparing the CIT cohort to the UCCC cohort a significant correlation was observed for TOP2A (r = 404 0.1275, P = 0.0020) and TCF4 (r = 0.1050, P = 0.011). Consistent with this result, it has been shown that TOP2A is a required component of the TCF-complex, promoting EMT in CRC. [Zhou et al., **2016**; Abraham et al., **2019**] Hence, CHD1L appears to be involved in TCF-transcription and EMT in CRC patients.

Example 2: CHD1L mediates TCF-transcription in CRC

Based on the correlation of CHD1L with TCF-complex members, CHD1L may have a mechanistic role in TCF-transcription. To assess this role, SW620 and DLD1 cell lines, which have high and low endogenous CHD1L expression, respectively, were utilized. Additional data for this example are found in Abbott et al, **2020**, and its Supplemetary Information. Small hairpin RNA (shRNA) was used to knockdown CHD1L in SW620 cells ($SW620^{CHD1L\text{-}KD}$). CHD1L was overexpressed in DLD1 cells ($DLD1^{CHD1L\text{-}OE}$). Using the TOPflash luciferase reporter [Morin et al., **1997**; Zhou et al., **2016**] transfected into $SW620^{CHD1L\text{-}KD}$ or $DLD1^{CHD1L\text{-}OE}$, it was determined that overexpression of CHD1L produced a significant increase in TCF-transcription (P<0.0001) (Abbott et al., **2020**). Conversely, $SW620^{CHD1L\text{-}KD}$ cells displayed a significant decrease in TCF-transcription (P = 0.0006). These results indicate that CHD1L is a potential factor directly involved in TCF-transcription. Each of Morin et al., **1997** and Zhou

et al., **2016** is incorporated by reference herein in its entirety for descriptions of the TOPflash reporter and assays employing it.

CHD1L directly interacts with the TCF-transcription complex

Activation of TCF-transcription is a dynamic process that involves the shedding of co-repressor proteins, binding of co-activator proteins, and remodeling of the chromatin landscape. [Lorch et al. **2010**, Shitashige et al., **2008**] Co-immunoprecipitation (Co-IP) studies with TCF4 were performed, demonstrating that CHD1L directly binds to the TCF-complex [Abbott et al, **2020**].

CHD1L has been well characterized as a binding partner with PARP1 in DNA damage response. [Pines, **2012**; Ahel et al., **2009**] PARP1 is also a component of the TCF-complex binding to TCF4 and β-catenin. [Idogawa et al. **2005**] The results herein demonstrate that CHD1L binds to the TCF-complex, which is likely through interactions between TCF4 and PARP1.

To further characterize CHD1L as a component of the TCF-complex, chromatin immunoprecipitation (ChIP) of CHD1L to TCF-complex WNT response elements (WREs) was performed in SW620 cells. [Abbott et al., **2020**] CHD1L was enriched at c-Myc, vimentin, slug, LEF1, and N-cadherin WREs, further supporting that CHD1L is functioning directly with the TCF-complex. Taken together, the data implicate CHD1L as a critical component of the TCF-transcription.

CHD1L mediated TCF-transcription promotes EMT and CSC stemness in CRC

Previously, TCF-transcription was characterized as a master regulator of EMT in CRC. [Zhou et al., **2016**] In addition, CHD1L localizes at WREs of EMT effector genes. [Abbott et al, **2020**] Therefore, biomarker expression in $SW620^{CHD1L\text{-}KD}$ and $DLD1^{CHD1L\text{-}OE}$ cells was measured to determine whether knockdown or overexpression of CHD1L modulates EMT. Knockdown of CHD1L induced reversion of EMT, decreasing vimentin and slug while increasing E-cadherin expression. [Abbott et al, **2020**] Conversely, EMT was induced in $DLD1^{CHD1L\text{-}OE}$ cells, evidenced by a decrease in E-cadherin and an increase in vimentin and slug expression. [Abbott et al, **2020**] These results indicate that CHD1L is an EMT effector gene involved in promoting the mesenchymal phenotype in CRC. A hallmark of EMT is an increase in CSC stemness.

Clonogenic colony formation assays [Abbott et al, **2020**] were performed to characterize the impact of CHD1L expression on stemness. [Franken et al., **2006**] CSC stemness increased in DLD1$^{CHD1L\text{-}OE}$ (P = 0.0001) and decreased in SW620$^{CHD1L\text{-}KD}$ (P = 0.002) cells measured by colony formation.

Example 3: Identification of Small Molecule Inhibitors of CHD1L

As established in Examples 1 and 2, herein, CHD1L is a driver of TCF-mediated EMT. Based on this, an assay to identify small molecule inhibitors of CHD1L is described herein. The drug discovery goal was to target CHD1L DNA translocation or interactions with DNA, which are dependent on CHD1L's catalytic domain ATPase activity. [Ryan & Owen-Hughes, **2011**; Flaus et al., **2011**]

CHD1L belongs to the SNF2 (sucrose non-fermenter 2) ATPase superfamily of chromatin remodelers that contains a two-lobe ATPase domain. [Abbott et al, **2020** and its Supplemental Information] CHD1L also has a macro domain that is unique relative to other chromatin remodelers, which promotes an auto-inhibited state through interactions between the macro and the ATPase domains. [Lehmann et al., **2017**; Gottschalk et al., **2009**] However, the macro domain binds to PARP1, the major activator of CHD1L, alleviating auto-inhibition. [Lehmann et al., **2017**; Gottschalk et al., **2009**]

Using the methodology of Lehmann et al., **2017**, full-length CHD1L (fl-CHD1L) and the catalytic ATPase domain (cat-CHD1L) were purified. [Abbott et al, **2020** and its Supplementary Information] Protein constructs were used for recombinant expression and purification of CHD1L for *in vitro* HTS, as illustrated in Abbott et al., **2020**. An SDS page gel showed purified cat-CHD1L (68 kD) and fl-CHD1L (101 kD). Enzyme kinetics of cat-CHD1l versus fl-CHD1L were compared. The cat-CHD1L provides for a more robust ATPase assay compared to fl-CHD1L, which is consistent with the report from Lehman et al., **2017**. Therefore, to identify direct inhibitors of CHD1L ATPase, an exemplary High-through-put screening (HTS) assay in the context of TCF-transcription is described which includes: cat-CHD1L, c-Myc DNA, ATP, and phosphate-binding protein that fluoresces upon binding inorganic phosphate (Pi).

This assay was validated and pilot screening was preformed against clinically relevant kinase inhibitors. [Abbott et al, **2020** and its Supplemental Information]. The pilot screen found no hits, demonstrating that CHD1L is not a likely target for kinase inhibitors. Once validated, a primary HTS was preformed using 20,000 compounds from the Life Chemicals Diversity Set, which were screened at 20 μM in 1% DMSO with 10 mM EDTA as a positive

control [Abbott et al, **2020** and its Supplementary Information] The screen provided robust statistics with an average Z'-factor value of 0.57 ± 0.06 over 64 plates. The average compound activity was 92.3% ± 17.8. As a result, the hit limit was set to be 3 standard deviations from the mean at 39% ATPase activity. This stringent hit limit identified 64 hits, of which 53 hits were confirmed against recombinant CHD1L ATPase activity.

Example 4: Exemplary Inhibitors

A subset of seven confirmed hits (compounds **1-7,** see Scheme 1) were purchased, representing a range of pharmacophores with greater than 50% inhibition against cat-CHD1L ATPase. Compounds **1-7** were subjected to dose response studies against cat-CHD1L ATPase, which validated these hits as potent CHD1L inhibitors with activity between 900 nM to 5 μM (**Figure 1A**). Structures of additional exemplary compounds **8-73** are provided in Scheme 1, where SEM represents the protecting group trimethylsilylethoxy methyl. Note that in a number of cases in Scheme 1, an additional compound number is given in parenthesis which may be employed in Tables and Figures herein or in Abbott et al., **2020** and its Supplementary Information.

Compounds **1-7** were tested in HCT116, SW620, and DLD1$^{CHD1L\text{-}OE}$ cells for their ability to inhibit TCF-transcription using the TOPflash reporter system (**Figure 1B**). Compounds **1-3** were shown to have no significant activity in cells. Compound **4** was shown to have modest activity in cells with no dose dependent inhibition of TCF-activity. However, compounds **5-7** demonstrate superior dose dependent activity against TCF transcription in all three CRC cell lines. Notably, decreased inhibition of TCF-transcription was observed for **5-7** at the low 2 μM dose in DLD1$^{CHD1L\text{-}OE}$ cells, which is evidence of cellular CHD1L target engagement.

<u>CHD1L inhibitors reverse EMT and malignant properties in CRC.</u>

After validating hits **5-7** against CHD1L mediated TCF transcription, the ability of these compounds to reverse EMT and other malignant properties in CRC were evaluated. E-cadherin and vimentin are putative biomarkers for the epithelial and mesenchymal phenotypes, respectively. [McDonald et al., **2015**] Loss of E-cadherin and gain of vimentin are also clinical biomarkers of poor prognosis. [Yun et al., **2014**; Richardson et al., **2012**; Dhanasekaran et al., **2001;** Kashiwagi et al., **2010;** Toiyama et al., **2013**] Accordingly, lentiviral promoter driven reporters for E-cadherin (pCDH1-EcadPro-RFP) and vimentin (pCDH1-VimPro-GFP) were developed, which faithfully report E-cadherin and vimentin protein expression, respectively. [Zhou et al., **2016**; Abraham et al., **2019**] SW620 cells transduced with either EcadPro-RFP or VimPro-GFP were cultured as tumor organoids for 72 h, reaching a diameter of 600 μm. Tumor organoids were treated with compounds **5-7** for

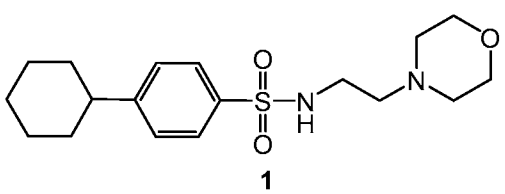
an additional 72 h to determine the effective concentration 50 percent (EC_{50}) for modulating promoter activity. Changes in promoter expression was quantified using a 3D confocal image 507 based high-content analysis algorithm **(Figure 2A-2B)**. [Zhou et al., **2016**; Abraham et al., **2019**]

Compounds **5-7** effectively downregulated vimentin promoter activity with EC_{50} values of 15.6 ± 1.7 μM (**5**), 4.7 ± 510 1.5 μM (**6**), and 12.8 ± 1.3 μM (**7**). Conversely, E-cadherin promoter activity was upregulated with EC_{50} values of 11.9 ± 0.3 μM (**5**), 11.4 ± 0.3 μM (**6**), and 28 ± 0.003 μM (**7**). Representative images exhibiting reversion of EMT by compound **6** in SW620 tumor organoids measured by EMT reporter assays are shown in Abbott et al., **2020**. These results indicate that small molecule inhibitors of CHD1L reverse TCF-driven EMT in CRC.

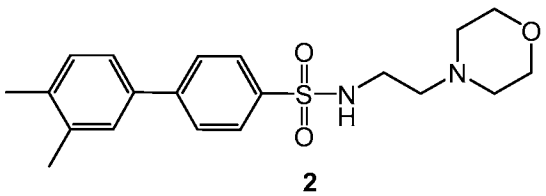
To confirm that CHD1L inhibitors reverse EMT, protein expression of two additional putative biomarkers of EMT, slug (mesenchymal) and zona occludens-1 (ZO-1, epithelial) were evaluated. Changes in slug and ZO-1 are considered major criteria for EMT. [Zeisberg & Neilson, **2009**] SW620 tumor organoids treated with CHD1L inhibitors downregulate slug and upregulate ZO-1, further indicating a reversion of EMT. Western blot analysis showing protein expression changes of additional EMT biomarkers slug and ZO1 is shown in Abbott et al., **2020**.

A hallmark of EMT is an increase in CSC stemness and cell invasion. Therefore, the ability of compounds **5-7** to inhibit migration and invasion in HCT-116 and $DLD1^{CHD1L\text{-}OE}$ cells was tested. All three compounds demonstrated a significant inhibition of CSC stemness (**Figure 2C**). However, compounds **5** and **6** display more potent dose dependent inhibition. Note that $DLD1^{CHD1L\text{-}OE}$ cells form two times more colonies than HCT-116 cells, which have moderate CHD1L expression. This observation is consistent with CHD1L's oncogenic and tumorigenic properties. Next, using HCT-116 cells with uniform scratch wounds imbedded in 50% Matrigel® matrix (Corning Life Sciences, Corning, NY) cells were treated with CHD1L inhibitors at concentrations indicated and invasion was monitored over 72 h. Compounds **5-7** exhibited a dose dependent inhibition of invasion (**Figure 2D**), with compound **6** displaying the most potent activity.

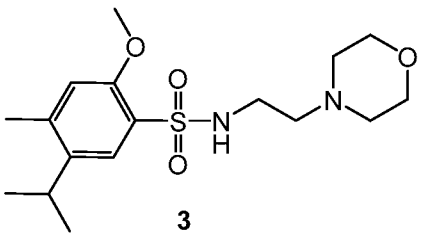
Scheme 1: Exemplary Compounds of Formula I or Formula XX



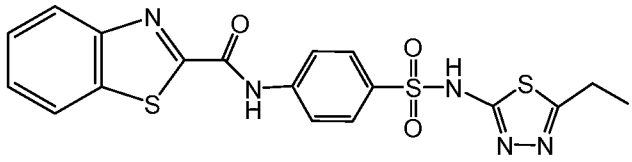
1



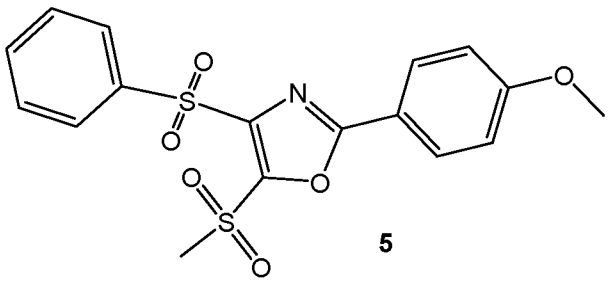
2



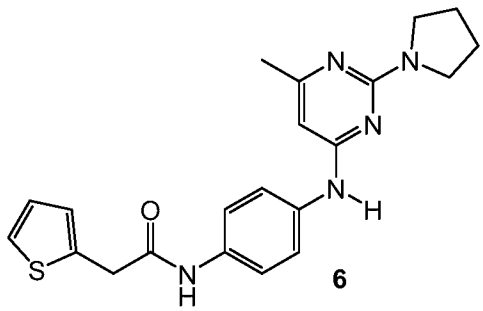
3



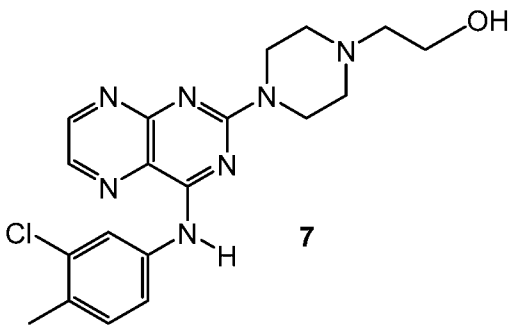
4



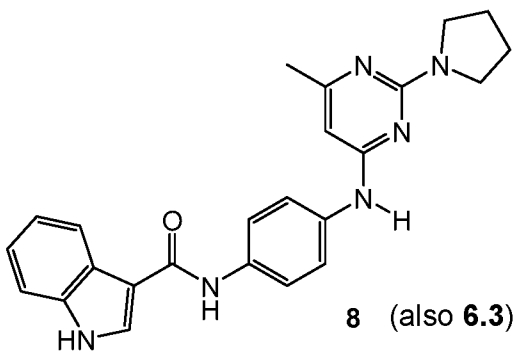
5



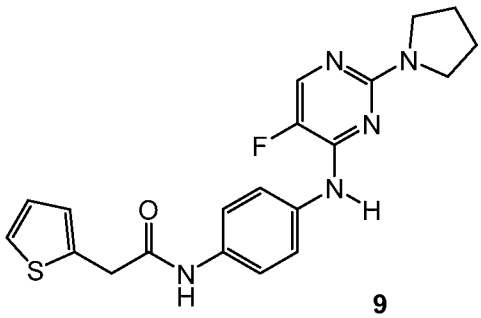
6



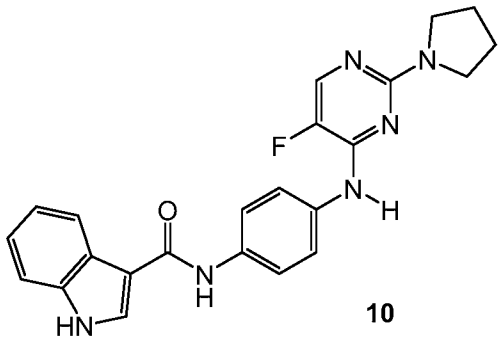
7



8 (also **6.3**)

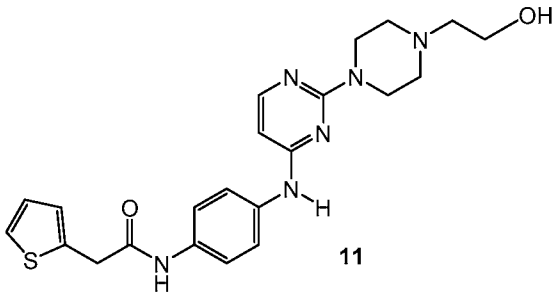


9

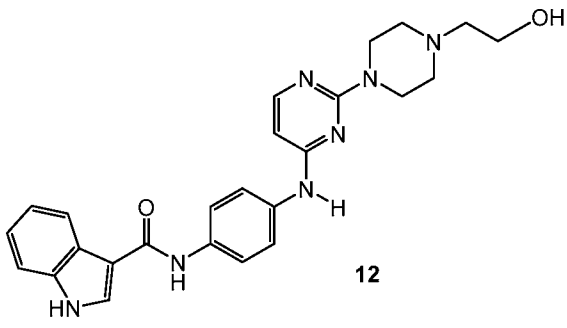


10

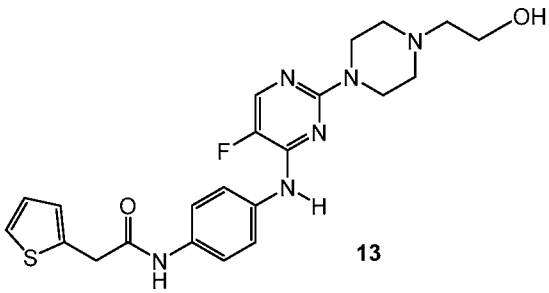
(**6.2**)



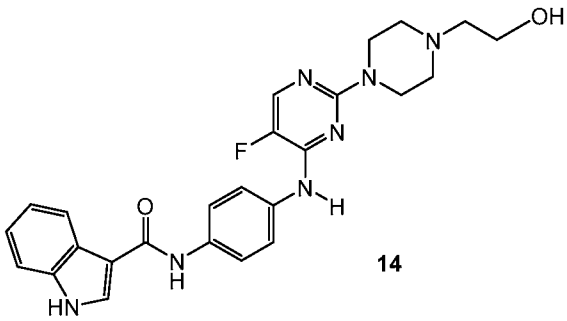
11



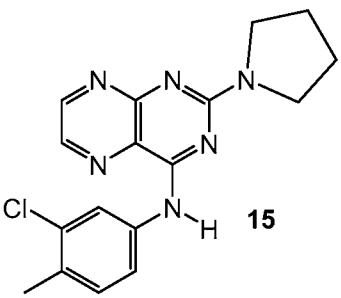
12



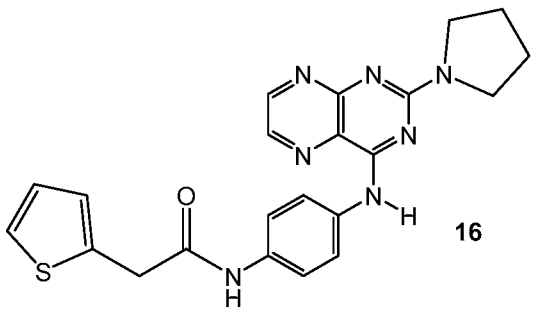
13



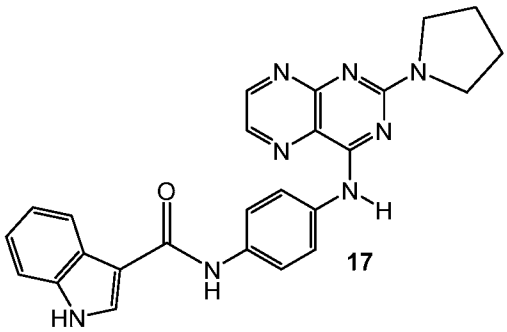
14



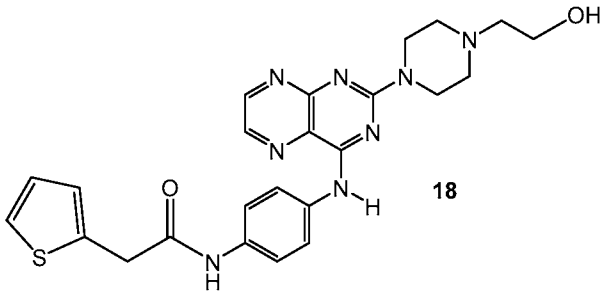
15



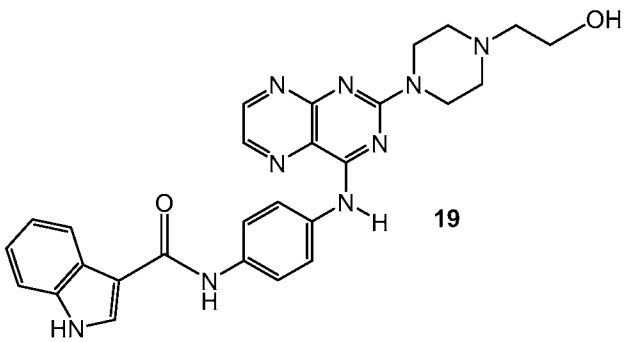
16



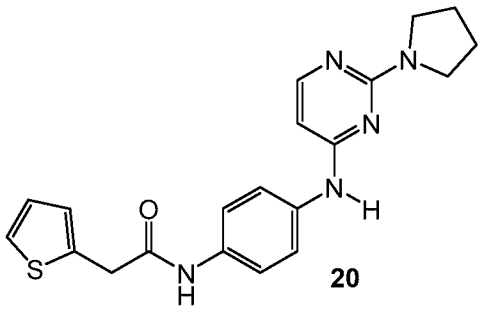
17



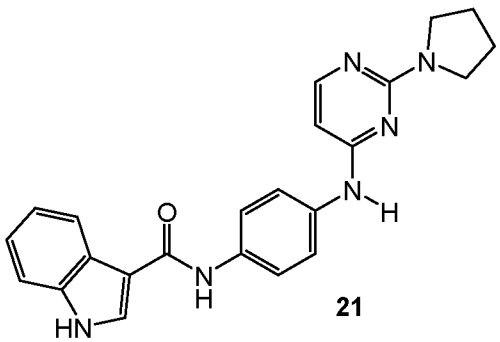
18



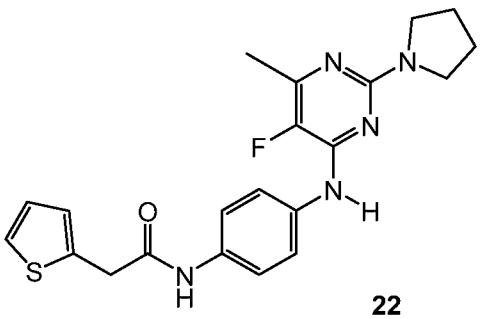
19



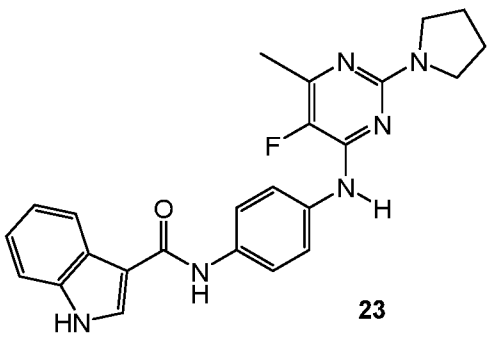
20



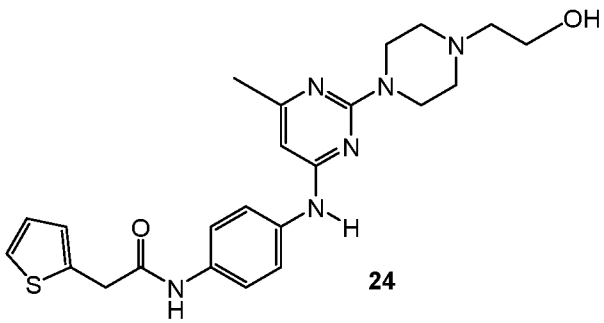
21



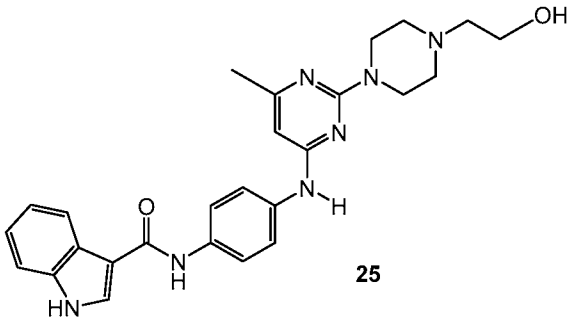
22



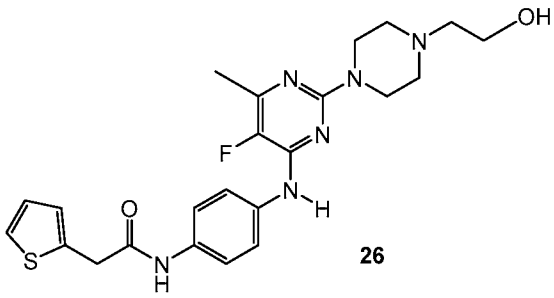
23



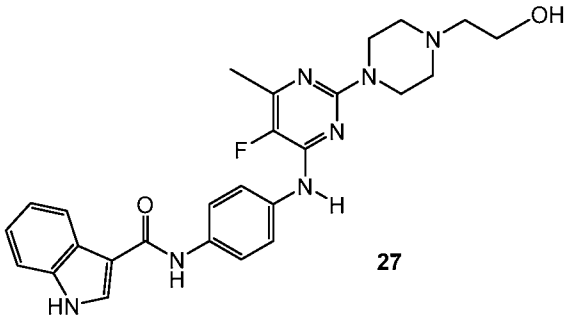
24



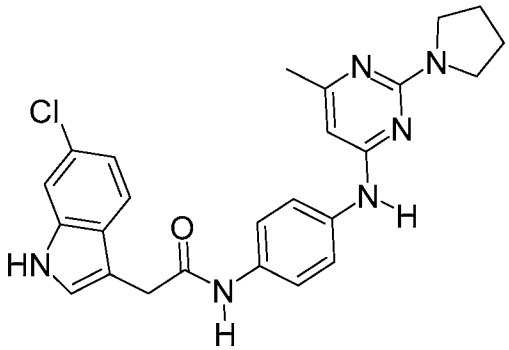
25



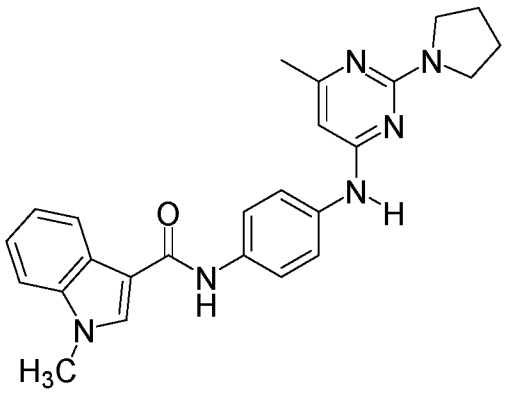
26



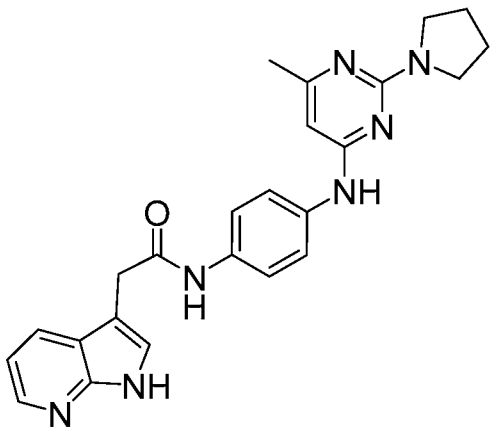
27



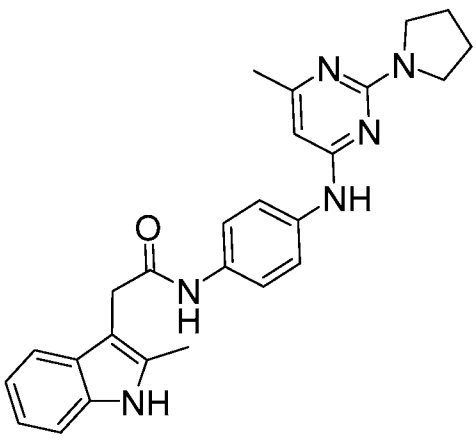
28 (6.18)



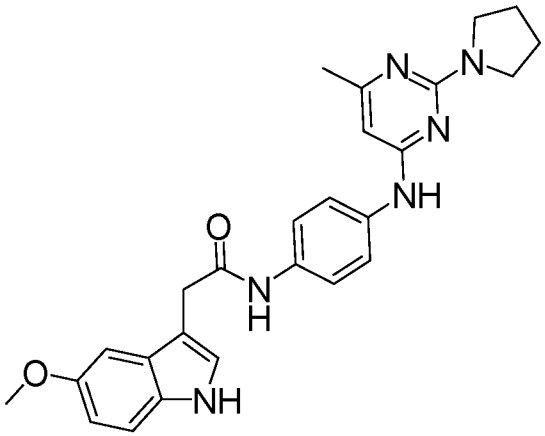
29 (6.19)



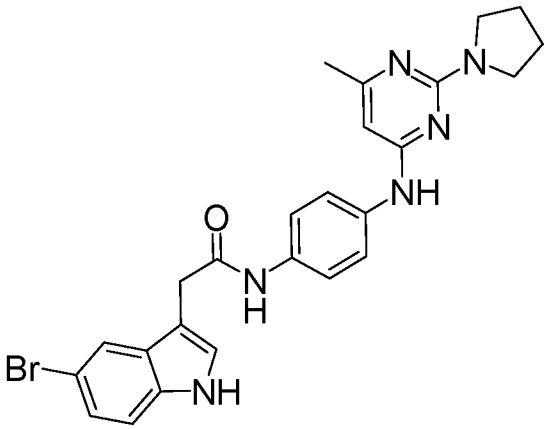
30 (6.20)



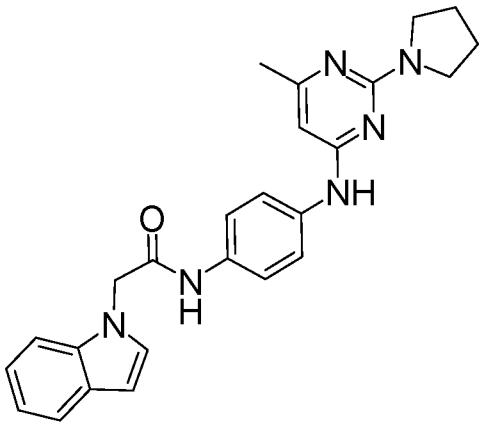
31 (6.21)



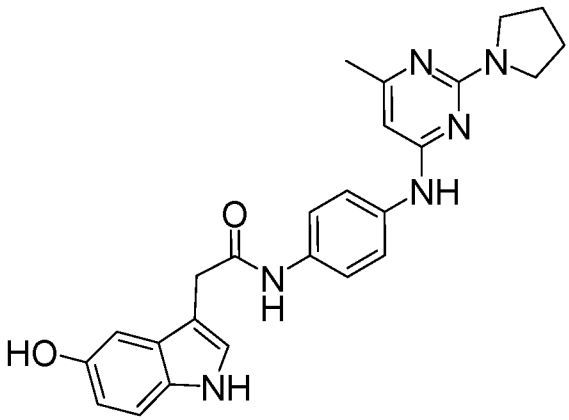
32 (6.22)



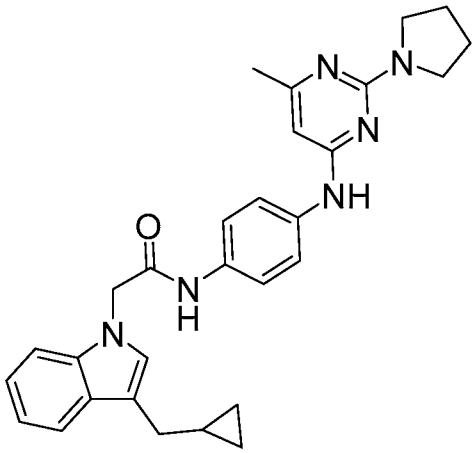
33 (6.23)



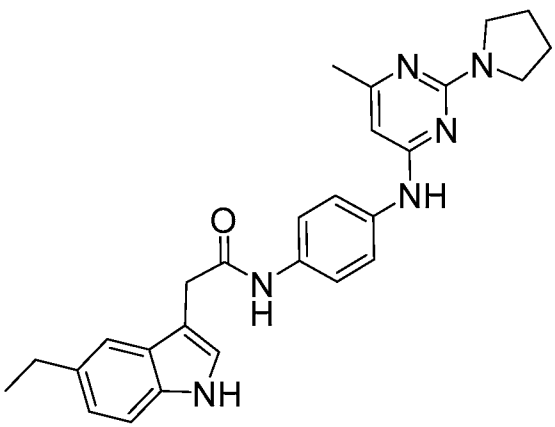
34 (6.24)



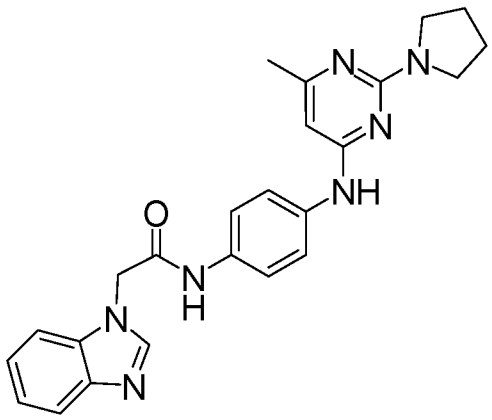
35 (6.25)



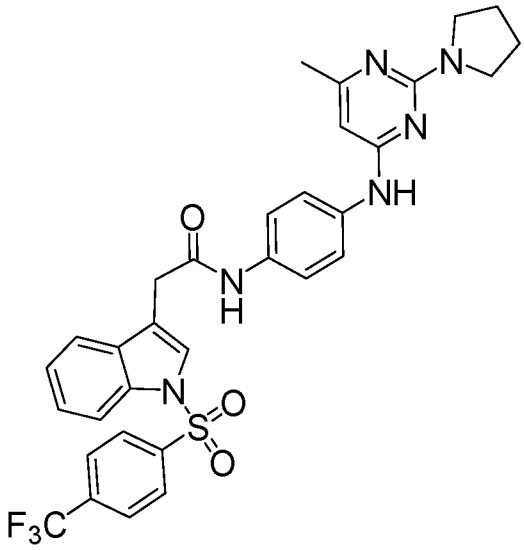
36 (6.26)



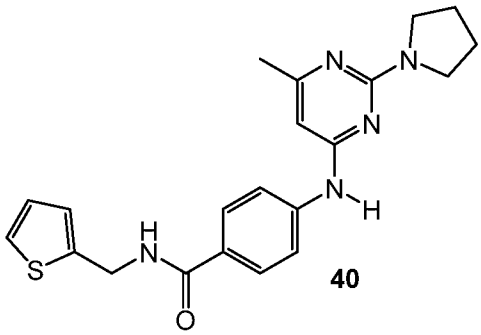
37 (6.27)



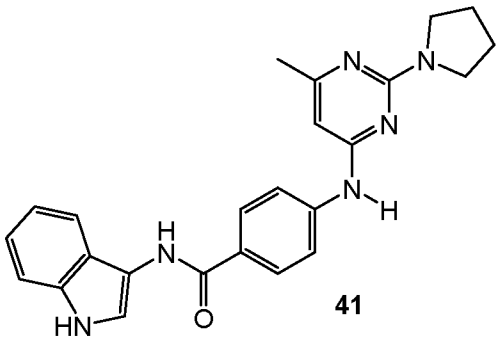
38 (6.28)



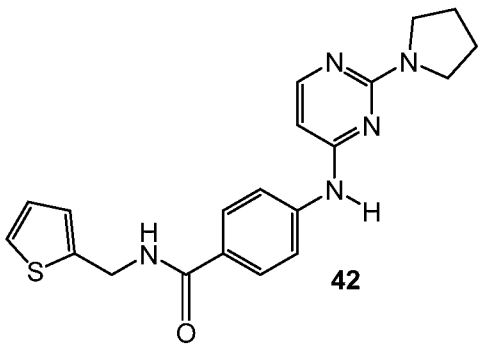
39 (6.29)



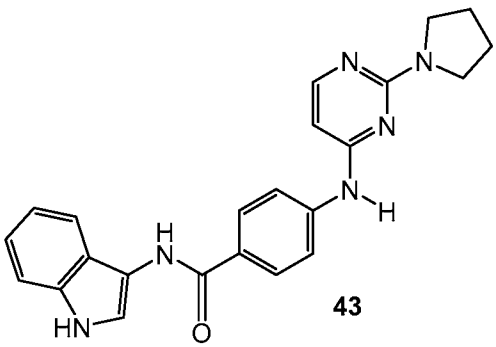
40



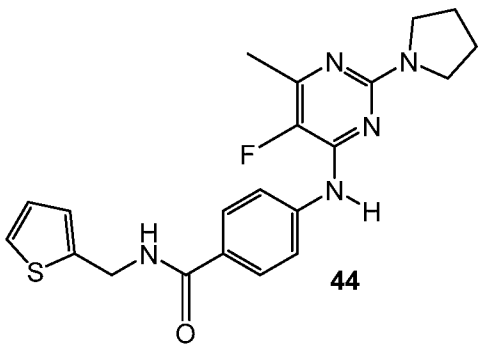
41



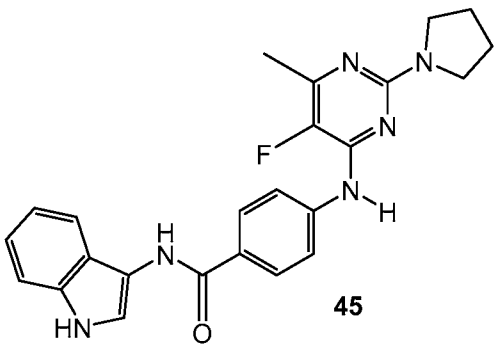
42



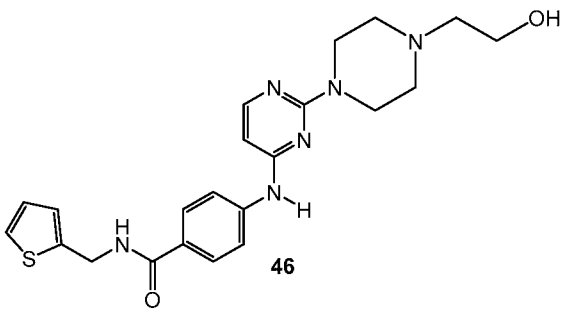
43



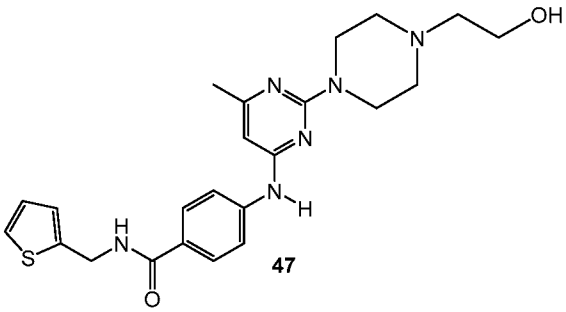
44



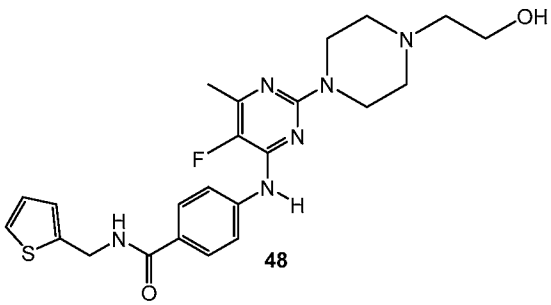
45



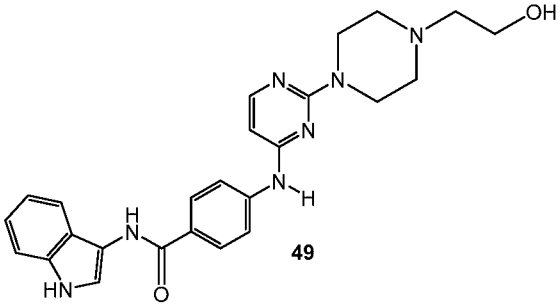
46



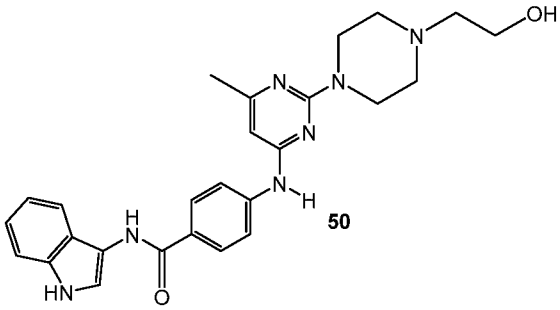
47



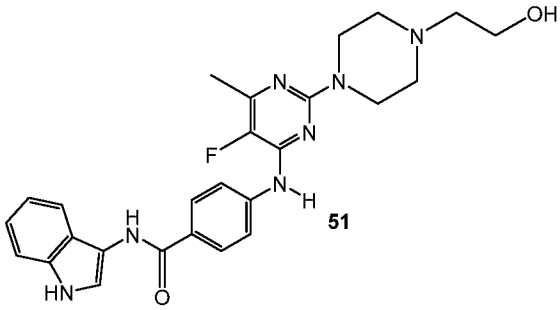
48



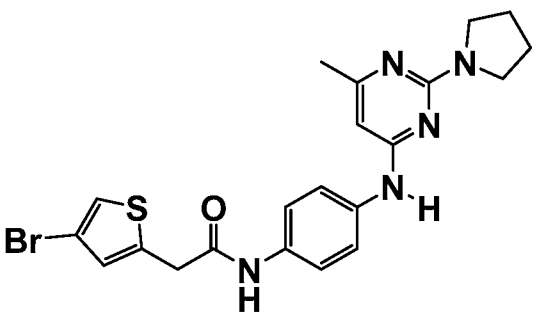
49



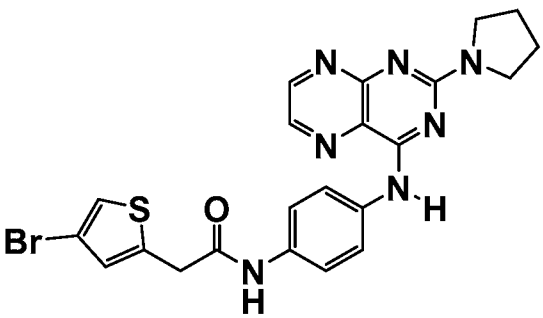
50



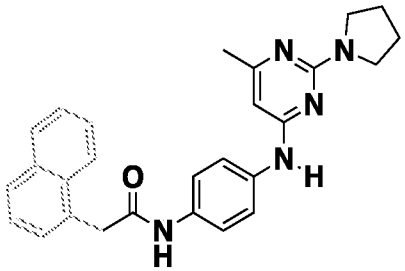
51



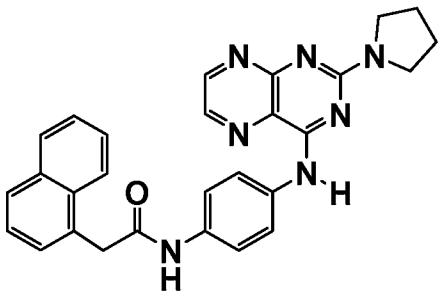
52 (6.11)



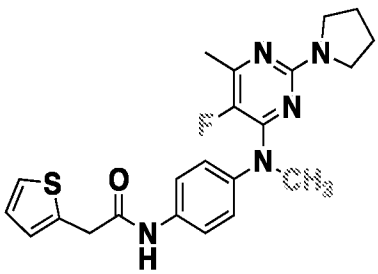
53



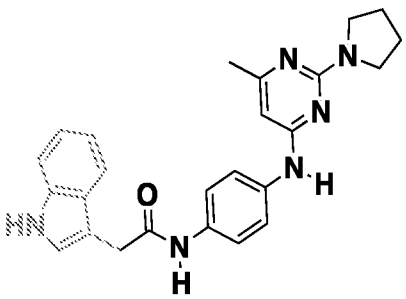
54 (6.16)



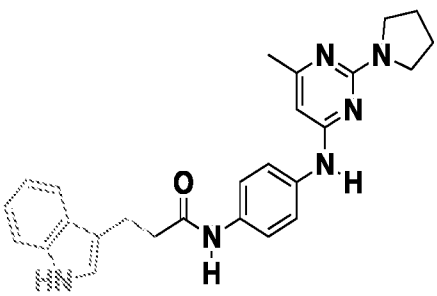
55



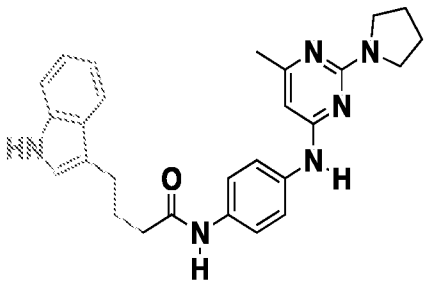
56 (6.4)



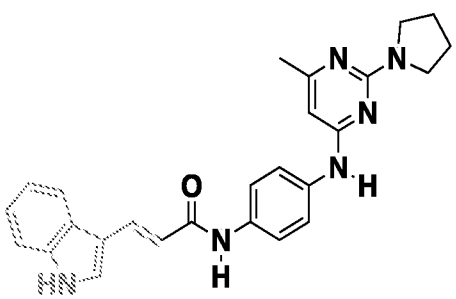
57 (D)



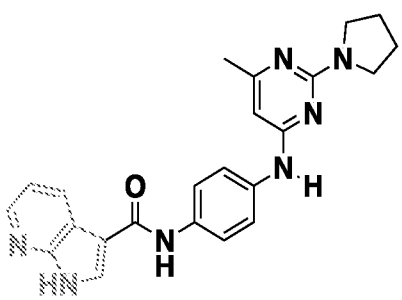
58 (6.6)



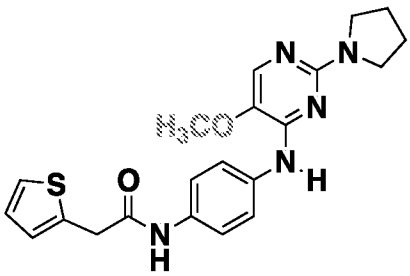
59 (6.7)



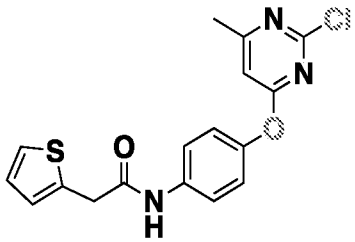
60 (6.8)



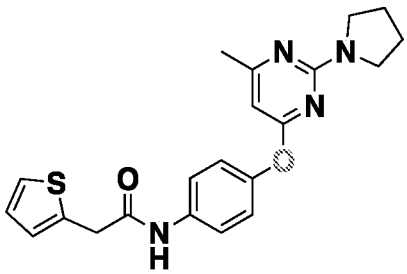
61 (6.9)



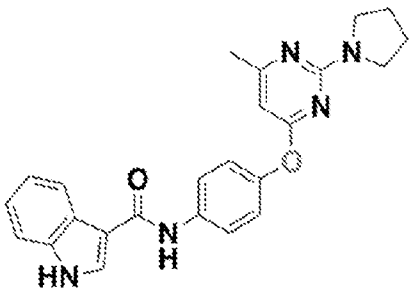
62 (6.10)



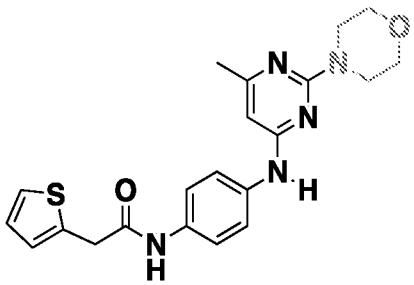
63 (6.12)



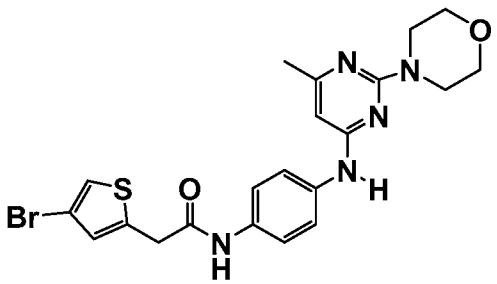
64 (6.13)



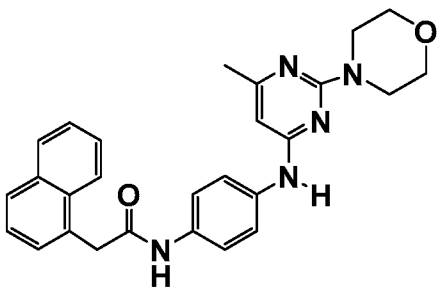
65 (6.14)



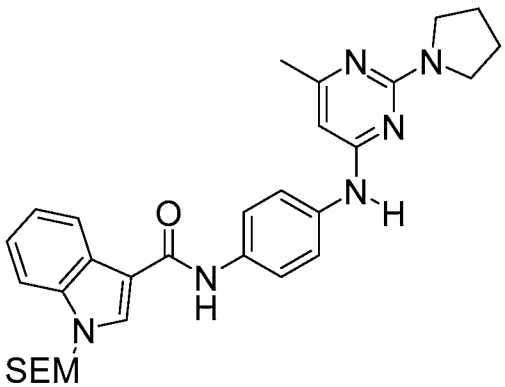
66 (6.15)



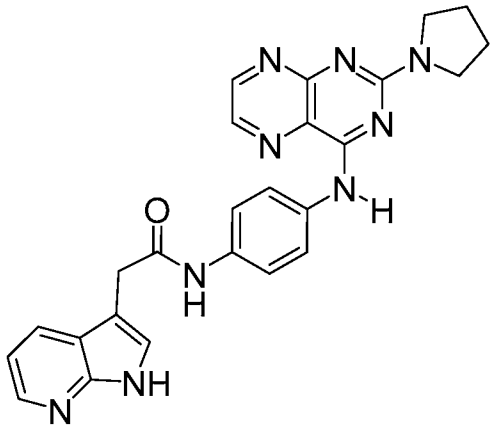
67



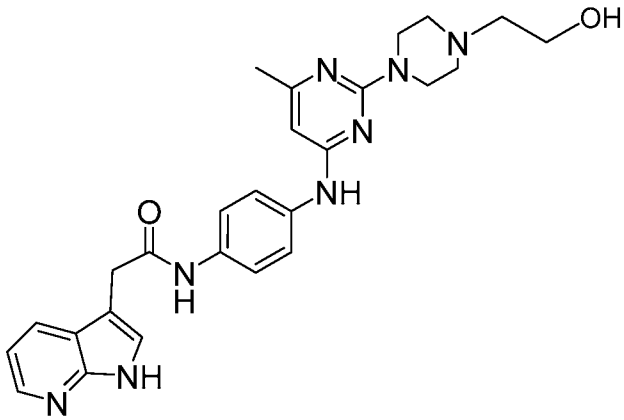
68



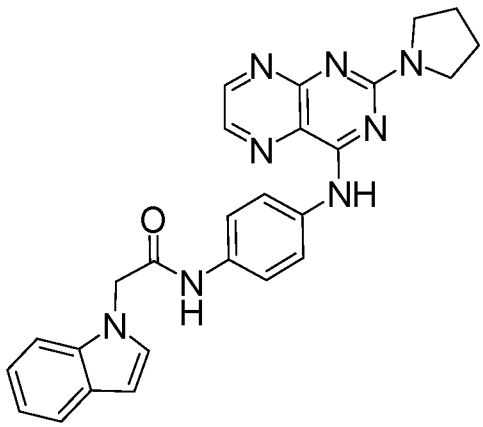
69



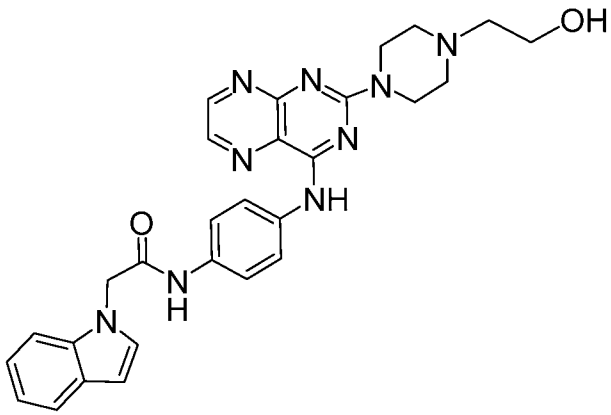
70



71



72



73

5

Example 5: Inhibition of CHD1L Efficacy of DNA Damaging Drugs

CHD1L is known to function in PARP1 mediated DNA damage response repair, which is a mechanism of with increased drug resistance to DNA damaging chemotherapy [Li et al., **2019**; Ahel et al., **2009**; Gottschalk et al., **2009**]. For example, drug resistance to cisplatin in lung cancer was observed in cells overexpressing CHD1L. The efficacy of cisplatin was restored after CHD1L knockdown. [Li Y., et al., **2019**] In addition, knockdown of CHD1L alone does not increase DNA damage. [Ahel D, et al., **2009**] In order to determine if CHD1L inhibitors could increase the efficacy of DNA damaging drugs against low CHD1L expressing DLD1 cells transduced with empty vector (DLD1CHD1L-EV) and overexpressing DLD1CHD1L-OE in CRC cells, compound **6** was evaluated alone as a single agent and in combination with SN-38 (active pharmacophore of prodrug irinotecan), oxaliplatin, and etoposide. To assess DNA damage, the phosphorylation of H2AX (γ-H2AX) by immunofluorescence, a biomarker for DNA damaging chemotherapy, [Ahel D, et al., **2009**], was measured as shown in Abbott et al., **2020** and its Supplementary Information. Compound **6** alone showed no significant DNA damage when treating cells at 10 μM and measuring γ-H2AX activity, which is consistent with previously reported CHD1L knockdown studies. [Ahel D, et al., **2009**]. However, combination treatments in DLD1CHD1L-OE cells with compound **6** synergized with etoposide (10 μM) and SN-38 (1 μM), significantly increasing DNA damage compared to etoposide and SN-38 alone. In DLD1CHD1L-EV cells only the combination of etoposide and compound **6** displayed significant synergy. Under the experimental conditions used we observed no synergy was observed with oxaliplatin. Nevertheless, SN-38 (i.e. irinotecan) combination therapy, known as FOLFIRI, is a standard of care in the treatment of CRC. Therefore, the enhanced DNA damage that occurs with compounds **6** in combination with SN-38 supports the hypothesis that CHD1L inhibitors can increase the efficacy of CRC standard of care DNA damaging chemotherapies.

Example 6: CHD1L inhibitors reverse EMT prior to the induction of cell death.

CHD1L has been reported to confer anti-apoptotic activity by inhibiting activation of caspase-dependent apoptosis. [Li et al., 2**013**; Sun et al., 2**016**] Additionally, reversal or inhibition of EMT is known to restore apoptotic activity of cancer cells. [Lu et al., **2014**] To determine if CHD1L inhibitors reverse EMT prior to induction of cell death, E-cadherin expression by EcadPro-RFP reporter activity was monitored and cytotoxicity was measured using the CellTox™ Green assay. Cells were treated with CHD1L inhibitors for 72 h and imaged every 2 h. A significant increase in E-cadherin expression prior to induction of cytotoxicity for compound **6** relative to DMSO (**Figure 3A**).

To determine if CHD1L inhibitors are able to induce apoptosis in CRC, western blots from SW620 tumor organoids were performed and it was observed that E-cadherin is cleaved after treatment with **5** and **6** [Abbott et al., **2020**]. Cleavage of E-cadherin is a marker of apoptosis [Steinhusen et al., **2001**]

The more potent CHD1L inhibitor **6**, exhibited increases in cleaved PARP1, cleaved caspase 8, and cleaved caspase 3 relative to DMSO control [Abbott et al., **2020**] These results indicate that compound **6** induces extrinsic apoptosis that is consistent with E-cadherin mediated apoptosis through death receptors. [Lu et al., **2014**]

To further characterize the apoptotic activity of CHD1L inhibitors, annexin-V staining in SW620 cells over 12 h was examined. Compound **6** induced significant apoptosis compared to DMSO alone and had similar activity to the positive control SN-38, the active metabolite of irinotecan (**Figure 3B**)

CHD1L inhibitors are effective against patient-derived tumor organoids (PDTOs). The use of PDTOs in preclinical drug development has been established as a predictive *in vitro* cell model for clinical efficacy. [Drost J & Clevers H, **2018**] After establishing the ability of compound **6** to reverse EMT and induce apoptosis using cell line based models, the efficacy of compound **6** was evaluated in PDTOs produced from patient sample CRC102 obtained from the University of Colorado Cancer Center (UCCC) gastrointestinal (GI) tissue bank (**Figure 3C**). Consistent with the results in CRC cell lines, compound **6** showed potent cytotoxicity in PDTOs with an EC50 of 11.6 ± 2 μM

Example 7: ***In vitro and in vivo PK, PD, and liver toxicity of Exemplary Inhibitor Compound 6.***

To assess the drug-like potential and properties of compound **6** *in silico*, *in vitro,* and *in vivo* PK studies were conducted assessing CLogP, aqueous solubility, stability in mouse liver microsomes, and PK in CD-1 mice.

Table 1 provides a *s*ummary of *in vivo* and *in vitro* pharmacokinetic parameters of compound **6**. The consensus LogP (CLogP) values were obtained using the SwissADME web tools. [Daina et al., **2017**] Compound **6** was administered by i.p. injection to athymic nude mice QD for 5 days to measure accumulation in SW620 xenograft tumors (**FIG. 4**) and to assess histopathology of liver toxicity. Representative H&E-stained photomicrograph sections (5x magnification) of liver in both vehicle and compound **6** treated animals are shown in Abbott et al., **2020**. The images demonstrate normal hepatic cord and lobule architecture, with no evidence of hepatocyte degeneration, necrosis, hyperplasia, or parenchymal inflammation. Compound **6** has an excellent balance of lipophilicity (CLogP = 3.2) and aqueous solubility

that is relatively stable to liver metabolizing enzymes, and an excellent PK disposition when administered to CD-1 mice. Compound **6** reaches a high plasma drug concentration C_{Max} (~30,000 ng/mL) and AUC (~80,000 ng/mL/h) with a relatively long half-life ($T_{1/2\lambda}$) of 3 h after intraperitoneal (i.p.) administration.

In an initial study, compound **6,** exhibited a half-life in liver microsomes of less than 20 minutes. In subsequent analogous *in vitro* liver microsome half-life experiments conducted with a different liver microsome preparation (data not shown), compound **6** exhibited a longer half-life of 67 minutes and compound **6.3** exhibited an improved (over **6**) *in vitro* half-life of 98 minutes and compound **6.11** exhibited a further improved (over **6**) *in vitro* half-life of 130 minutes. The initial half-life studies with compound **6** were conducted with a different liver microsome preparation and not comparable to later *in vitro* microsome half-live experiments. The results of the second series of *in vitro* and *in vivo* half-life measurements is provided in Table 2 which includes data for several additional compounds as indicated.

A second acute *in vivo* experiment was conducted using a maximum tolerated dose of **6** (50 mg/kg) administered to athymic nude mice by i.p. QD over five days. The goals of this experiment were to (1) determine if compound **6** causes acute toxicity to livers, (2) accumulates in VimPro-GFP SW620 xenograft tumors, and (3) to determine PD effects. Compound **6** accumulates in SW620 tumors at a concentration of 10,533 ± 5,579 ng/mL (n=4). As expected, when comparing the ratio of compound **6** accumulation in tissue/plasma, 2.7 times more accumulation in liver compared to tumor was observed (**FIG. 4**). However, there was no apparent liver toxicity resulting from compound **6** at the dose and schedule administered (Table 3). Overall, there were no significant histological differences between the livers of vehicle or compound **6** treated mice. The primary histological changes observed were minimal fibrosis and inflammation of the hepatic capsule in both vehicle and compound **6** treated animals. This suggests a very low grade, sub-clinical peritonitis, and is consistent with being secondary to i.p. drug administration.

In accordance with accumulation of compound **6** in tumors, PD effects on tumor tissue were measured by Western blot analysis, indicating a significant downregulation of mesenchymal markers vimentin, vimentin reporter (VimPro-GFP), and slug [Abbott et al., **2020**]. Although not statistically significant, upregulation of the epithelial marker ZO-1 and induction of cleaved caspase 3 (the putative biomarker of apoptosis) were also observed. Taken together, these observations of PD effects by compound **6** indicate the reversion of EMT and apoptosis *in vivo* that were consistent with *in vitro* cell-based antitumor activity of compound

6. Compound **6** displays good PK drug-like properties and the ability to alter EMT and induce apoptosis *in vivo* with no observed liver toxicity.

In contrast, compound **6.11** exhibits significantly longer half-life ($T_{1/2\lambda}$) compared to that of compound **6** of much greater than 6 h after intraperitoneal (i.p.) administration.

Table 1: PK Parameters Compound 6

PK Parameters		*In Vitro*				*In Vivo*		
Comp.	ClogP	PBS Solubility (mg/mL)	Microsomal Half-life (min)	C_{Max} (ng/mL)	V_2 (L/kg)	AUC_{0-t} (ng/mL x h)	CL (L/h/kg)	T1/2λ (h)
6	3.2	0.70	17.0	29,900	2.7	80,333	0.62	2.97

Table 2: CHD1L Inhibitor Pharmacokinetics

CHD1L Inhibitor	**Pharmacokinetics (PK)**	
	***In Vitro* Half-Life (Min)**	***In Vivo* Half-Life (hr)**
6	67	3
6.1	34.9	–
6.2	26.1	–
6.3	98	–
6.4	31.4	–
6.9	295	–
6.10	16.7	–
6.11	130	8
6.12	9.3	–
6.13	43.1	–
6.14	70.4	–
6.15	22.7	–

Table 3: Histological evaluation raw scores of livers from athymic nude mice treated with vehicle or compound 6 (50 mg/kg) QD for 5 days.

Animal Groups	Organ	Assessment[1]	Inflammation score[2]
Vehicle - 1	Liver	N	—
Vehicle - 2	Liver	A	1
Vehicle - 3	Liver	N	—
Vehicle - 4	Liver	A	1
Compound 6 - 1	Liver	N	—
Compound 6 - 2	Liver	A	1
Compound 6 - 3	Liver	N	—
Compound 6 - 3	Liver	A	1

[1]Assessment: N = normal background lesion for mouse strain; A = abnormal. [2]Inflammation score (performed if abnormal tissue assessment): 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Example 8: Biological Evaluation of Compound 8

Compound **8** was evaluated in a number of biological assays described above. Results are presented in **Figures 7A-E**. Compound **8** displays more potent dose dependent inhibition of CHD1L-mediated TCF-transcription (**Fig. 7A**) compared to compound **6**. Likewise, compound **8** reverses EMT, evidenced by the downregulation of vimentin and upregulation of E-cadherin promoter activity (**Figs. 7B** and **7C**, respectively). Compound **8** significantly inhibits clonogenic colony formation over 10 days (**Fig. 7D**). Compound **8** significantly inhibits HCT116 invasive potential over 48 h (**FIG. 7E**).

Example 9: Methods Applied in Examples herein

Additional Materials and Methods

Antibodies. Monoclonal mouse anti-TCF4 antibody was purchased from EMD Millipore (Billerica, MA, USA) (catalog# 05-511), a 1:1000 dilution was used for Western blot and 2 µg antibody per 300 µg of protein was used for IP. Monoclonal rabbit anti-CHD1L antibody was purchased from Abcam (Cambridge, MA, USA) (catalog #ab197019), a 1:5000 dilution was used for Western blot, and 1.5 µg antibody per 300 µg of protein was used for IP. Monoclonal rabbit anti-Vimentin (catalog# 5741), anti-Slug (catalog #9585), anti-E-cadherin (catalog

#3195), anti-ZO-1 (catalog #8193), anti-Histone H3 (catalog #4620) were purchased from Cell Signaling (Danvers, MA, USA) and mouse anti-α-tubulin (catalog# 3873) were purchased from Cell Signaling and a 1:1000 dilution was used for Western blot. Monoclonal rabbit anti-β-catenin (catalog #9582) were purchased from Cell Signaling, a 1:1000 dilution was used for Western blot. Monoclonal rabbit anti-phospho-β-catenin was purchased from Cell Signaling (catalog# 5651). Monoclonal rabbit anti-TCF4 (catalog #2569) and anti-Histone H3 (catalog #4620) were purchased from Cell Signaling and 2 µg antibody per 1 mg of protein was used for ChIP. Anti-rabbit IgG HRP-linked secondary antibody (catalog #7074) was purchased from Cell Signaling and a 1:3000 dilution was used for Western blot. Anti-goat and anti-mouse IgG HRP-linked secondary antibodies (catalog #805-035-180 and #115-035-003) were from Jackson ImmunoResearch (West Grove, PA), a 1:10,000 dilution was used for Western blot.

Clinicopathological Characterization of CHD1L

Transcriptome expression data of 585 CRC patients from the CIT cohort (GEO: GSE39582) were used for *in silico* validation (GSE39582). [Marisa et al., **2013**] Gene expression analyses were performed by the Affymetrix GeneChip™ Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific, Waltham, MA). Robust Multi-Array Analysis (RMA) was used for data preprocessing and ComBat (empirical Bayes regression) for batch correction. Signal intensity was log2 normalized. The CHD1L cutoff for CRC risk stratification based on disease specific survival was determined by the receiver operating characteristic (ROC) curve. Cutoff for CHD1L expression was set to 6.45. Differences in OS were estimated by the Kaplan-Meier method and compared using the log-rank test. The Fisher's exact test was used for the comparison of categorical variables. The Mann-Whitney U test was used for 2 groups of continuous variables. In case of more than two groups, data was analyzed by the Kruskal-Wallis test. For all 2-sided P-values, the unadjusted significance level of 0.05 was applied.

The CHD1L cutoff and clinicopathologic characteristics were evaluated by multiple cox regression analysis. Only variables that were significant in univariate analyses were integrated in the cox regression model using the Wald forward algorithm for significance determination. All variables including more than 2 groups were categorized and the stepwise entry criterion for covariates was $P<0.05$ and the removal criterion was $P>0.1$. Statistical analysis was performed using IBM® SPSS Statistics (IBM, Armonk, NY), Prism8 (GraphPad Software, San Diego, CA), JMP® (SAS Institute, Cary, NC), and RStudio™IDE (RStudio Inc, Boston, MA).

UCCC Patient sample RNA-seq analysis

RNA-seq data from CRC patient tumor xenograft explants were obtained from the UCCC (University of Colorado Cancer Center) GI tumor tissue bank, and analyzed as previously described. [Scott et al, **2017**] Briefly, gene expression was Log2 normalized and measured by FPKM (Fragments Per Kilobase of transcript per Million mapped reads). The Wnt signaling pathway defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG) was used as the gene set in this study. Samples with expression of CHD1L <1 FPKM were considered low expression and were removed from this study. Genes with significant Spearman's correlations ($P<0.05$) were displayed as heatmap using matrix2png (gene-wise Z-normalized) [See: Abbott et al, **2020** and its Supplementary Information]

CHD1L overexpression and shRNA knockdown

Full length CHD1L was synthesized in a pGEX-6P-1 plasmid (GenScript, Piscataway, NJ). The CHD1L sequence flanked by EcoR*I* and Not*I* was digested out and ligated to a lentiviral backbone to create pCDH1-CMV-CHD1L-EF1-puro plasmid for overexpression of CHD1L in human CRC cells. Mission® shRNA (Sigma-Aldrich Co. LLC, St. Louis, MO) (scrambled) and TRCN0000013469 and TRCN0000013470 (sh69 and sh70) specific for CHD1L were purchased from Sigma-Aldrich (St. Louis, MO). Virus was produced in HEK293T cells using TransIT®-293 reagent (Mirus, Madison, WI), and plasmids pHRdelta8.9 and pVSV-G. CRC cells were transduced with overexpression or shRNA knockdown virus and selected with 2 µg/ml puromycin for 7 days.

Western Blots

CRC cell lines and homogenized tumor tissue samples from mice were resuspended in RIPA lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM b-glycerophosphate, 1 mM $Na_3 VO_4$, 0.1 mM PMSF. Protein concentration was determined using the Pierce™ BCA protein assay kit (ThermoFisher, Waltham, MA). Forty micrograms of sample were run on 10% Bis-Tris gels. Following electrophoresis, the proteins were transferred to a nitrocellulose membrane. The membranes were blocked at room temperature with 5% non-fat milk in TBS/Tween® 20 (TBST contains 20 mM Tris, 150 mM NaCl, and 0.1% Tween® 20 (Croda International PLC, Snaith, UK) for 1 hour at room temperature. Membranes were washed three times with TBST. Blots were incubated with the appropriate primary antibody in 5% nonfat milk in TBST overnight at 4 °C. Membranes were washed three times with TBST and then incubated with appropriate secondary antibody for one hour. Membranes were washed again with TBST three times. Blots were

exposed using SuperSignal™West Pico PLUS Chemiluminescent Substrate (ThermoFisher, Waltham, MA) and imaged using a ChemiDoc imaging system (Bio-Rad, Hercules, CA). [See: Abbott et al., **2020** and its Supplementary Information for Western Blots]

TOPflash TCF-transcriptional reporter assay

TOPflash assay (Millipore, Billerica, MA) was used to evaluate TCF transcriptional activity in CRC cells. A total of 20,000 cells per well were plated into 96-well white plates and transfected with TransIT®-LT1 transfection reagent (Mirus, Madison, WI). Cells were incubated with transfection mix for 24 h. Next, cells were washed with phosphate-buffered solution (PBS) and a 1:1 ratio of PBS: ONE-Glo™ luciferase reagent Promega (Madison, WI) was added and the luminescence was detected within 10 min. A duplicate experiment was conducted to measure cell viability using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI), which was used to normalize TOPflash luminescence to obtain the fold change in TCF activity. Experiments were replicated 2x (n = 3 for each experiment).

Co-ImmunoPrecipitation (Co-IP)

Nuclear cell lysates were generated 138 from untreated SW620 cells. For the input control, 100 µL of 1 mg/mL nuclear extract was saved and used as the input. ImmunoPreciptation (IP) was conducted with Dynabeads™ Protein A IP Kit (ThermoScientific, Waltham, MA). Briefly, 300 µg of lysate incubated with 2 µg of the anti-TCF4 and anti-CHD1L IP antibody, anti-rabbit IgG and anti-mouse IgG were used as nonspecific binding controls and were rotated at 4 °C for 2 h. After preincubation, 50 µL of beads were transferred to the preincubated antibody/lysate mixture followed by overnight incubation at 4 °C. The flow through was collected and the beads were washed 3x with PBST. Proteins were eluted with 20 µL of 50 mM glycine (pH = 2.8) at 70 °C for 10 min.

Chromatin Immunoprecipitation (ChIP)

Using detailed methods previously described [Zhou et al., **2016**], cells were cross-linked with 1.42% formaldehyde for 15 min and quenching with 125 mM glycine for 5 min. Cells were lysed with Szak's RIPA (Radioimmunoprecipitation assay buffer) buffer and sonicated. The IP steps were conducted at 4 °C as follows: 50 µL of protein A/G agarose beads were prewashed with cold Szak's RIPA buffer and incubated with 1 mg of lysate for 2 h. 0.3 mg/mL of salmon sperm DNA was added and incubated for 2 h. Lysate (100 µL) was set aside as the input control. Anti-CHD1L (2 µg) was added to the remainder and incubated overnight. Beads were washed and the supernatant was aspirated to 100 µL followed by the addition of 200 µL of 1.5x-Talianidis elution buffer (70 mM Tris-Cl pH 8.0, 1 mM EDTA pH

8.0, 1.5% w/v SDS). To elute immunocomplexes and reverse crosslink, 12 µL of 5M NaCl was added and the mixture was incubated at 65 °C for 16 h. The supernatant was mixed with 20 µg of proteinase K and incubated for 30 min at 37 °C. DNA was extracted with phenol/chloroform and precipitated with ethanol. The IP product was amplified with PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Austin, TX) using known published primers. [Zhou et al., **2016**]

Clonogenic Assay

Colony formation was assessed after CHD1L knockdown in SW620 cells or overexpression in DLD1 cells as previously described. [Zhou et al., **2016**; Abraham et al., **2019**] Cells were plated at 1,000 cells/well in six-well plates and medium was changed 2x per week over a 10-day time course. Colony formation analysis was also performed as previously described. [Zhou et al., **2016**; Abraham et al., **2019**]

To assess CHD1L inhibitors for their ability to suppress CSC stemness, HCT-116 or CHD1L overexpressing DLD1 cell lines were pre-treated in monolayer cultures for 24 h with vehicle control (0.5% DMSO) or CHD1L inhibitors at the concentrations indicated in FIG. 2C. Pretreated viable cells were plated at 1,000 cells/well in 6-well plates or 200 cells/well in a 24-well plates. Colonies were analyzed using the IncuCyte® S3 2018A (Sartorius, France) software (with the following parameters modified from default: (1) for HCT116 cells segmentation adjustment = 0.6; Min area (µm2) = 3x104; Max area (µm2) = 1.6x106; Max eccentricity = 0.9; (2) for DLD1CHD1L OE cells segmentation adjustment = 1; Min area (µm2) = 1x104; Max area was not constrained; Max eccentricity = 0.95. Experiments were replicated 2x (n = 2 for each experiment).

Tumor organoid Culture

Cell lines were cultured [Zhou et al., **2016**; Abraham et al., **2019**] as tumor organoids using phenol red free RPMI-1640 containing 5% FBS and by seeding 5,000 cells/well into un-coated 96-well U-bottom Ultra Low Attachment Microplates (Perkin-Elmer, Hopkinton, MA) followed by centrifugation for 15 min at 1,000 rpm to promote cells aggregation. A final concentration of 2% Matrigel® matrix (Corning Incorporated, Corning, New York) was added and tumor organoids were allowed to self-assemble over 72 h under incubation (5% CO_2, 37 °C, humidity) before treatment, and maintained under standard cell culture conditions during treatment time courses.

VimPro-GFP and EcadPro-RFP reporter 3D high-content imaging assays

Stable VimPro-GFP or EcadPro-RFP SW620 reporter cells were generated using pCDH imPro-GFP-EF1-puro virus or pCDH-EcadPro-mCherry-EF1-puro virus as previously reported. [Zhou et al., **2016**; Abraham et al., **2019**] The stable fluorescently labeled reporter cells were used to generate tumor organoids as described herein. Tumor organoids were treated with CHD1L inhibitors at 10 μM for an additional 72 h. Following treatment, tumor organoids were stained with 16 μM of Hoechst 33342 for 1 h (nuclei stain). Images were taken with a 5x air objective. Z-stacks were set at 26.5 μm apart for a total of 15 optical slices. Imaging and high-content analysis were performed using an Opera Phenix™ and Harmony® software (PerkinElmer, Hopkinton, MA). Nuclei were identified within each layer and cells were found with either GFP or mCherry channel. The fluorescence intensities of each channel were calculated and thresholds were set based on the background intensities. Percentages of GFP or mCherry RFP positive cells were calculated and normalized to the DMSO treated group.

Tumor organoid cytotoxicity.

SW620 tumor organoids were cultured as described herein. CellTox™ Green cytotoxicity assay solution was prepared per manufacturer's protocol (Promega, Madison, WI). Briefly, tumor organoids were treated for 72 h with CellTox™ Green reagent (0.5X) and various doses of CHD1L inhibitors over a range of 0-to-100 μM. Organoids were imaged using the Opera Phenix™ 207 screening system (PerkinElmer Cellular Technologies, Hamburg, Germany) with excitation at 488 nm and emission at 500-550 nm. Mean intensity of the whole well was utilized for calculating cytotoxicity with Lysis Buffer (Promega, Madison, WI) as the 100% cytotoxicity control and 0.5% DMSO as the 0% cytotoxicity control. Intensity values were normalized to these controls using Prism8 (GraphPad, San Diego, CA).

Invasion assays.

HCT116 cells were plated at 60,000 cells/well into an IncuCyte ® ImageLock 96-well plate (Sartorius, France) and allowed to attach overnight. A wound was created in all wells using the IncuCyte® WoundMaker then washed 2x with PBS. The plate was brought to 4 °C using a Corning XT Cool Core to avoid polymerization of the Matrigel® matrix (Corning Life Sciences, Corning, NY) during the preparation of the invasion conditions. Wells were coated with 50 μL of 50% Matrigel® matrix in RPMI-1640 media. Plates were centrifuged at 150 rpm at 4 °C for 3 min, using a swing bucket rotor to ensure even matrix coating with no air bubbles. Afterwards, plates were placed on a Corning XT CoolSink module prewarmed inside a cell culture incubator (5% CO_2, 37 °C, humidity) for 10 min to evenly polymerize the matrix, followed by the addition of CHD1L inhibitors dissolved in 50 μL of RPMI-1640 media

containing 5% FBS. Finally, the plate was placed in an IncuCyte® S3 live cell imager (Sartorius, France) for 48 h. The wound was imaged every hour using the phase contrast channel and 10x objective in wide mode.

Cloning and purification of recombinant human CHD1L

Cat-CHD1L (residues 16-61) and fl-CHD1L (residues 16-879) constructs were a generous gift from Helena Berglund at the Karolinska Institute, Department of Medical Biochemistry and Biophysics. Proteins were expressed in Rosetta™ 2 (DE3) pLysS cells (Novagen available from Sigma-Aldrich, St. Louis, MO) in Terrific Broth (ThermoFischer, Waltham, MA). Cultures were induced with 0.2 mM IPTG at OD600 = 2.0 at 18 °C for 16 h. Cells were harvested and resuspended in buffer-A, containing 20 mM HEPES, pH 7.5, 500 mM NaCl, 50 mM KCl, 20 mM imidazole, 10 mM $MgCl_2$, 1 mM TCEP (tris(2-carboxyethyl)phosphine), 10% glycerol and 500 µM PMSF. Cells were lysed by sonication and cellular debris was removed by centrifugation. The supernatant was loaded onto a Ni-NTA resin column (Qiagen, Hilden, Germany). Protein bound to the column was washed with 1x with buffer-A, 1x with buffer-A containing 10 mM ATP, and washed an additional time with buffer-A. Proteins were eluted using buffer-B (buffer-A with 500 mM imidazole) with a gradient from 20 to 500 mM imidazole. Following affinity purification, Cat-CHD1L was dialyzed overnight into 50 mM Tris, pH 7.5, 200 mM NaCl, and 1 mM DTT. Similarly, fl-CHD1L was dialyzed overnight into 20 mM MES, pH 6.0, 300 mM NaCl, 10% glycerol, and 1 mM DTT. Protein was then purified by ion-exchange chromatography. cat-CHD1L was bound to a Q-sepharose column (GE Healthcare, Chicago, IL) and fl-CHD1L was bound to a S-sepharose column (GE Healthcare, Chicago, IL), and proteins were eluted using a NaCl gradient of 0.2 – 1M for cat-CHD1L and 0.3 -1M for fl-CHD1L. Pure fractions were pooled, concentrated, and further purified by size-exclusion chromatography using a Superdex™ 200 column (GE Healthcare, Chicago, IL) with 20 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM TCEP, and 10% Glycerol. Protein purifications were conducted using an ÄCTA Start FPLC (GE Healthcare, Chicago, IL).

CHD1L ATPase assay

All reactions were carried out using low volume non-binding surface 384-well plates (Corning Inc., Corning NY). cat-CHD1L or fl-CHD1L (100 nM) and 200 nM c-Myc DNA or mononucelosome (Active Motif, Carlsbad, CA) were added to a buffer containing 50 mM Tris pH 7.5, 50 mM NaCl, 1 mM DTT, 5% glycerol, and the reaction was initiated by the addition of 10 µM ATP (New England Biolabs, Ipswich, MA) to a total volume of 10 µL and incubated at 37 °C for 1 h. ATPase activity was assayed by adding 500 nM of Phosphate Sensor (Life Technologies, Carlsbad, CA), containing labeled phosphate-binding protein, specifically

labeled with the fluorophore MDCC, and measuring excitation (430 nm) and emission (450 nm) immediately on an EnVision® plate reader (PerkinElmer, Hopkinton, MA). An inorganic phosphate standard curve was used to convert the fluorescence to [Pi], and enzyme kinetics were determined using Prism8 (GraphPad Software, San Diego, CA).

HTS drug discovery for inhibitors of CHD1L

Assay composition was the same as described above using cat-CHD1L, except that the reaction mixture volume was modified to accommodate addition of drug or DMSO. Using a Janus® liquid handler (PerkinElmer, Hopkinton, MA), a selected amount of compounds dissolved in 100% DMSO were mixed with 50 mM Tris pH 7.5, 50 mM NaCl, 1 mM DTT, 5% glycerol buffer to 200 µM in 10% DMSO. Next, 1 µL of each compound was added to the enzyme mixture to give a final concentration of 20 µM. The negative control used was 1% DMSO and 10 mM EDTA was used as a positive control. Reactions were initiated with the addition of 10 µM ATP and incubated at 37 °C for 1 h. ATPase activity was measured by fluorescence by adding 500 nM Phosphate Sensor. Cat-CHD1L was screened against a 20,000-compound diversity set from Life Chemicals (Woodbridge, CT) and a Kinase Inhibitor library from Selleck Chemicals (Houston, TX). Both libraries were prescreened before purchase to remove Pan-assay interference compounds (PAINS) which tend to react nonspecifically with many biological targets rather than selectively with a desired target. [Baell & Nissink, **2018;** Baell & Holloway, **2010]**

Patient derived tumor organoid (PDTO) culture and viability assay

CRC patient tumor tissues were obtained from the UCCC GI tissue bank and expanded following established protocols. [Morin et al., **1997**]. Briefly, cells were seeded at 5,000 cells per well in 96-well plates and cultured by established methods [Franken et al., **2006**] allowing PDTO formation over 72 h. PDTOs were treated with DMSO (0.5%) or compound **6** with various concentrations for an additional 72 h to obtain a dose response. PDTO cell viability was measured using CellTiter-Blue® reagent (Promega, Madison, WI). Media (80 µL) was aspirated from wells and 80 µL of the reagent was added and incubated for 4 h and cell viability was measured by fluorescence intensity using excitation 560 excitation and 590 emission.

Evaluation of apoptosis

SW620 cells were plated at 30,000 cells/well in 96-well plates. Cells were treated with DMSO (negative control), SN-38 (apoptosis positive control), and compound **6** at concentrations indicated for 12 h. Cells were then rinsed 2x with cold PBS, 1x with cold Annexin-V staining buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$), and then

incubated with Annexin-V FITC at 1:100 for 30 min in the dark. Cells were then rinsed 2x with Annexin-V staining buffer and FITC intensity was measured using an EnVision® plate reader (PerkinElmer, Hopkinton, MA).

Evaluation of DNA damage by γ-H2AX

DLD1$^{CHD1L-OE}$ cells were seeded into a 96-well PerkinElmer Cell Carrier plate and allowed to adhere overnight. Cells were then treated with the appropriate compound at 10 μM (0.5% DMSO) or with CHD1L inhibitor in combination SN-38 (1 μM), oxaliplatin (10 μM), and etoposide (10 μM). Cells were treated for 6 h. Media was aspirated and cells were washed with cold PBS. Cells were then fixed with 3% paraformaldehyde for 15 min at room temperature, fixed cells were washed with PBS three times. Cells were blocked for 1 hour at room temperature in 5% BSA, 0.3% Triton X-100 in PBS. Cells were then immunostained with phospho-(S139)-g-H2AX rabbit mAb using a 1:800 dilution in 1% BSA, 0.3% Triton X-100 in PBS at 4 °C overnight. Primary antibody was aspirated and cells were washed with PBS. Cells were incubated for 2 h at room temperature with goat anti-rabbit Alexa Fluor PlusTM 647 fluorescent secondary antibody at a concentration of 5 μg/mL in 1% BSA, 0.3% Triton X-100 in PBS. Cells were then washed with PBS, Hoechst 33342 stain was diluted to a concentration of 1:1000 in PBS, and added to cells for 15 min at room temperature. Cells were then imaged using a 20X water objective on the Opera Phenix™ HCS imaging system (PerkinElmer**).** Synergy was determined using the coefficient of drug interaction (CDI) equation, CDI = (A+B)/(AB). Synergy was defined in these experiments with a CDI < 0.8. additivity was 0.8-1.2 and antagonism was defined by a CDI > 1.2.

Aqueous solubility and CLogP

Using a recently reported detailed method [Abraham et al., **2019**], aqueous solubility was measured for compound **6**. The PBS UV absorption spectra were compared to a fully saturated solution in 1-propanol and the solubility of compound **6** in 10% DMSO in PBS (pH 7.4) was determined using linear regression analysis. The measurement of solubility in PBS was conducted in duplicate experiments. The consensus LogP (CLogP) values were obtained using the SwissADME web tools. [Daina et al., **2017**]

Microsome stability studies

The microsomal stability of compound **6** was determined using female CD-1 mouse microsomes (M1500) purchased from Sekisui XenoTech (Kansas City, KS), following the recently reported method. [Abraham et al., **2019**] Samples were centrifuged at 20,000*g* for 10 min and the supernatant was transferred to an autosampler vial for LCMS analysis. The

following mass transition (*m/z*, amu) was monitored for compound **6** (molecular weight = 393.5).

In vivo pharmacology

All animal studies were conducted in accordance with the animal protocol procedures approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Colorado Anschutz Medical Campus (Aurora, CO) and Colorado State University (Fort Collins, CO).

Pharmacokinetics

Nine-week old female CD-1 mice, purchased from Charles River (Wilmington, MA), were used for PK studies using recently reported methods [Abraham et al., **2019**] Briefly, the PK studies were designed to cover a range of 0.25-to-24 h with 3 mice/time point for a total of 21 mice/compound **6**. Each mouse was dosed with a single i.p. injection of compound **6** at 50 mg/kg prepared in 100% DMSO. Whole blood was harvested at specific time points and the separated plasma frozen at -80 °C for storage or used for LC-MS/MS analysis.

Pharmacodynamics and liver toxicity

Two million VimPro-GFP SW620 cells suspended in 100 µL of a 1:1 mixture of Matrigel® matrix (Corning Life Sciences, Corning, NY) and RPMI 1640 were injected into the flanks of 9-week old female athymic nude mice (Nude-*Foxn1nu* (069)) (Envigo, Huntingdon, Cambridgeshire, UK). Growth was monitored with caliper measurements 3x per week. At four weeks, mice were randomized into 2 groups and treated with 50 mg/kg of compound **6** in 200 µL of vehicle (10% DMSO, 40% PEG 400, 50% PBS pH=7.4) or with vehicle control. Treatments were administered i.p. QD over five days. Mice were sacrificed 2 h after the final dose on day five of the treatment. Tumors and livers were collected for analysis of compound **6** accumulation measured by LCMS, Western blot analysis measuring effects on EMT and apoptosis, and liver toxicity.

Statistical Analysis

Data were subjected to unpaired two-tailed Student's t-test with Welch's correction statistical analysis or as otherwise stated using Prism8 (GraphPad, LaJolla, CA). All experiments were replicated 3x (n=3) or as described in the methods.

Example 10: Additional Experimental Methods for Assessment of Compound Activities

Microsome stability. CD-1 mouse microsomes were commercially purchased and the reactions were performed as previously desribed. Briefly, a master mix was prepared as follows: Microsomes (0.5 mg/mL), 10 μM CHD1Li solubilized in DMSO (0.1%), 5 mM UDPGA, 25 μg alamethicin, and 1 mM MgCl2 in 100 mM phosphate buffer (pH 7.4). The master mix was pre-incubated at 37°C for 5 min, then 1 mM NADPH was added to start the microsomal activity reaction and maintained at 37°C throughout the time course. Reactions were stopped at 0, 5, 15, 30, 45, and 60 min by adding 200 μL acetonitrile and analyzed by mass spectrometry. The appropriate microsome controls were also performed in the same reaction conditions.

γ-H2AX DNA damage combination studies with irinotecan (SN38). CHD1L inhibitor **6** alone and in combination with SN38 was assessed for DNA damage as previously reported [Abbott et al., **2020**; Abraham et al., **2019**]. Using DLD1 colorectal cancer cells that have low CHD1L endogenous expression and DLD1 cells engineered to overexpress CHD1L, DNA damage studies were conducted measuring the immunofluorescence of γ-H2AX, a well-established biomarker of DNA damage [Ji et al., **2017**; Ivashkevich et al., **2012**]. Cells were seeded into a 96-well plate as monolayers and treated with compound 6 at 10 μM (0.5% DMSO) or SN-38 (1 μM), or the combination of 6 and SN38 over 6 hours. Media was aspirated and cells were washed with cold PBS. Cells were then fixed with 3% paraformaldehyde for 15 min at room temperature and washed with PBS three times. Cells were blocked for 1 hour at room temperature in 5% BSA, 0.3% Triton X-100 in PBS. Cells were then immunostained with phospho-(S139)-γ-H2AX rabbit mAb using a 1:800 dilution in 1% BSA, 0.3% Triton X-100 in PBS at 4 °C overnight. Primary antibody was aspirated, and cells were washed with PBS. Cells were incubated for 2 hours at room temperature with goat anti-rabbit Alexa Fluor Plus™ 647 fluorescent secondary antibody at a concentration of 5 μg/mL in 1% BSA, 0.3% Triton X-100 in PBS. Cells were then washed with PBS; Hoechst 33342 stain was diluted to a concentration of 1:1000 in PBS and added to cells for 15 min at room temperature. Cells were then imaged using a 20X water objective on the PerkinElmer Phenix HCS imaging system. We observed synergy between compound 6 and SN38 in inducing damage in DLD1 cells that overexpress CHD1L, determined using the coefficient of drug interaction (CDI) equation. CDI = (A+B)/(AB), synergy was determined with a CDI < 0.8, additivity was 0.8-1.2, and antagonism was defined by a CDI > 1.2. Welch's t-test statistical analysis was used to determine significance, where **= $P \leq 0.01$.

Cell based cytotoxicity dose response and combination studies. CHD1L inhibitors and SN38 (the active pharmacophore of irinotecan) were assessed for antitumor activity against colorectal cancer cell lines alone or in combination. Cell lines were cultured as monolayers or 3D tumor organoids using RPMI-1640 containing 5% fetal bovine serum as previously reported [Abbott et al. , **2020**]. For 3D SW620 tumor organoid cytotoxicity studies, 2,000 cells in 100 µL were plated into each well of the 96-well U-bottom ultra-low attachment microplates (Corning Inc., Corning, NY, USA). Plates were centrifuged at 1,000 rpm for 15 minutes to promote cell aggregation. A final 2% of Matrigel concentration was reached by coating the centrifuged cells with 25 µL of 10% Matrigel per well. Plates were then incubated for 3 days before treatment. 3D organoids were treated with 25 µL of various concentrations of drugs. 3 days after treatment, organoids with 40 µL of medium were manually transferred to 96-well white solid bottom plates. An equal amount of Celltiter-glo 3D (Promega) was added, and the plates were kept on a plate shaker for 45 minutes at 400 rpm before luminescence was read with Envision plate reader (PerkinElmer). For combination studies, synergy scores were determined using Combenefit analysis [De Veroli et al., **2016**].

In vivo studies. CHD1L inhibitors compound **6** and **6.11** were assessed pharmacokinetically to determine the plasma half-life in nine-week-old female CD-1 mice as previously reported [Abbott et al., **2020**]. Compound **6** was further assessed for antitumor activity alone and in combination with irinotecan against SW620 tumor xenografts in athymic nude mice. Xenografts were generated using the methodology as previously reported [Zhou et al., **2016**]. Briefly, compound **6** was administered at 5 mg/kg by intraperitoneal injection (i.p.) 2x/day 7 days/week for a total of 5 weeks. irinotecan was administered i.p. at 60 mg/kg 1x/week for 3 weeks, starting after the first week of compound **6** treatment. Body weight and tumor volumes were monitored 2x/week. Mice were sacrificed and tissues collected when single tumors reached 2000 mm^3 or the total tumor volume reached 3000mm [Ji et al., **2017**]. Compound **6.11** was analogously assessed for antitumor activity alone and in combination with irinotecan against SW620 tumor xenografts. It was recently reported [Esquer et al., **2021** and it Supplementary Information] that the CRC M-phenotype is significantly more tumourogenic than other CRC EMT-phenotypes and that the M-phenotype also has significantly higher TCF-transcription. The xenografts used in this study were generated using isolated dual-reporter mesenchymal cells (M-phenotype) as described in Esquer et al, **2021**. The half-life of compound **6.11** is 8 hours in CD-1 mice, which is 2.7-fold more stable compared to compound **6** (half-life = 3 hours). Thus, the number of treatments was reduced from 2x/day to 1x/day. In addition, irinotecan was administered i.p. at 50 mg/kg.

FIGs. 7A and 7B illustrate representative single agent cytotoxicity dose response studies in SW620 colorectal cancer (CRC) tumor organoids and provide IC_{50} for exemplary compounds as indicated. Tables 4A and 4B below provides a summary of cytotoxicity data for exemplary compounds. Table 4A provides cytotoxicity data for representative single compounds in several different CRC tumor organaoids.

Table 4A: Tumor Organoid Cytotoxicity

CHD1L Inhibitor	Tumor Organoid Cytotoxicity IC_{50} (μM)			
	SW620 (μM)	HCT116 (μM)	CRC042 (μM)	CRC102 (μM)
6	4.74	4.93	18.61	22.6
6.1	>40	>30	–	–
6.2	>7	–	–	–
6.3	3.62	3.72	–	–
6.4	28.3	>30	–	–
6.5	1.18	2.2	–	–
6.6	>40	>30	–	–
6.7	13	17.6	–	–
6.8	5.48	6.85	–	–
6.9	8.28	19.6	–	–
6.10	24.1	>30	–	–
6.11	2.63	3.48	4.5	8.38
6.12	>20	>30	–	–
6.13	>20	>30	–	–
6.14	11.1	15.3	–	–
6.15	15.33	19.7	–	–
6.16	1.52	2.43	–	–
6.17	5.47	3.86	–	–
6.18	1.5	2.95	–	–
6.19	7.97	>30	–	–
6.20	2.16	–	–	–
6.21	1.73	–	–	–
6.22	11.34	–	–	–
6.23	6.01	–	–	–

6.24	3.24	–	–	–
6.25	13.0	–	–	–
6.26	3.55	–	–	–
6.27	2.84	–	–	–
6.28	>30	–	–	–

Table 4B provides results of combination treatments of the indicated representative CHD1L Inhibitors (CHD1Li) with SN38 or Olaparib. Treatments are performed in four different CRC tumor organoid types. The concentration of CHD1L inhibitor is varied as indicated. IC_{50} for the combination treatment are generally decreased compared to SN38 and Olaparib alone.

Table 4B: Tumor Organoid Cytotoxicity Combination Treatments

CHD1L Inhibitor	Tumor Organoid Cytotoxicity Combination Treatments					
	SW620 CHD1Li (μM) + SN38 (nM)	HCT116 CHD1Li (μM) + Olaparib μM	CRC042 CHD1Li (μM) + SN38 nM	CRC042 CHD1Li (μM) + Olaparib μM	CRC102 CHD1Li (μM) + SN38 nM	CRC102 CHD1Li (μM) + Olaparib μM
6	(0 μM) - 356nM; (3 μM) - 191nM; (4 μM) - 42nM; (5 μM) - 8nM;	(0 μM) - 377.8 μM; (5 μM) - 114.4 μM; (6 μM) - 64.7 μM; (7 μM) - 17.4 μM;	(0 μM) - 52nM; (14 μM) - 26nM; (18 μM) - 23nM; (22 μM) - 6nM;	(0 μM) - 134μM; (22 μM) - 116μM; (25 μM) - 58μM; (28 μM) - 42μM;	(0 μM) - 340nM; (20 μM) - 111nM; (22 μM) - 56nM; (25 μM) - 26nM;	(0 μM) - 202μM; (22 μM) - 73μM; (25 μM) - 70μM; (28 μM) - 62μM;
6.3	(0 μM) - 356nM; (2 μM) - 96nM; (3 μM) - 6nM; (4 μM) - 1nM;	–	–	–	–	–
6.11	(0 μM) - 356nM; (2.5 μM) - 9nM; (3 μM) - 8nM;	(0 μM) - 261.6 μM; (2.5 μM) - 300.8 μM; (3.5 μM) - 16.59 μM; (4.5 μM) - 8.44 μM;	(0 μM) - 52nM; (3.5 μM) - 43nM; (4.5 μM) - 8nM; (5 μM) - 1nM;	(0 μM) - 134μM; (4 μM) - 52μM; (5 μM) - 23μM; (6 μM) - 10μM;	(0 μM) - 340nM; (6 μM) - 305nM; (8 μM) - 16nM; (10 μM) - 6nM;	(0 μM) - 202μM; (6 μM) - 50μM; (8 μM) - 37μM; (10 μM) - 11μM;
6.15	(0 μM) - 356nM; (8 μM) - 228nM; (9 μM) - 197nM; (10 μM) - 224nM;	–	–	–	–	–
6.16	(0 μM) - 356nM; (1.25 μM) - 205nM; (1.5 μM) - 90nM; (1.75 μM) - 5nM;	–	–	–	–	–

FIG. 8B presents a graph of γ-H2AX intensity (relative to DMSO) for compound **6** alone, irinotecan (SN38) alone, and a combination of the two in DLD1 empty vector (EV) cells and DLD1 (OE) overexpressing cells. **FIG. 8A** is a Western Blot showing relative expression of CHD1L in DLD1(EV) cells compared to DLD1(OE) cells compared to control expression of α-tubulin in these cells. CHD1L is known to be essential for PARP-1 Mediated DNA Repair, causing resistance to DNA damaging chemotherapy [Ahel et al., 2009; Tsuda et al., 2017]. Data in **FIG. 8B** demonstrate CHD1L inhibitor "on target" effects that synergize with SN38 inducing DNA damage.

FIGs. 9A-9C illustrate the results of synergy studies with exemplary CHD1L Inhibitors **6**, **6.3**, **6.9** and **6.11** in SW620 Colorectal Cancer (CRC) Tumor Organoids. SN38 combinations with **6,** and **6.3** are 50-fold, and 150-fold more potent, respectively, than SN38 alone in killing colon SW620 tumor organoids. SN38 combinations with **6.9** and **6.11** are both over 100-fold more potent than SN38 alone. Each of compounds **6, 6.3, 6.9** and **6.11** shows synergism with irinotecan (and SN38) for killing SW620 tumor organoids.

Synergy scores for exemplary CHD1L inhibitors where scores are determined as described in De Veroli et al. 2016 are provided in Table 5. For interpreting the value of synergy scores, as SynergyFinder has normalized input data as percentage inhibition, they can be directly interpreted as the proportion of cellular responses that can be attributed to the drug interactions. (e.g., synergy score 20 corresponds to 20% of response beyond expectation). According to our experience, the synergy scores near 0 gives limited confidence on synergy or antagonism. When the synergy score is:

Less than -10: the interaction between two drugs is likely to be antagonism;

From -10 to 10: the interaction between two drugs is likely to be additivity;

Larger than 10: the interaction between two drugs is likely to be synergy.

Table 5: Exemplary Synergy Scores of SN38 with Representative Compounds

LOEWE Synergy Scores (Compound No.(IC_{50} of Compound))			
SN38(nM)	Cpd **6** (5μM)	Cpd **6.3** (3μM)	Cpd **6.11** (3μM)
0.64	-5	4	46
3.2	36	31	54
16	35	36	52
80	45	54	49
400	41	48	48
2000	28	35	36
10000	16	25	25

FIG. 10 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 28 days) of treatment with Compound **6** alone, irinotecan alone or a combination thereof. The combination of irinotecan and Compound **6** significantly inhibit colon SW620 tumor xenografts to almost no tumor volume within 28 days of treatment compared to the single agent treatment groups or control.

FIG. 11 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 28 days) of treatment with irinotecan alone (1) or a combination of Compound **6** and irinotecan (2). The combination of irinotecan and Compound **6** significantly inhibits colon SW620 tumors to almost no tumor volume beyond the last treatment compared to irinotecan alone. Within 2-weeks of the last treatment of irinotecan alone tumor volume rose to above the volume of the last treatment, signifying tumor recurrence. In contrast the combination maintained a lower tumor volume.

FIG. 12 shows that Compound **6** alone and in combination with irinotecan (4) significantly increases the survival of CRC-tumor-bearing mice compared to vehicle (1), Compound **6** alone (2) and irinotecan alone (3).

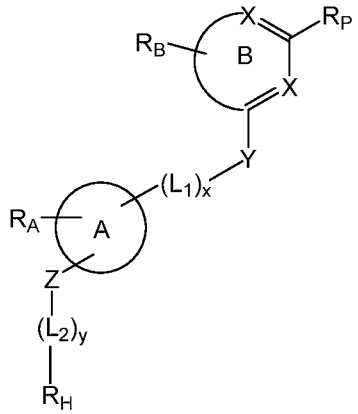
FIG. 13 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 20 days) of treatment with Compound **6.11** alone, irinotecan alone or a combination thereof. The combination of irinotecan and Compound **6.11** significantly inhibits colorectal cancer SW620 tumor xenografts compared to irinotecan alone or control.

FIG. 14 includes a graph of tumor volume (fold) SW620 tumor xenografts as a function of days (up to 33 days) of treatment with irinotecan alone or a combination of compound **6.11** with irinotecan. The combination of irinotecan and compound **6.11** significantly inhibits colorectal SW620 tumors beyond the last treatment (day 33) compared to irinotecan alone. Eight days post treatment (Tx Released), tumor volume with irinotecan treatment alone rose ~3-fold, signifying tumor recurrence. Conversely, tumor volume with treatment of the combination of 6.11 and irinotecan continued to drop (by ~1.5-fold) post treatment. The difference in tumor volume between treatment with irinotecan alone and treatment with the combination of **6.11** and irinotecan 8 days post treatment is 3.4-fold.

FIG. 15 shows that Compound **6.11** in combination with irinotecan significantly increases the survival of CRC-tumor-bearing mice compared to irinotecan alone and control.

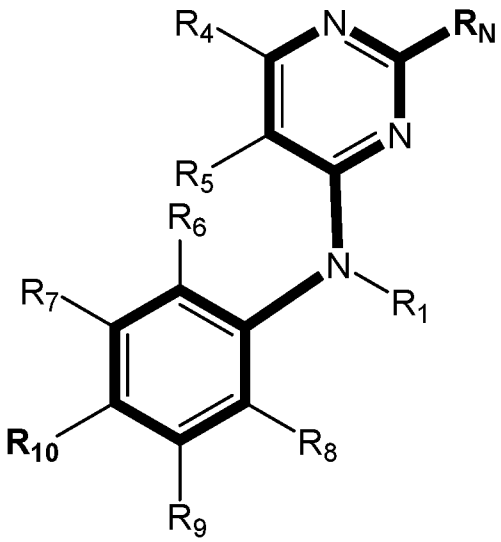
Example 11: Summary of Currently Preferred Structure Activity Relationships for Inhibitors.

The currently preferred structure activity relationship based on formula I for CDH1L Inhibitors of this invention is as follows:



For the B ring, it is currently preferred the ring is a 6-member aromatic or fused 6, 6-member aromatic ring and that both X are N. The second fused ring, if present, can contain one or two additional N. Preferred R_B (B ring substitution), if present, are other than electronegative groups. Preferred R_B are hydrogen or C1-C3 alkyl. The preferred A ring is optionally substituted phenyl, with unsubstituted phenyl (where R_A is hydrogen) more preferred. The R_P group is believed to be associated with water solubility, with -$N(R_2)(R_3)$ groups generally preferred and more particularly preferred optionally substituted N-containing heterocycles, where R_2 and R_3 together with the N to which they are attached form a 5- to 8-member ring which may contain one or more additional heteroatoms and which may be saturated (no double bond) or contain one or more double bonds. R_H is believed associated with activity and potency as well as metabolic stability. RH is preferably an aromatic group and more particularly a heteroaromatic group with ring substitution that stabilizes the aromatic or heteroaromatic ring. Preferred Y in NR with R that is hydrogen more preferred. Preferably x is 0. Preferred Z is –CO-NH-. Preferred L_2 is $–CH_2-$ or $–CH_2-CH_2-$.

In an embodiment, HTS screening for CHD1L identified a phenylamino pyrimidine pharmacophore illustrated in formula XX:



and salts thereof, where R_1-R_9 represent hydrogen or optional substituents, R_{10} is a moiety believed to be associated with potency; and R_N is a moiety believed to be associated with physicochemical properties such as solubility. In embodiments, R_5 is a substituent other than hydrogen which is believed to be associated with metabolic stability. In specific embodiments, R_5 is a halogen, particularly F or Cl, a C1-C3 alkyl group, particularly a methyl group. In embodiments, R_4 is a substituent other than hydrogen and in particular is a C1-C3 alkyl group, and more particularly is a methyl group. In a specific embodiment, R_5 is F and R_4 is methyl. In embodiments, R_6-R_9 are selected from hydrogen, C1-C3-alkyl, halogen, hydroxyl, C1-C3 alkoxy, formyl, or C_1-C_3 acyl. In embodiments, one or two of R_6-R_9 are moieties other than hydrogen. In an embodiment, one of R_6-R_9 is a halogen, particularly fluorine. In specific embodiments, all of R_6-R_9 are hydrogen. In embodiments, R_N is an amino moiety $–N(R_2)(R_3)$. In specific embodiments, R_N is an optionally substituted heterocyclic group having a 5- to 7- member ring optionally containing a second heteroatoms (N, S or O). In embodiments, R_N is optionally substituted pyrrolidin-1-yl, piperidin-1-yl, azepan-1-yl, piperazin-1-yl, or morpholino. In R_N is substituted with one substituent selected from C1-C3 alkyl, formyl, C1-C3 acyl (particularly acetyl), hydroxyl, halogen (particularly F or Cl), hydroxyC1-C3 alkyl (particularly $–CH_2$-CH_2-OH). In embodiments, R_N is unsubstituted pyrrolidin-1-yl, piperidin-1-yl, azepan-1-yl, piperazin-1-yl, or morpholino.

In embodiments, R_{10} is –NRy-CO-(L_2)y-R_{12} or –CO-NRy--(L_2)y-R_{12}, where y is 0 or 1 to indicate the absence of presence of L_2 which is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two, carbons of the linker are optionally replaced with O, NH, NRy or S, where Ry is hydrogen or a 1-3 carbon alkyl, and R_{12} is an aryl group, cycloalkyl group, heterocyclic group, or heteroaryl group, each of which is optionally substituted. I_N embodiments, y is 1. L_2 is $–(CH_2)$p-, where p is 0-3. In

embodiments, R_{12} is thiophen-2-yl, thiophen-3-yl, furany-2-yl, furan-3-yl, pyrrol-2-yl, pyrrol-3-yl, oxazol-4-yl, oxazol-5-yl, oxazol-2-yl, indol-2-yl, indol-3-yl, benzofuran-2-yl, benzofuran-3-yl, benzo[b]thiophen-2-yl, benzo[b]thiophen-3-yl, isobenzofuran-1-yl, isoindol-1-yl, or benzo[c]thiophen-1-yl. In embodiments, R_1 is hydrogen or methyl. In embodiments, R_{12} is thiophen-2-yl, furany-2-yl, pyrrol-2-yl, oxazol-4-yl, indol-2-yl, benzofuran-2-yl, or benzo[b]thiophen-2-yl. In embodiments, R_{12} is thiophen-2-yl or indol-2-yl. In embodiments, R_1 is hydrogen or methyl.

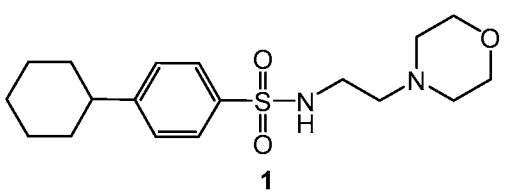
Exemplary compounds of the invention are illustrated in Scheme 1.

Exemplary R_P and -$N(R_2)(R_3)$ groups are illustrated in Scheme 2.

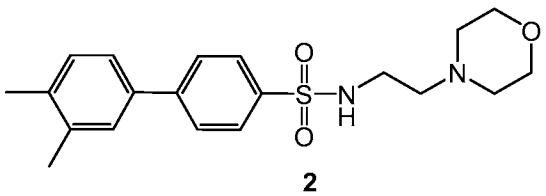
Exemplary R_{12} and R_H groups are illustrated in Scheme 3.

Exemplary B rings for formula I are illustrated in Scheme 4.

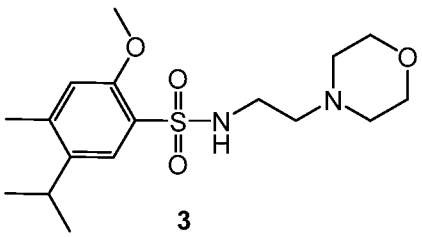
Scheme 1: Exemplary Compounds of Formula I or Formula XX



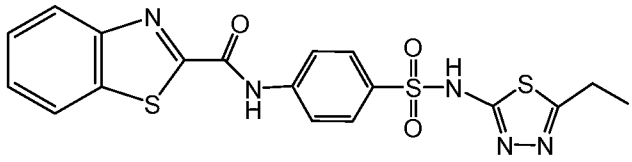
1



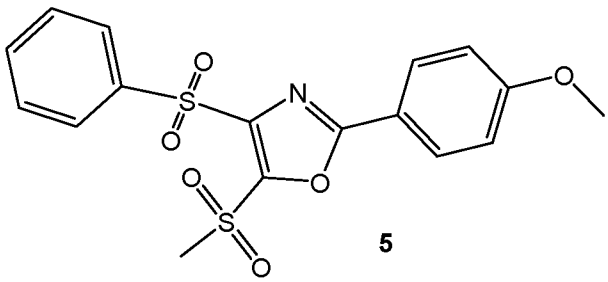
2



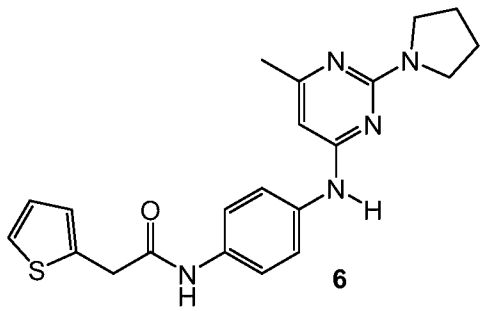
3



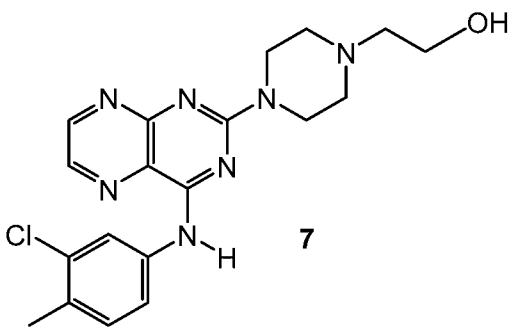
4



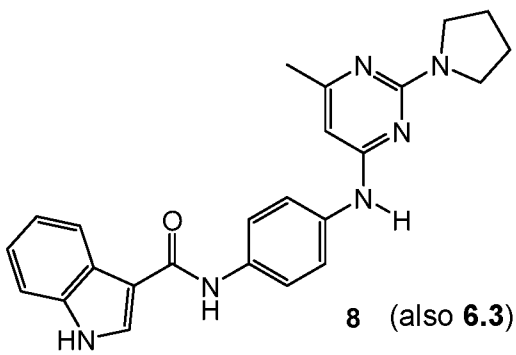
5



6

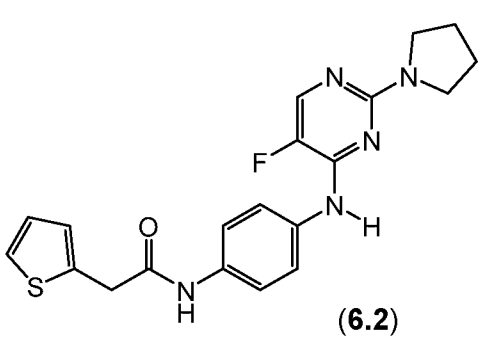


7

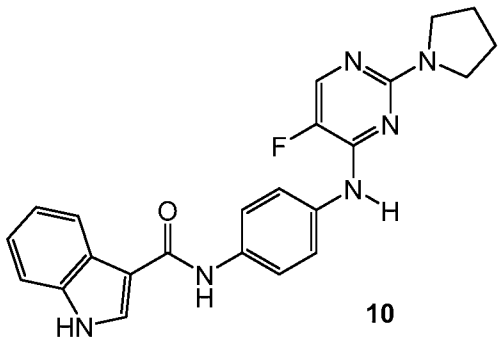


8 (also **6.3**)

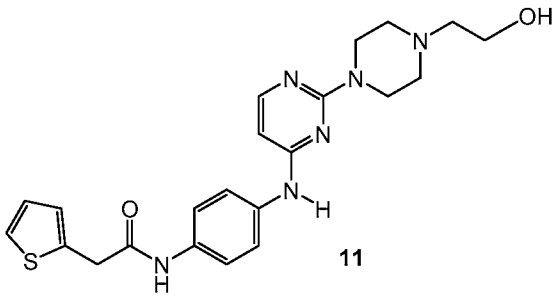
Scheme 1 (continued)



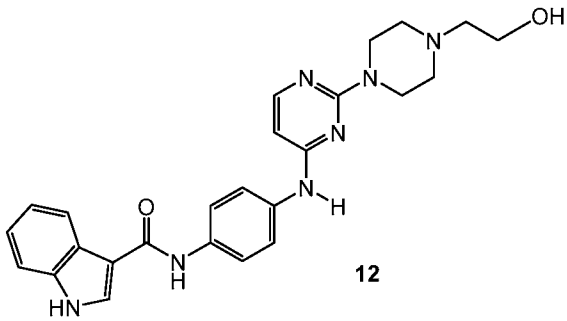
(**6.2**)



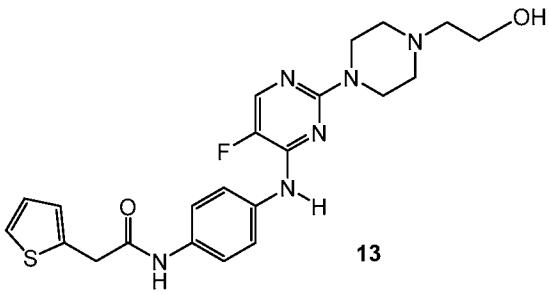
10



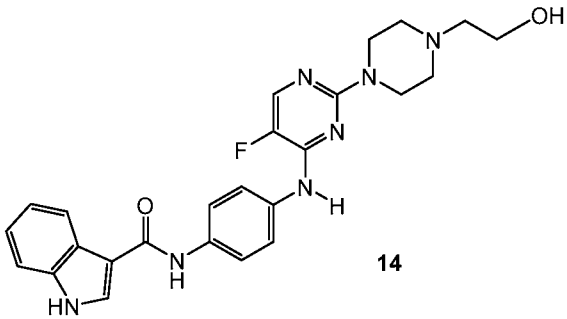
11



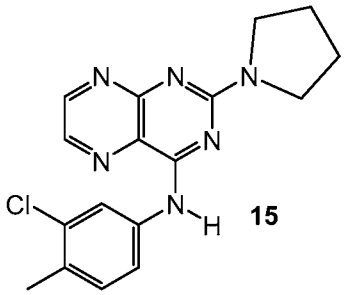
12



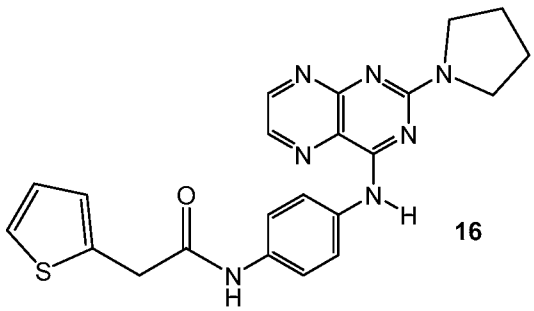
13



14

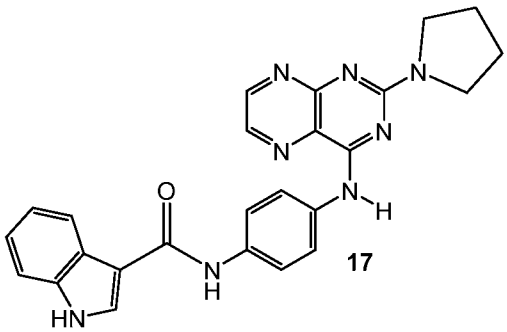


15

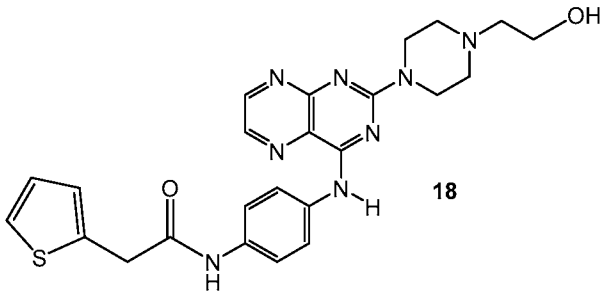


16

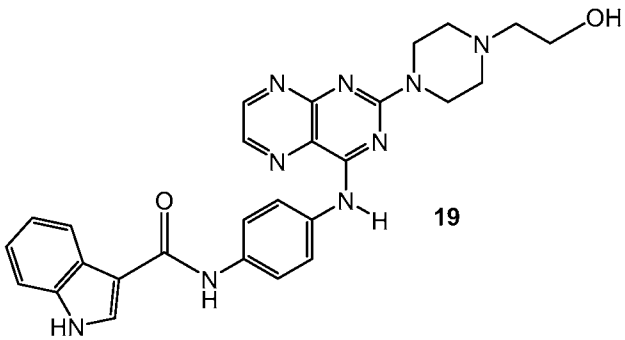
Scheme 1 (continued)



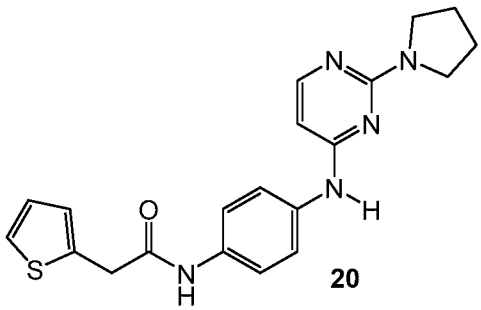
17



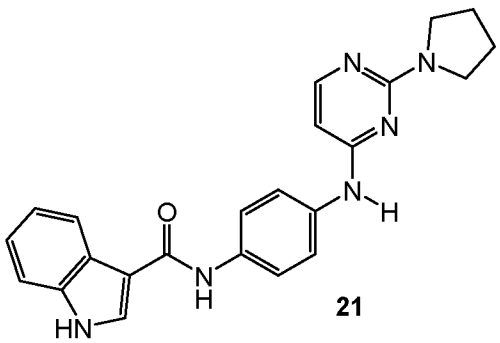
18



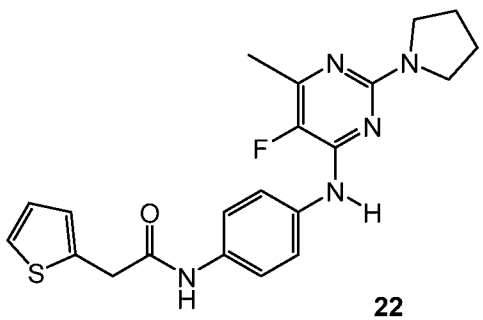
19



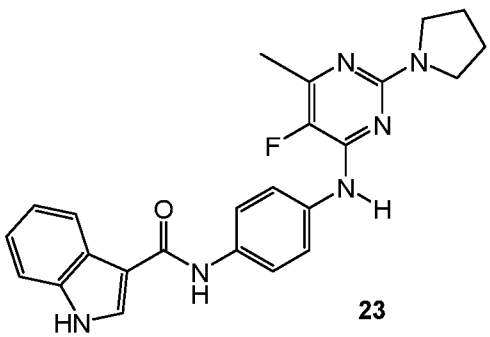
20



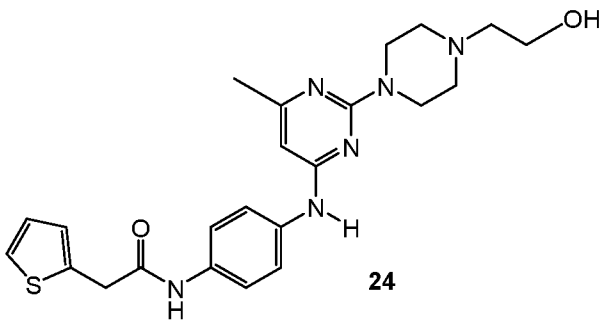
21



22

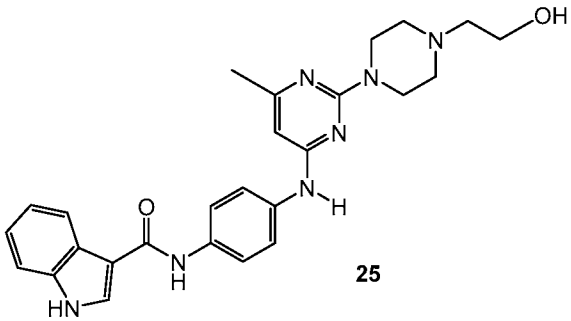


23

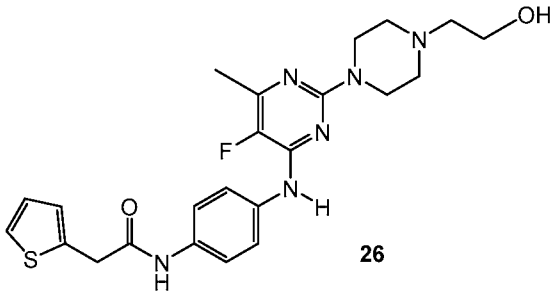


24

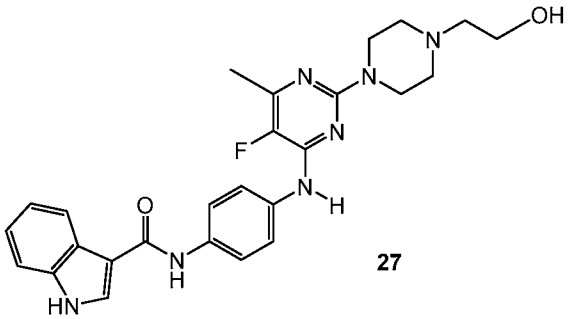
Scheme 1 (continued)



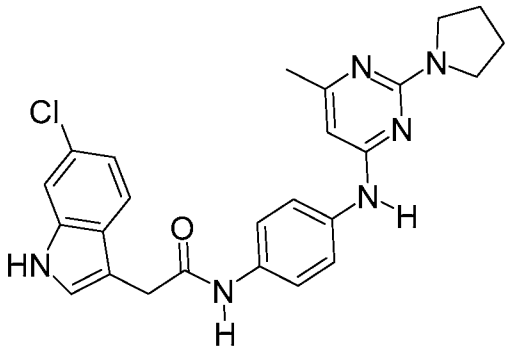
25



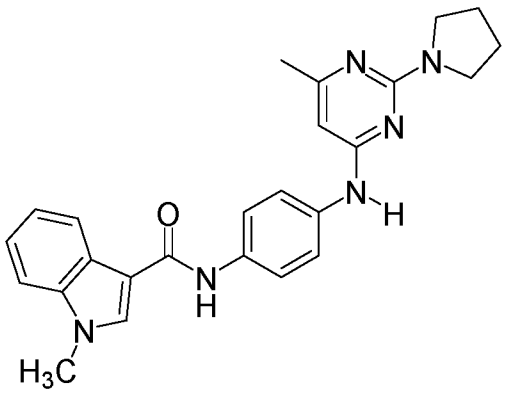
26



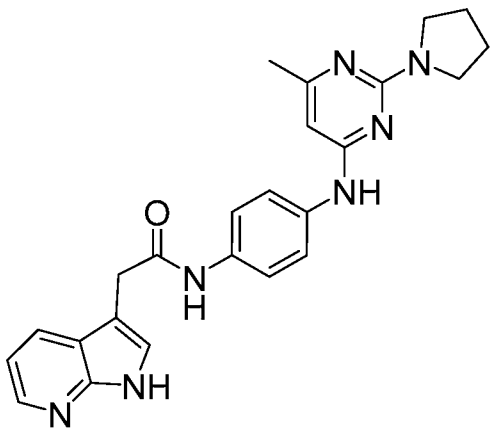
27



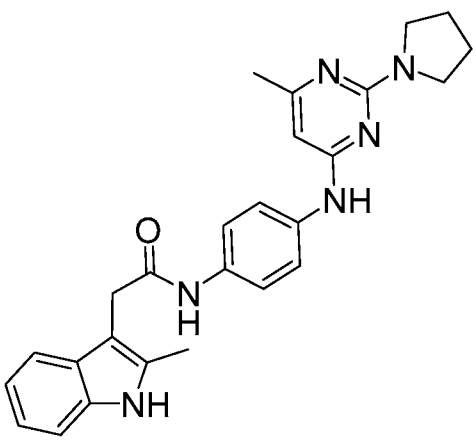
28 (6.18)



29 (6.19)

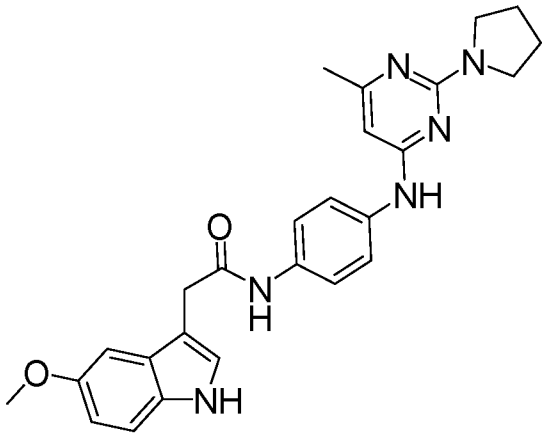


30 (6.20)

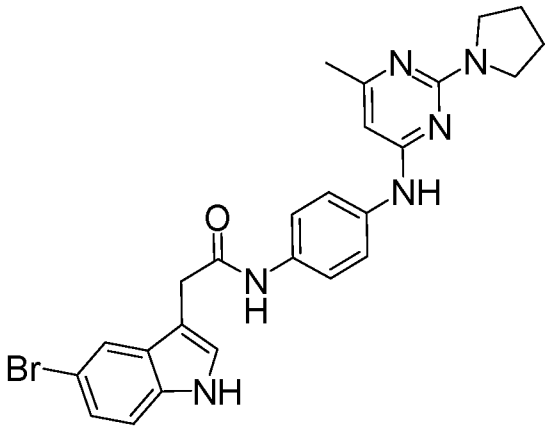


31 (6.21)

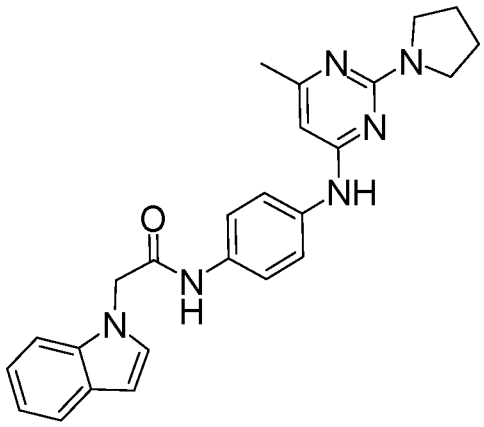
Scheme 1 (continued)



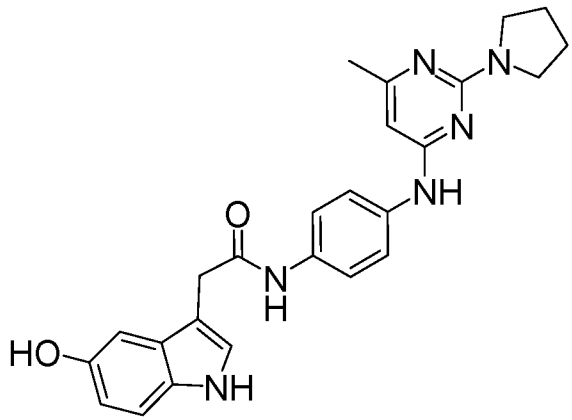
32 (6.22)



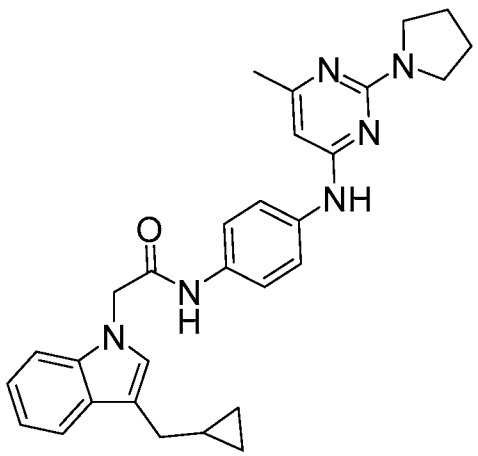
33 (6.23)



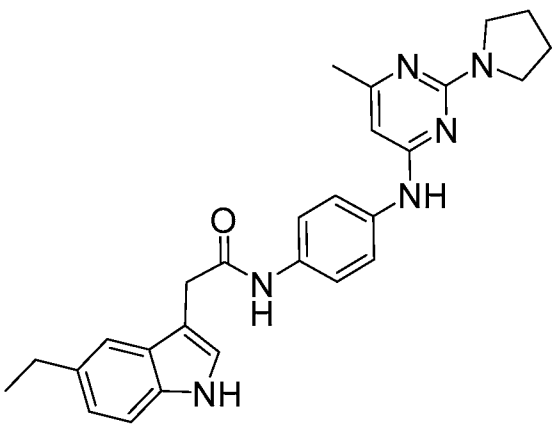
34 (6.24)



35 (6.25)

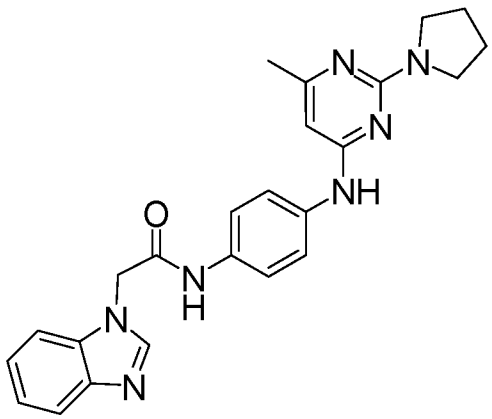


36 (6.26)

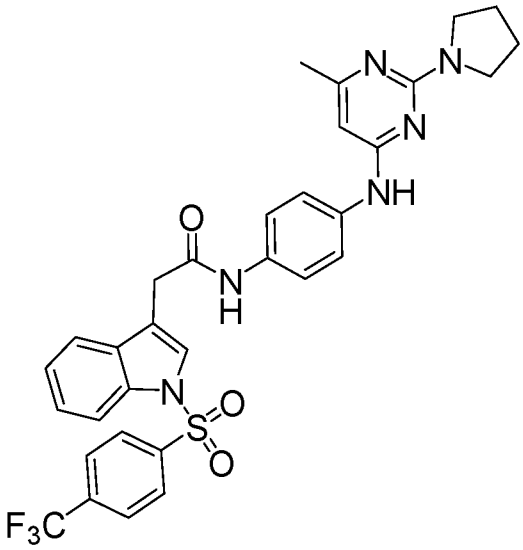


37 (6.27)

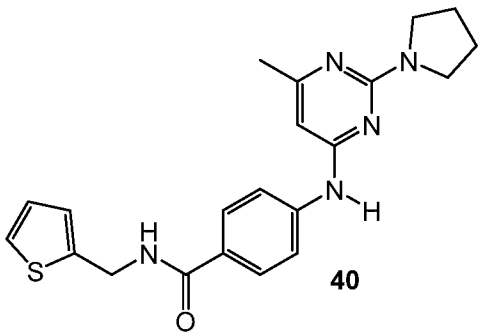
Scheme 1 (continued)



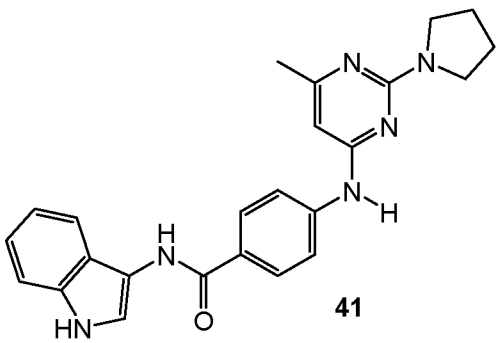
38 (6.28)



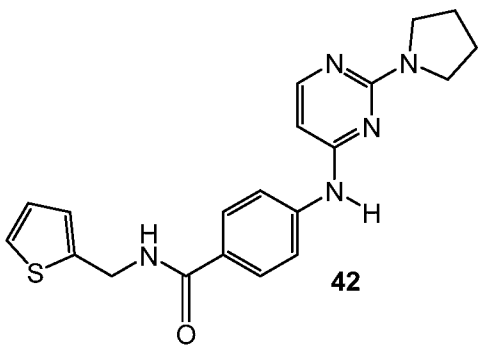
39 (6.29)



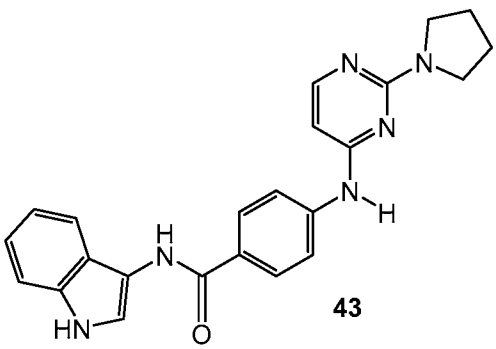
40



41

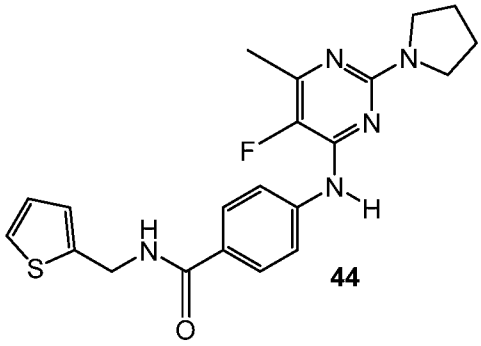


42

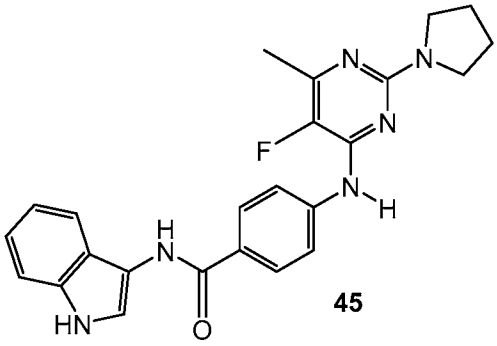


43

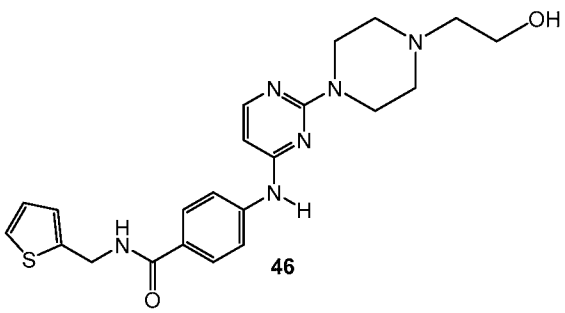
Scheme 1 (continued)



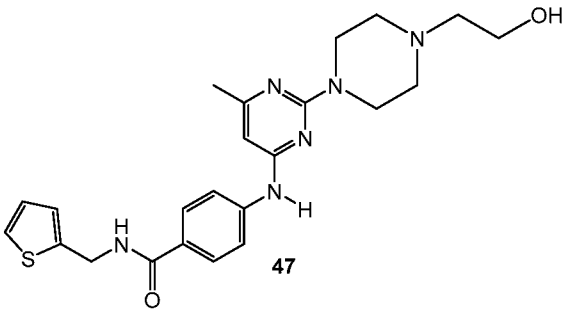
44



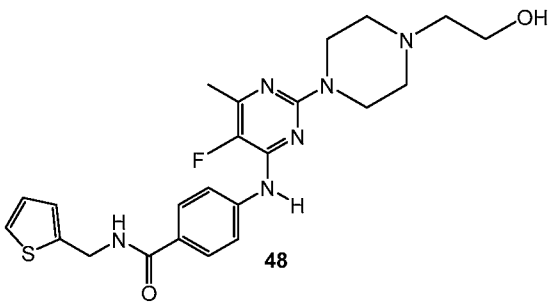
45



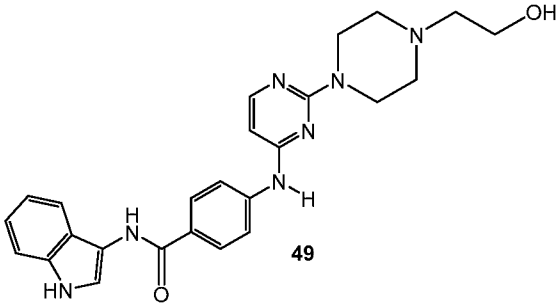
46



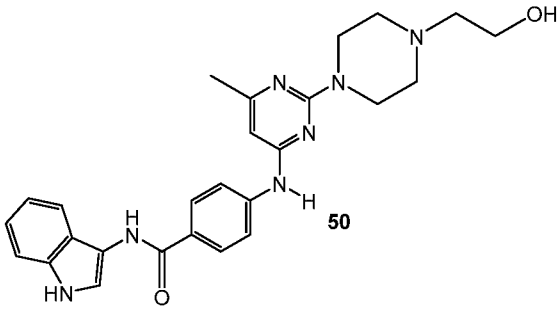
47



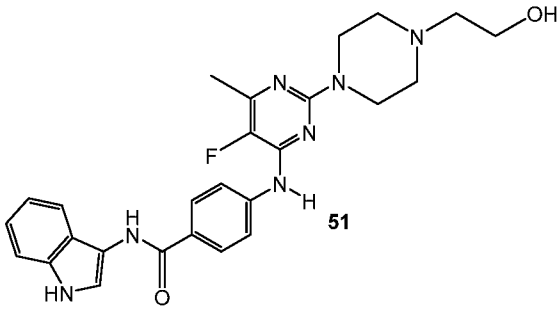
48



49

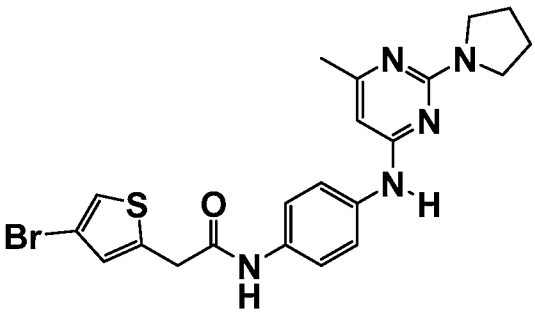


50

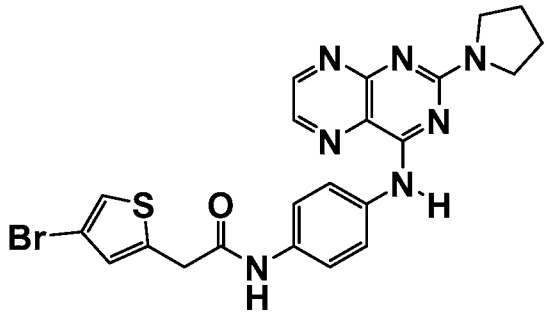


51

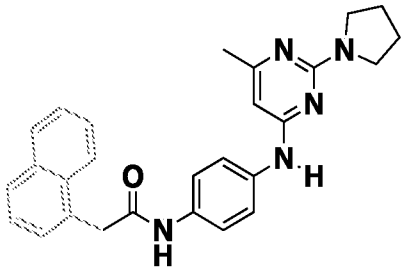
Scheme 1 (continued)



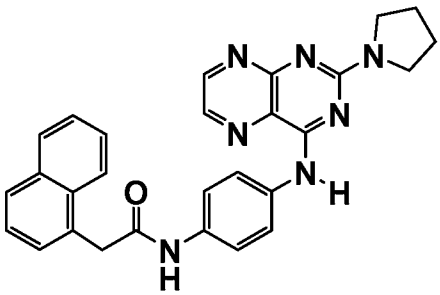
52 (6.11)



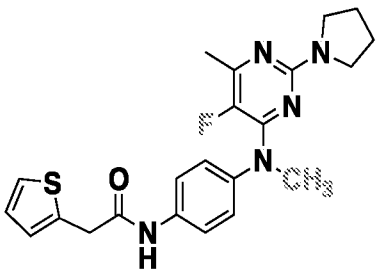
53



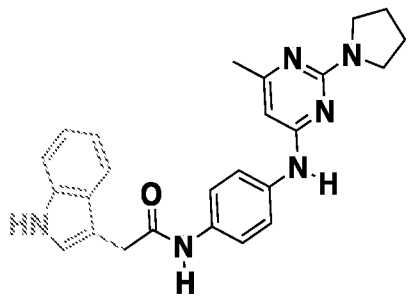
54 (6.16)



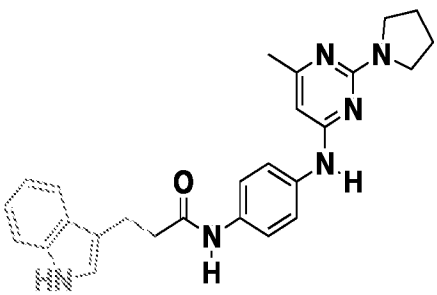
55



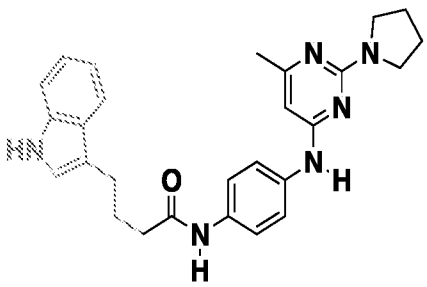
56 (6.4)



57 (6.5)

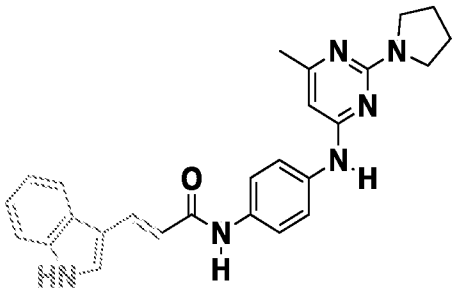


58 (6.6)

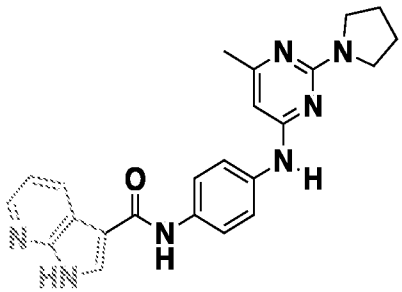


59 (6.7)

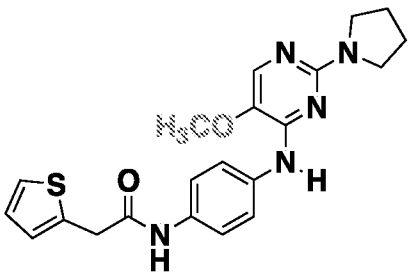
Scheme 1 (continued)



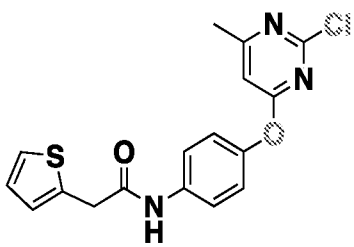
60 (6.8)



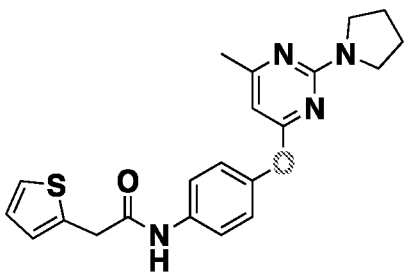
61 (6.9)



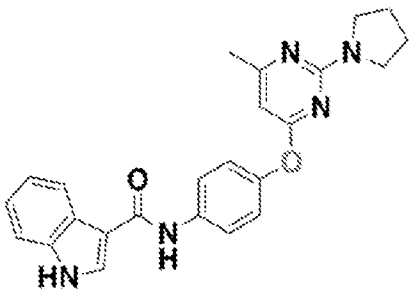
62 (6.10)



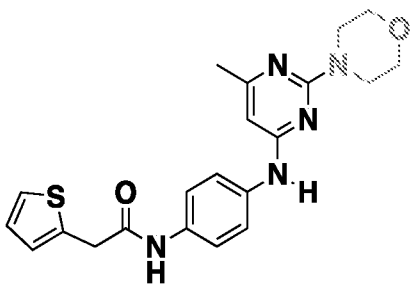
63 (6.12)



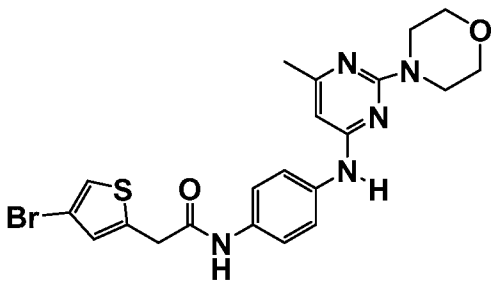
64 (6.13)



65 (6.14)

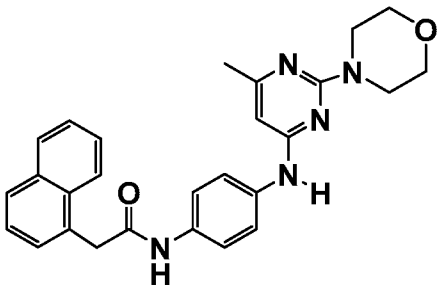


66 (6.15)

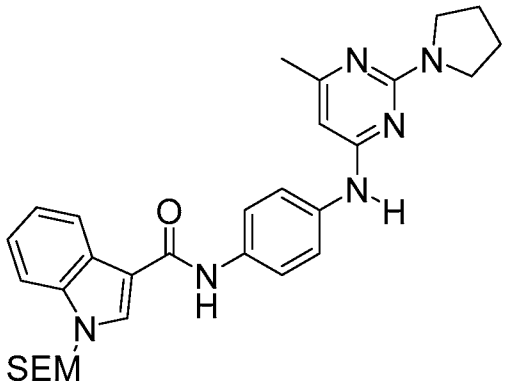


67

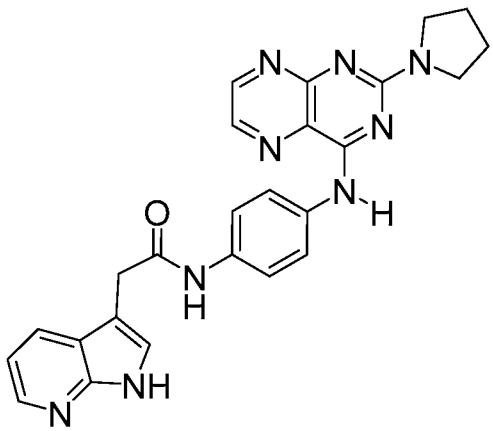
Scheme 1 (continued)



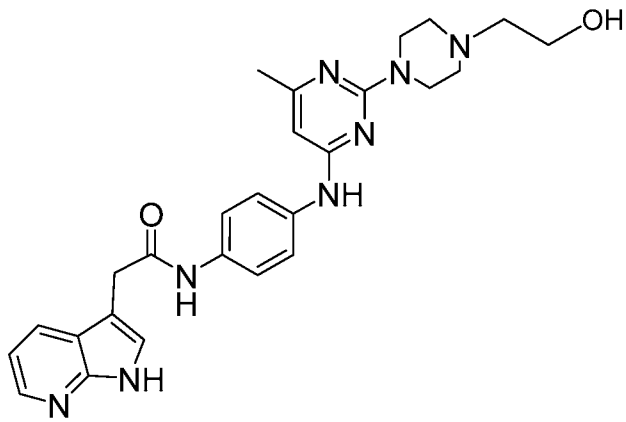
68



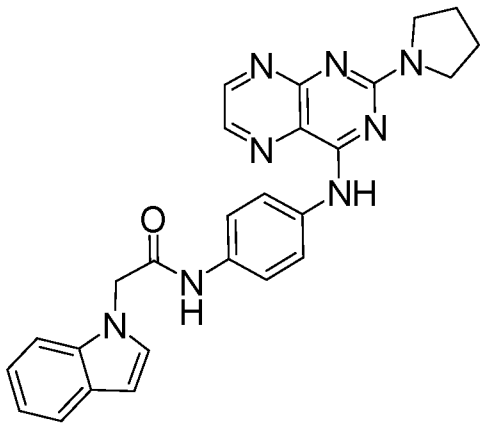
69



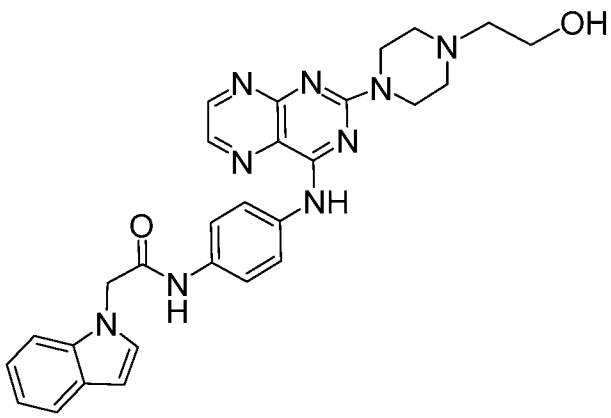
70



71

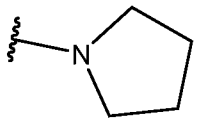


72

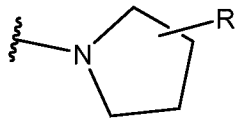


73

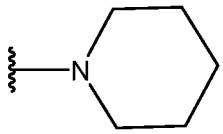
Scheme 2 (Exemplary R_P and -N(R_2)(R_3) groups)



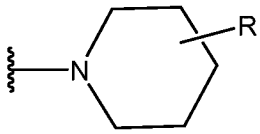
R_N1



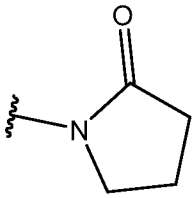
R_N2,
R = alkyl, OH, acyl, halogen, trifluormethyl, hydroxalkyl, -CH_2CH_2-OH



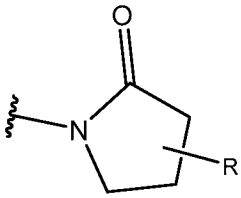
R_N3



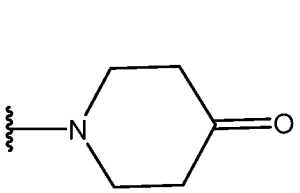
R_N4,
R = alkyl, OH, acyl halogen, trifluormethyl, hydroxalkyl, -CH_2CH_2-OH



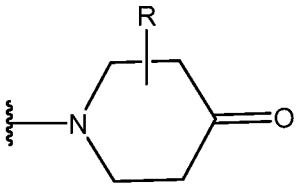
R_N5



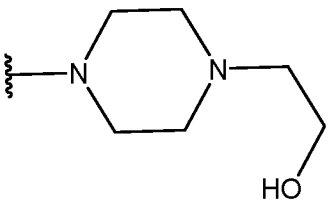
R_N6,
R = alkyl, OH, acyl, halogen, trifluormethyl, hydroxalkyl, -CH_2CH_2-OH



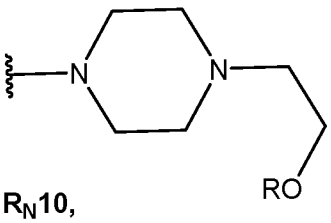
R_N7



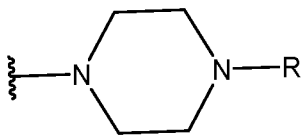
R_N8,
R = alkyl, OH, acyl, halogen, trifluormethyl, hydroxalkyl, -CH_2CH_2-OH



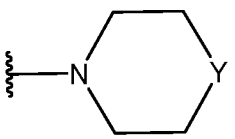
R_N9



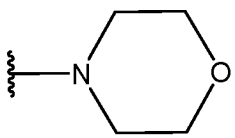
R_N10,
R = alkyl, -COalkyl, -$CONH_2$, -CONH(alkyl), -CON(alkyl)2



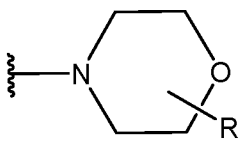
R_N11,
R = H, alkyl, acyl, OH, SO_2alkyl



R_N12, Y = S, SO_2

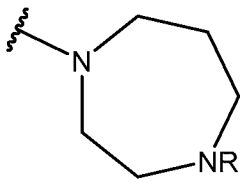


R_N13

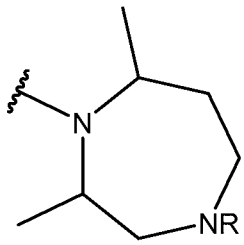


R_N14,
R= alkyl, acyl, OH, hydroxyalkyl

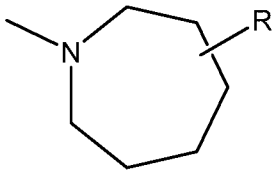
Scheme 2 (continued)



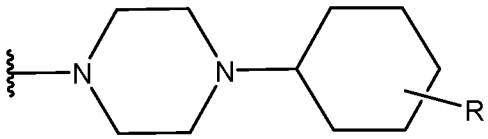
R_N15, R = H, alkyl, acyl, OH, hydroxyalkyl



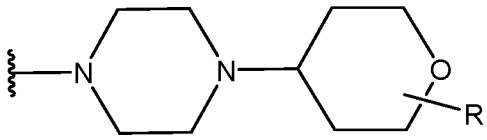
R_N16, R = H, alkyl, acyl, OH, hydroxyalkyl



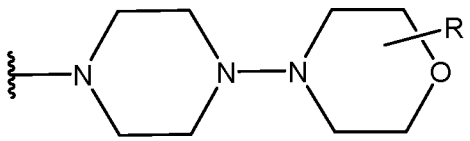
R_N25, R = H, alkyl, acyl, OH, hydroyxalkyl



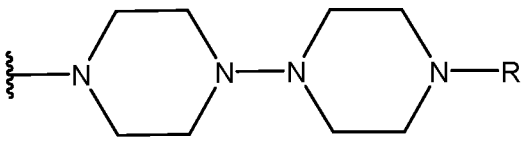
R_N17, R = H, alkyl, OH, hydroxyalkyl, acyl



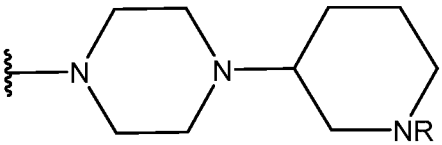
R_N18, R = H, alkyl, OH, hydroxyalkyl, acyl



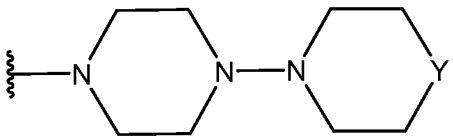
R_N19, R = H, alkyl, OH, hydroxyalkyl, acyl



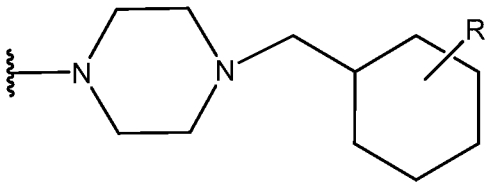
R_N20, R = H, alkyl, OH, acyl, SO_2-alkyl



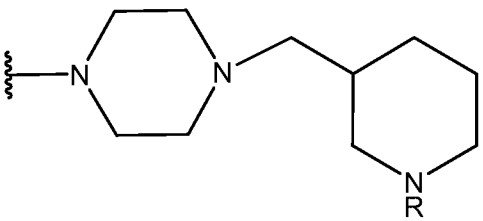
R_N21, R = H, alkyl, OH, acyl, SO_2-alkyl



R_N22, Y = S, SO_2

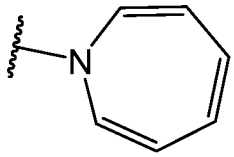


R_N23, R = H, alkyl, acyl, OH, hydroxyalkyl

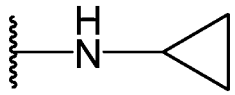


R_N24, R = H, alkyl, acyl, OH, hydroxyalkyl, SO_2-alkyl

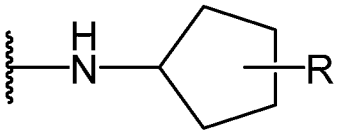
Scheme 2 (continued)



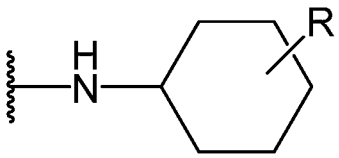
R_N25



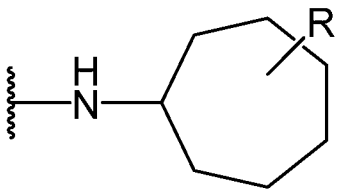
R_N26



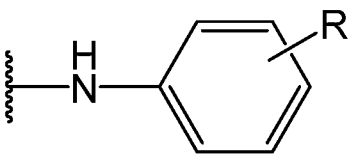
R_N27 R = H, alkyl, acyl, OH, hydroxyalkyl



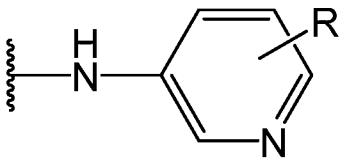
R_N28 R = H, alkyl, acyl, OH, hydroxyalkyl



R_N29 R = H, alkyl, acyl, OH, hydrxyalkyl

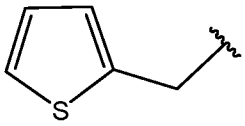


R_N30 R = H, alkyl, acyl, OH, hydroxyalkyl

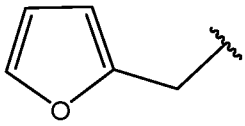


R_N31 R = H, alkyl, acyl, OH, hydroxyalkyl

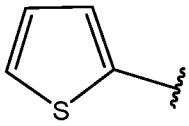
Scheme 3; Exemplary R_{12} and R_H Groups



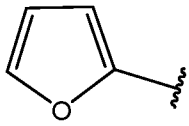
R12-1



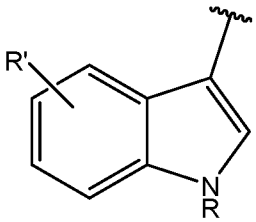
R12-2



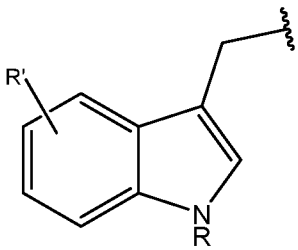
R12-3



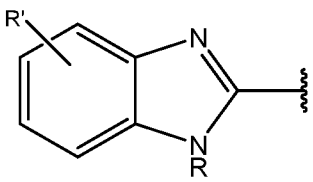
R12-4



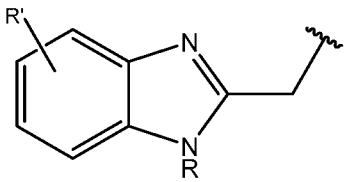
R12-5,
R is H, alkyl
R' is H, alkyl, acyl, halogen



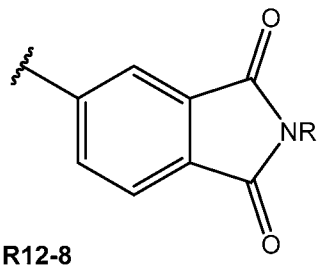
R12-6,
R is H or alkyl
R' is H, alkyl, acyl, halogen



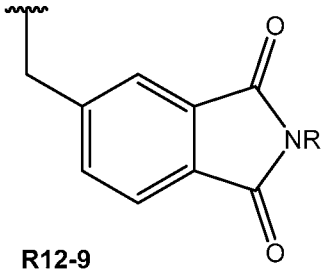
R12-7
R is H or alkyl
R' is H, alkyl, acyl, halogen



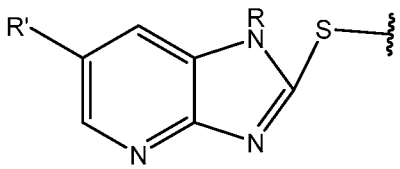
R12-7
R is H or alkyl



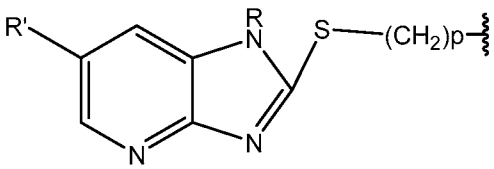
R12-8
R is H or alkyl



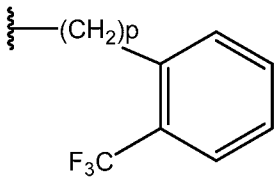
R12-9
R is H or alkyl



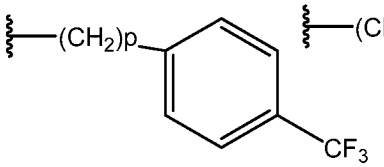
R12-10,
R is H or alkyl,
R' is H, alkyl, acyl, halogen



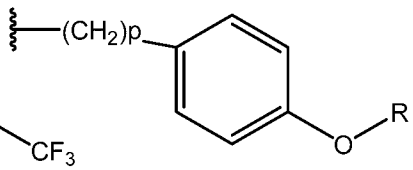
R12-11,
R is H or alkyl,
R' is H,alkyl, acyl, halogen,
p is 1-5



R12-12, p is 0-3

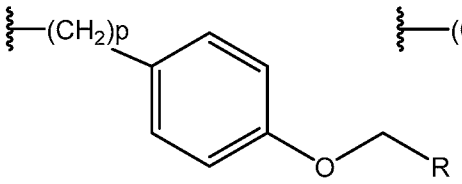


R12-13, p is 0-3

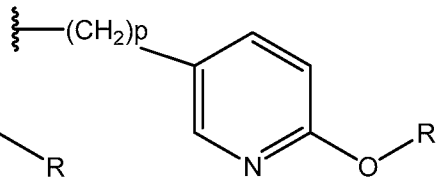


R12-14,
R is H, alkyl, phenyl, -COH, acyl
p is 0-3

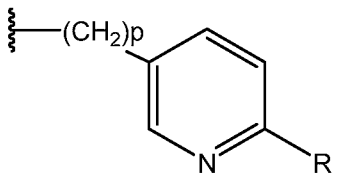
Scheme 3 (continued)



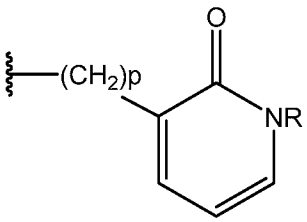
R12-15,
R is H, alkyl, phenyl, acyl
p is 0-3



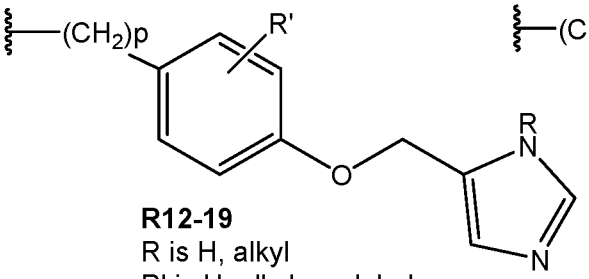
R12-16,
R is H, alky. phenyl, -COH, acyl
p is 0-3



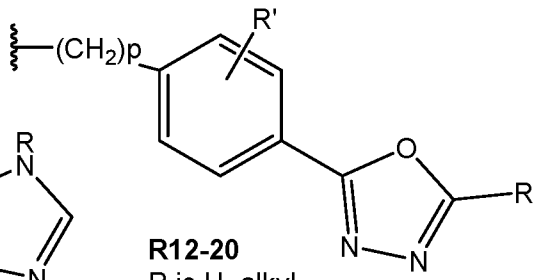
R12-17,
R is H, alkyl, acyl, phenyl
p is 0-3



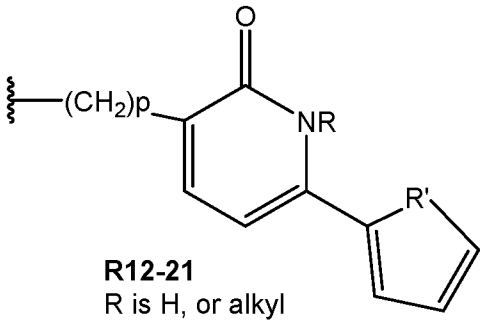
R12-18,
R is H, alkyl
p is 0-3



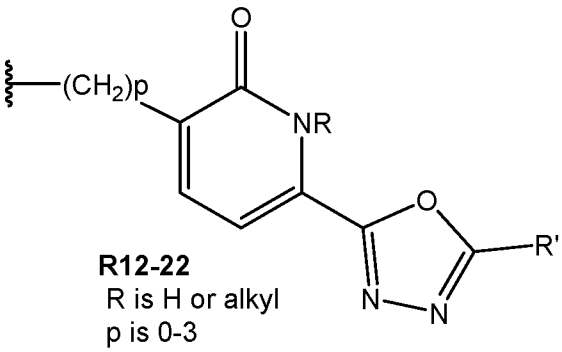
R12-19
R is H, alkyl
R' is H, alkyl, acyl, halogen
p is 0-3



R12-20
R is H, alkyl
R' is H, alkyl, acyl, halogen
p is 0-3

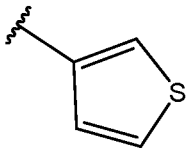


R12-21
R is H, or alkyl
p is 0-3

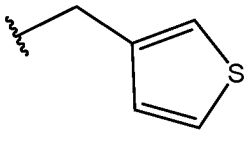


R12-22
R is H or alkyl
p is 0-3

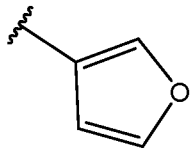
Scheme 3 (continued)



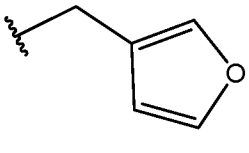
R12-23



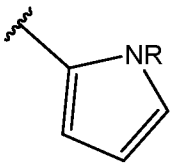
R12-24



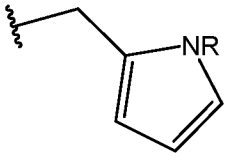
R12-25



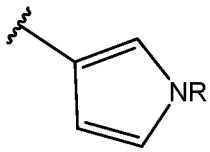
R12-26



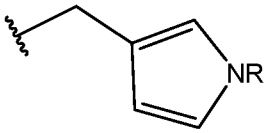
R12-27
R is H, or alkyl



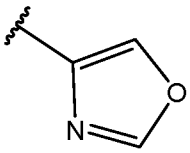
R12-28
R is H, or alkyl



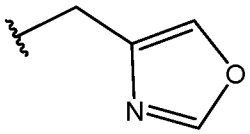
R12-29
R is H or alkyl



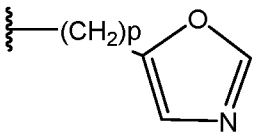
R12-30
R is H or alkyl



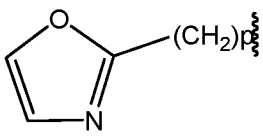
R12-31



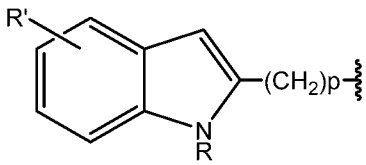
R12-32



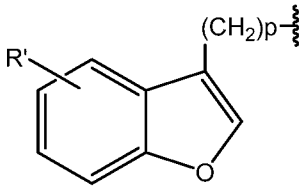
R12-33
p is 0, 1 or 2



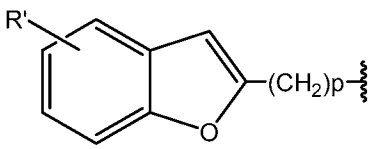
R12-34
p is 0, 1 or 2



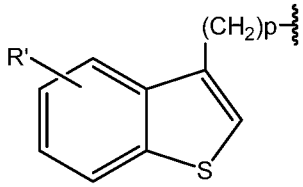
R12-35
R is H or alkyl
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2



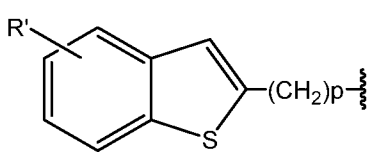
R12-36
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2



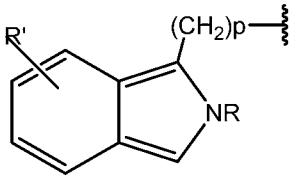
R12-37
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2



R12-38
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2



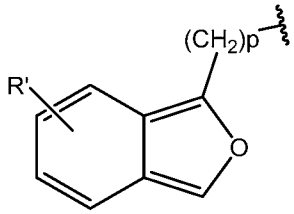
R12-39
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2



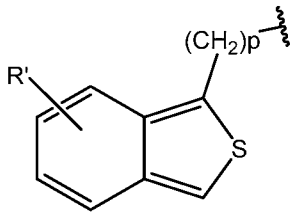
R12-40
R is H or alky
R' is H, alkyl, acyl
or halogen
p is 0, 1 or 2

5

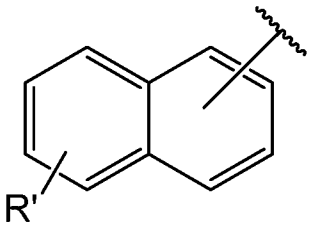
Scheme 3 (continued)



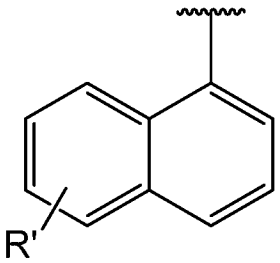
R12-41
R' is H, alkyl, acyl or halogen
p is 0, 1 or 2



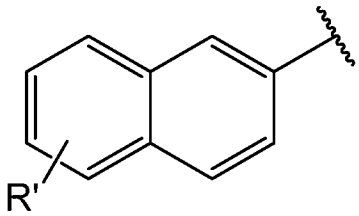
R12-42
R' is H, alkyl, acyl or halogen
p is 0, 1 or 2



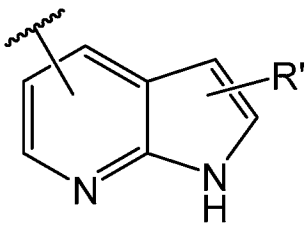
R12-43,
R' is H, alky, acyl, halogen



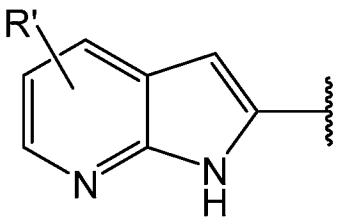
R12-44,
R' is H, alky, acyl, halogen



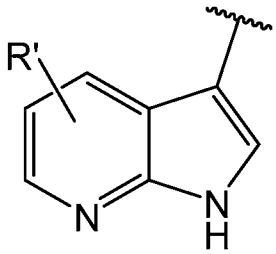
R12-45,
R' is H, alky, acyl, halogen



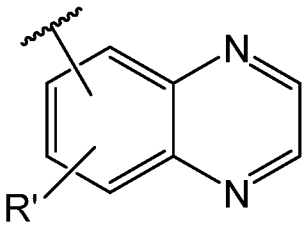
R12-46,
R' is H, alky, acyl, halogen



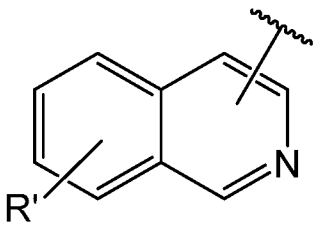
R12-47,
R' is H, alky, acyl, halogen



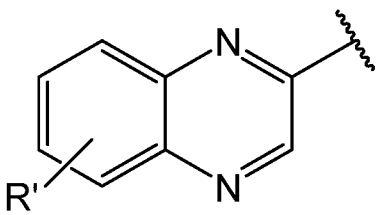
R12-48
R' is H, alky, acyl, halogen



R12-49,
R' is H, alky, acyl, halogen

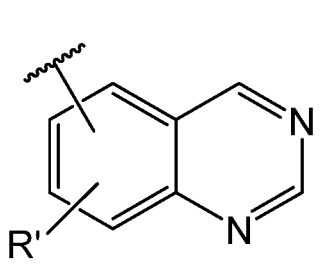


R12-50,
R' is H, alky, acyl, halogen

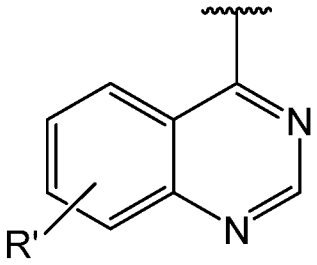


R12-51,
R' is H, alky, acyl, halogen

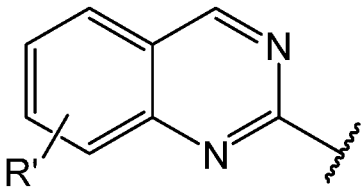
Scheme 3 (continued)



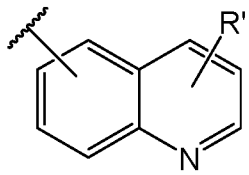
R12-52,
R' is H, alky, acyl, halogen



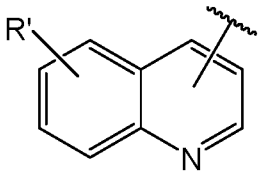
R12-53,
R' is H, alky, acyl, halogen



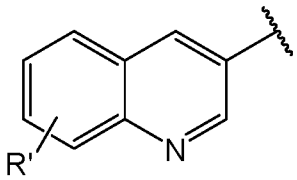
R12-54,
R' is H, alky, acyl, halogen



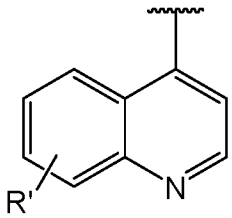
R12-55,
R' is H, alky, acyl, halogen



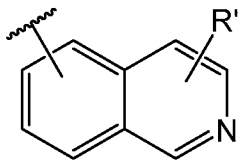
R12-56,
R' is H, alky, acyl, halogen



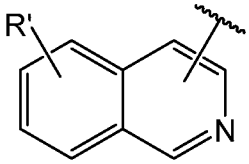
R12-57,
R' is H, alky, acyl, halogen



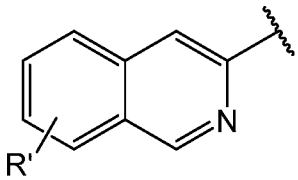
R12-58,
R' is H, alky, acyl, halogen



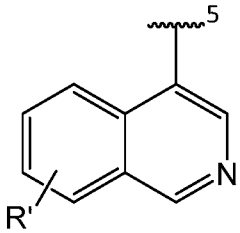
R12-59,
R' is H, alky, acyl, halogen



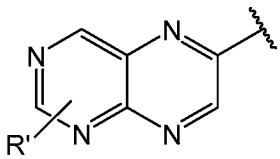
R12-60,
R' is H, alky, acyl, halogen



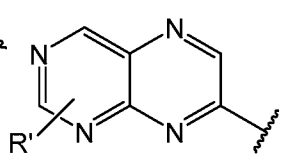
R12-61,
R' is H, alky, acyl, halogen



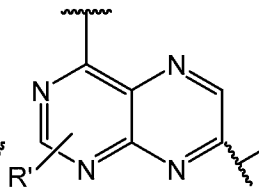
R12-62
R' is H, alky, acyl, halogen



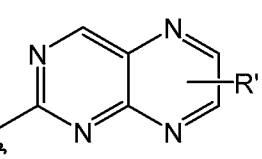
R12-63,
R' is H, alky, acyl, halogen



R12-64,
R' is H, alky, acyl, halogen

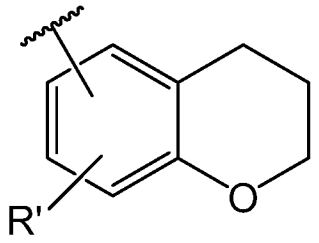


R12-65,
R' is H, alky, acyl, halogen

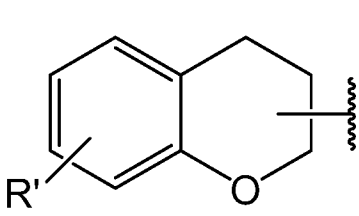


R12-66,
R' is H, alky, acyl, halogen

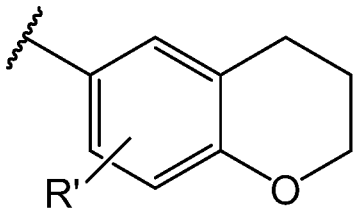
Scheme 3 (continued)



R12-67,
R' is H, alky, acyl, halogen

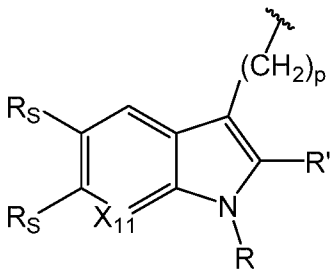


R12-68,
R' is H, alky, acyl, halogen

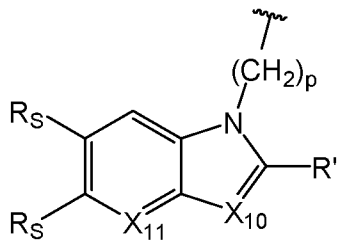


R12-69,
R' is H, alky, acyl, halogen

5

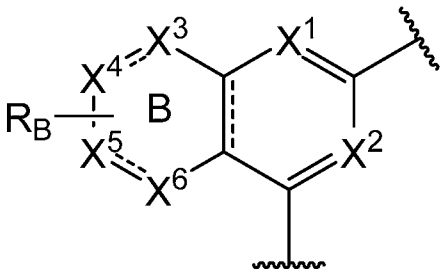


R12-70,
X_{11} is CH or N; p is 0, 1 or 2
R is hydrogen, C1-C6 alkyl, C4-C7 cycloalkylalkyl, -SO_2-R', phenyl, or benzyl;
R' is hydrogen, C1-C6 alkyl, C4-C7, cycloalkylalkyl, phenyl, or benzyl;
each Rs, independently, is hydrogen, halogen, hydroxide, C1-C6 alkyl, C4-C7cycloalkylalkyl, phenyl or benzyl or C1-C3 alkoxide

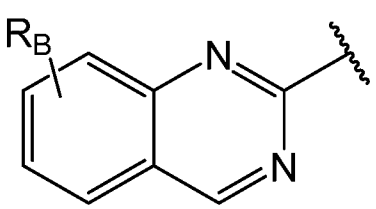


R12-71,
X_{11} is CH or N; X_{10} is CR or N;
P is 0, 1 or 2;
R is hydrogen, C1-C6 alkyl, C4-C7, cycloalkylalkyl, -SO_2-R', phenyl, or benzyl;
R' is hydrogen, C1-C6 alkyl, C4-C7, cycloalkylalkyl, phenyl, or benzyl;
each Rs, independently, is hydrogen,
halogen, hydroxide, C1-C6 alkyl, C4-C7cycloalkylalkyl, phenyl or benzyl or C1-C3 alkoxide

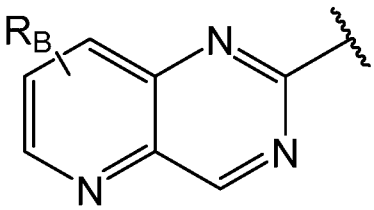
Scheme 4 (Exemplary B rings for formula I)



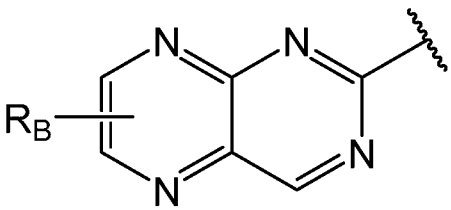
RBI



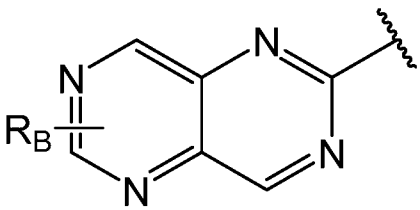
RB2



RB3



RB4



RB5

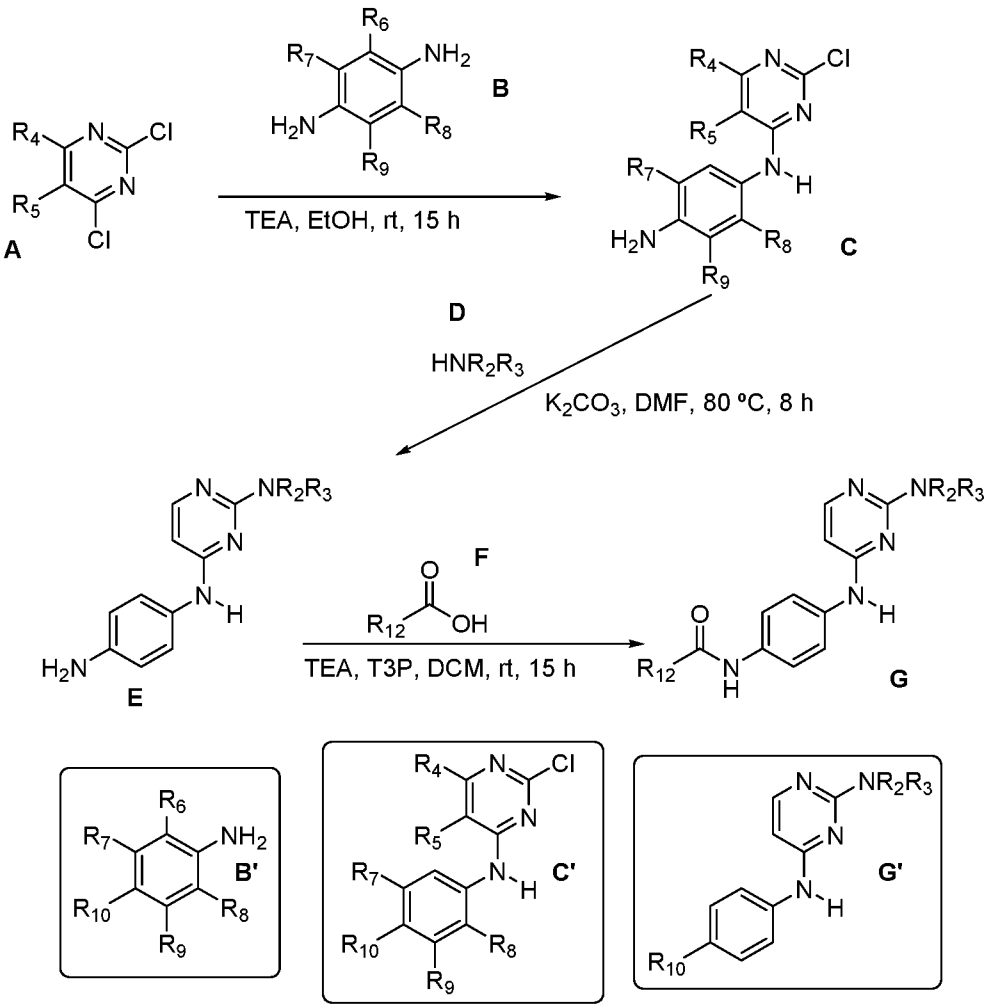
where X^1 and X^2 are selected from CH and N and at least one of X^1 and X^2 is N, X^3-X^6 are selected from CH_2, CH, O, S, N, and NH and the B ring is saturated, partially unsaturated or aromatic dependent upon choice of X^3-X^6, and R_B represents optional substitution as defined for formula I at ring carbons and or nitrogens.

Example 12: Exemplary Synthetic Methods

Compounds of Formula XX as well as many other compounds of this invention are prepared, for example, by the method illustrated in Scheme 5 where variables are as defined above. This three-step synthesis starts with selective aromatic nucleophilic substitution on the 4-position of a 2,4-dichloro-pyrimidine **A** (e.g., 2,4-dichloro-6-methylpyrimidine, where R_4 is methyl or 2,4-dichloro-5-fluoropyrimidine, where R_5 is fluorine) with a p-phenylenediamine **B** to form the intermediate **C**. Exemplary reaction conditions are shown in Scheme 5 where reactants are added with trimethylamine to ice cold ethanol and stirred at rt for 15 h. [Kumar et al., **2014**; Odingo et al., **2014**]. Chlorinated intermediate **C** is then reacted with any amine HNR_2R_3 **D** by amination to generate intermediate **E**. Exemplary amination conditions are shown in Scheme 5, where reactants are reacted in DMF in the presence of K_2CO_3 at elevated temperature. Step three couples the R_{10} group employing acid **F** to intermediate **E**. Various known synthetic methods can be employed to introduce a selected R_{10} group, for example, cross coupling, click chemistry or substation reactions (e.g., SN2, aromatic, electrophilic) [Li et al., **2014a**; Li et al., **2014b**; LaBarbera et al., **2007**]. Scheme 5 illustrates coupling of the amine group of **E** with a selected carboxylic acid **F** to form R_{10} which is –NH-CO-R_{12} in compound **G**. Exemplary R_{12} are aryl, aryl-substituted alkyl, heteroaryl and heteroaryl-substituted alkyl. Exemplary coupling conditions are illustrated in Scheme 5, where coupling proceeds in the presence of propylphosphonic anhydride (T3P) and triethyamine at room temperature to form the desired compound **G**. The illustrated method has been employed, for example to prepare compound **6,** and compound **8 (see, Scheme 6).**

Various substituted starting materials **A**, **B**, **D** and **F** are commercially available or can be prepared using known methods. In embodiments, aniline derivatives already substituted with R_{10} (**B'**) can be used in place of p-phenylenediamine derivatives **B t**o form a corresponding R_{10}-substituted intermediate **C'**. Carrying out step 2 of the illustrated reaction, by reacting intermediate **C'** with **D** will result in desired corresponding compound **G'** (where R_{10} replaces R_{12}-CO-NH-). As will be appreciated by one of ordinary skill in the art, it may be useful or necessary to protect certain groups in the starting materials or intermediates during reactions shown to prevent undesired side-reactions. For example, ring N in reactants **F** may be protected with appropriate amine protecting groups. Use of appropriate protecting groups is generally routine in the art. A variety of primary or secondary amines (**D**) are commercially available or can be prepared by well-known methods. Alternatively, chlorinated intermediate **C** can be reacted with an appropriate nucleophile to add a selected –NR_2R_3 group at the 4-chloro position. For example, **D** can be a cyclic amine such as pyrrolidine. As another possible alternative, Suzuki coupling may be used to install an amine

containing group by C-C bond formation [Li et al., **2014a**]. As another possible alternative, Buchwald-Hartwig cross coupling can be used to form carbon and amine bonds in such intermediates.



Scheme 5

Detailed Synthesis of Compounds **6** and **8 (Scheme 6)**

N-(4-aminophenyl)-2-chloro-6-methyl-pyrimidin-4-amine (**102**). To 0.5 g (3.06 mmol) of 2,4-dichloro-6-methylpyrmidine (**100**) dissolved in 10 mL of ethanol at 0°C were added 513.7 µL (1.2 equivalents, 372.5 mg, 3.68 mmol) of triethyl amine (TEA), and 330.5 mg (3.06 mmol) of p-phenylenediamine (**101**). The reaction mixture was warmed to room temperature and stirred at that temperature overnight. The solvents were removed *in vacuo* and the resulting residue was chromatographed on silica gel using 40% hexane in ethyl acetate as the eluent to afford 500 mg (70 % yield) of the pure product **102**). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 7.19

(broad s, 1H), 7.02 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 6.18 (s, 1H), 3.77 (broad s, 2H), 2.26 (s, 3H).

N-(4-aminophenyl)-6-methyl-2-(pyrolidin-1-yl)pyrimidin-4-amine (**104**). To 1.2 g of N-(4-aminophenyl)-2-chloro-6-methyl-pyrimidin-4-amine (**102**) dissolved in 120 mL of DMF were added 777.3 mg (5.62 mmol) of potassium carbonate and 3.63 mg (4.12 mL, 51.1 mmol) of pyrrolidine (**103**) at room temperature. The reaction mixture was heated 80 °C for 8 h. The reaction was cooled to room temperature and diluted with water. The product was extracted with ethyl acetate (3 x 100 mL). The organic layers were combined and washed with brine, followed by drying over Na_2SO_4, filtered and concentrated to give an oily crude product that was chromatographed on silica gel using 10% methanol in DCM (with drops of TEA) to give 1.31 g (96 % yield) of the pure product (**104**). ^{1}H NMR: (400 MHz, CDCl3): δ 7.11 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 6.25 (broad s, 1H), 5.68 (s, 1H), 3.63 (broad s, 2H), 3.56 (t, J = 6.8 Hz, 4H), 2.19 (s, 3H), 1.93 (t, J = 6.8 Hz, 4H).

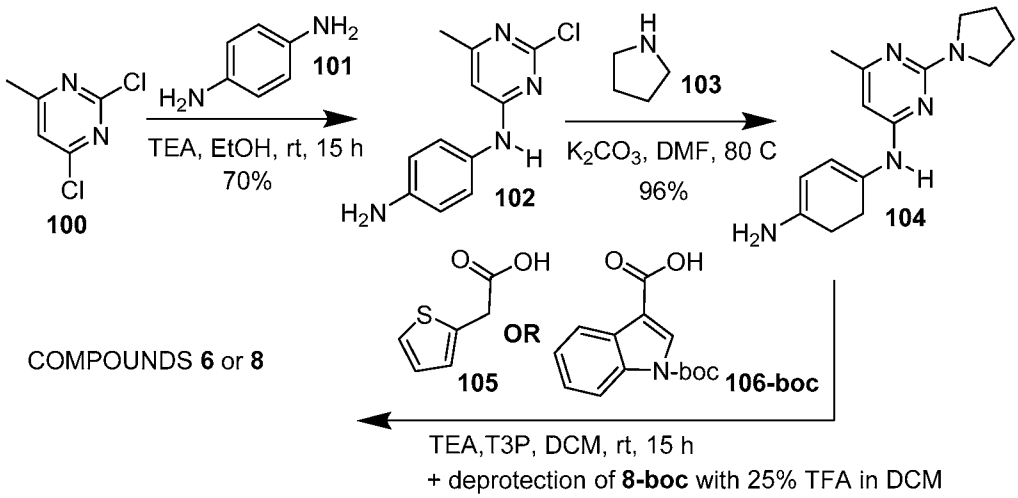
N-(4-((6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yl)amino)phenyl)-2-(thiophen-2-yl)acetamide (**6**). To 700 mg (2.60 mmol) of N-(4-aminophenyl)-6-methyl-2-(pyrolidin-1-yl)pyrimidin-4-amine (**104**) in 15 mL were added 406 mg (2.86 mmol) of 2-thiopheneacetic acid (**105**), 906.8 µL (657.5 mg, 6.50 mmol) of TEA, and 3.06 mL (1.65 mg, 5.20 mmol) of T3P (50% weight solution in ethyl acetate) at 0°C. The mixture was warmed to room temperature and stirred for 15 h. The reaction was quenched by gradual addition of water, and the product was extracted with DCM (3 x 150 mL), followed by washing with brine. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product that was purified using silica gel and 10% methanol in DCM to give 864.5 mg (84% yield) of the pure product (**6**). ^{1}H NMR: (400 MHz, DMSO-d6): δ 10.07 (s, 1H), 9.06 (broad s, 1H), 7.64 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 9.2 Hz, 2H), 7.39 – 7.37 (m, 1H), 6.98 – 6.96 (m, 2H), 3.84 (s, 2H), 3.47 (s, 4H), 2.13 (s, 3H), 1.89 (t, J = 6.6 Hz, 4H). HPLC: 98% pure.

Boc-protected indole-3-caboxylic acid **106-boc** was used in a peptide coupling methodology with compound **104** in the presence of T3P and TEA to achieve the synthesis of boc-protected indole derivative **8-boc,** which was converted to the indole derivative **8** in good yield via TFA deprotection of the boc-protecting group. Note that the boc protecting group is -COO-t-butylK2Co3,KI, EtOH

N-{4-{[6-Methyl-2-(1-pyrrolidinyl)-4-pyrimidinyl]amino}phenyl)-1-{[(2-Methyl-2-propanyl}oxy]carbonyl}-1H-indole-3-carboxamide (**8-boc**). To 80 mg (0.297 mmol) of

compound **104** in 8 mL DCM, were added 85.36 mg (0.3267 mmol of boc-protected indole-3-carboxylic acid (**106-boc**), 103.6 µL (75.13 mg, 0.742 mmol) of TEA, 350 µL (189 mg, 0.594 mmol) of T3P at 0°C. The mixture was warmed to room temperature and stirred for 20 h. The reaction was quenched by gradual addition of water, and the product was extracted with DCM (3 x 50 mL), followed by washing with brine. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product that was purified using silica gel and 10% methanol in DCM to give 76 mg (50% yield) of the pure product (**8-boc**). ^{1}H NMR: (400 MHz, $CDCl_3$): δ 8.28 (broad s, 1H), 8.24 - 8.13 (m, 3H), 7.57 (q, *J* = 4.8, 8.8 Hz, 4H), 7.40 -7.31 m, 3H), 5.92 (s, 1H), 3.54 (s, 4H), 2.23 (s, 3H), 1.86 (s, 4H), 1.66 (s, 9H).

N-(4-{[6-Methyl-2-(1-pyrrolidinyl)-4-pyrimidinyl]amino}phenyl)-1H-indole-3-carboxamide (8). 70 mg (0.136 mmol) of N-(4-{[6-Methyl-2-(1-pyrrolidinyl)-4-pyrimidinyl]amino}phenyl)-1H-indole-3-carboxamide (**8-boc**) was dissolved in 25% TFA in DCM (5 mL). The solution was stirred for 3 h at room temperature. The solvents were removed under reduced pressure and the crude product was purified using silica gel and 10% methanol in DCM to give 43.78 mg (78 % yield) of the pure product. ^{1}H NMR: (400 MHz, DMSO-d6): δ 11.7 (s, 1H), 9.65 (s, 1H),9.09 (s, 1H), 8.28 (broad s, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 7.67 (s, 4H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.20 -7.12 (m, 2H), 5.88 (s, 1H), 3.50 (s, 4H), 2.14 (s, 3H), 1.91 (s, 4H).

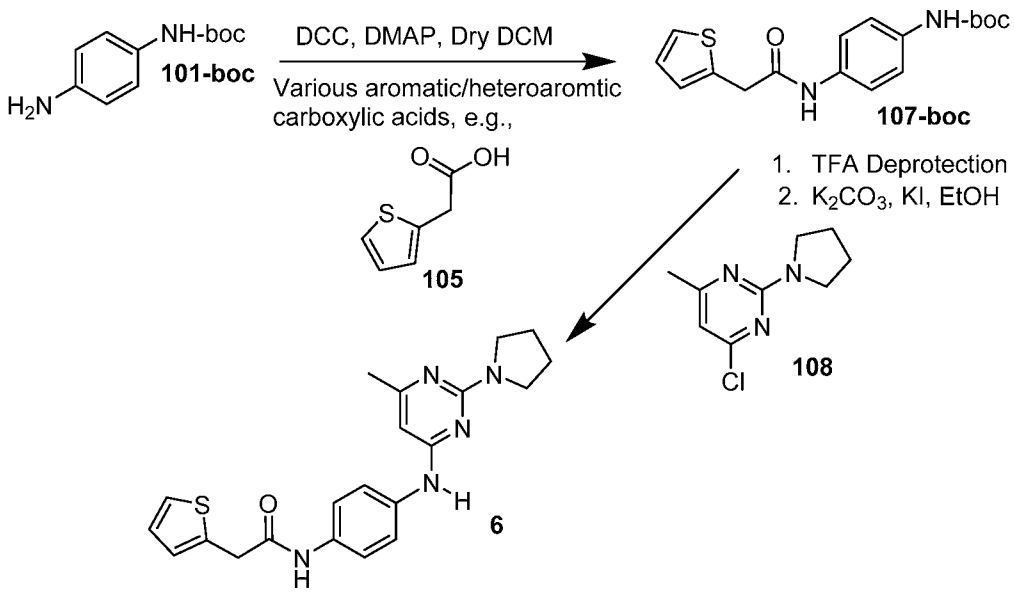


Scheme 6

Scheme 7 illustrates an alternative method of synthesis optimized for yield of compound **6.** In this method, a t-butyl protected carbamate, for example, compound **35** is reacted with a

selected aromatic carboxylic acid, for example, compound **36** to form a protected carbamate intermediate, for example, compound **37.** The intermediate is deprotected as known in the art, for example with trifluoroacetic acid (TFA) and the deprotected carbamate is reacted with a chlorinated heterocyclic group carrying a primary or secondary amine group (e.g., a pyrrolidinyl group), for example, compound **38** to form the desired compound of Formula XX, for example, compound **6.** This method can also be employed to prepare various compounds of formula XX by selection of starting aromatic carboxylic acids and chlorinated heterocyclic compound carrying a primary of secondary amine group.

In Scheme 7, reagents employed for synthesis of compound **6** are shown, where in the first reaction DCC is N,N'-dicyclohexylcarbondiimide, DMAP is dimethylaminopyridine and the solvent is DCM dichloromethane. In the second reaction, after TFA deprotection, potassium carbonate, and potassium iodide in ethanol is employed. One of ordinary skill in the art can readily adapt the reagents and reaction conditions employed to prepare desired compounds of formula XX.



Scheme 7

References

1. Lorch Y, Maier-Davis B, Kornberg RD. Mechanism of chromatin remodeling. Proc Natl Acad Sci U S A **2010**;107(8):3458-62.
2. Kumar R, Li DQ, Muller S, Knapp S. Epigenomic regulation of oncogenesis by chromatin remodeling. Oncogene **2016**;35(34):4423-36.
3. Swygert SG, Peterson CL. Chromatin dynamics: interplay between remodeling enzymes and histone modifications. Biochim Biophys Acta **2014**;1839(8):728-36 doi 10.1016/j.bbagrm.2014.02.013.
4. Erdel F, Krug J, Langst G, Rippe K. Targeting chromatin remodelers: signals and search mechanisms. Biochim Biophys Acta **2011**;1809(9):497-508.
5. Brownlee PM, Meisenberg C, Downs JA. The SWI/SNF chromatin remodelling complex: Its role in maintaining genome stability and preventing tumourigenesis. DNA Repair (Amst) **2015**;32:127-33.
6. Zhang C, Lu J, Zhang P. The Roles of chromatin remodeling proteins in cancer. Curr Protein Pept Sci **2016**;17(5):446-54.
7. Valencia AM, Kadoch C. Chromatin regulatory mechanisms and therapeutic opportunities in cancer. Nat Cell Biol **2019**;21(2):152-61.
8. Ma NF, Hu L, Fung JM, Xie D, Zheng BJ, Chen L, et al. Isolation and characterization of a novel oncogene, amplified in liver cancer 1, within a commonly amplified region at 1q21 in hepatocellular carcinoma. Hepatology **2008**;47(2):503-10.
9. Cheng W, Su Y, Xu F. CHD1L: a novel oncogene. Molecular Cancer **2013**;12(1):170.
10. Li Y, He LR, Gao Y, Zhou NN, Liu Y, Zhou XK, *et al.* CHD1L contributes to cisplatin resistance by upregulating the ABCB1-NF-kappaB axis in human non-small-cell lung cancer. Cell Death Dis **2019**;10(2):99.
11. Pines A, Vrouwe MG, Marteijn JA, Typas D, Luijsterburg MS, Cansoy M, et al. PARP1 promotes nucleotide excision repair through DDB2 stabilization and recruitment of ALC1. J Cell Biol **2012**;199(2):235-49.
12. Tsuda M, Cho K, Ooka M, Shimizu N, Watanabe R, Yasui 849 A, et al. ALC1/CHD1L, a chromatin-remodeling enzyme, is required for efficient base excision repair. PLoS One **2017**;12(11):e0188320.
13. Li Y, Chen L, Chan TH, Liu M, Kong KL, Qiu JL, *et al.* SPOCK1 is regulated by CHD1L and blocks apoptosis and promotes HCC cell invasiveness and metastasis in mice. Gastroenterology **2013**;144(1):179-91.
14. Chen L, Hu L, Chan TH, Tsao GS, Xie D, Huo KK, *et al.* Chromodomain helicase/adenosine triphosphatase DNA binding protein 1-like (CHD1l) gene suppresses the

nucleus-to-mitochondria translocation of nur77 to sustain hepatocellular carcinoma cell survival. Hepatology **2009**;50(1):122-9.

15. He LR, Ma NF, Chen JW, Li BK, Guan XY, Liu MZ, *et al.* Overexpression of CHD1L is positively associated with metastasis of lung adenocarcinoma and predicts patients' poor survival. Oncotarget **2015**;6(31):31181-90.

16. Hyeon J, Ahn S, Park CK. CHD1L Is a marker for poor prognosis of hepatocellular carcinoma after surgical resection. Korean J Pathol **2013**;47(1):9-15.

17. Su Z, Zhao J, Xian G, Geng W, Rong Z, Wu Y, *et al.* CHD1L is a novel independent prognostic factor for gastric cancer. Clin Transl Oncol: **2014**;16(8):702-7 (Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico).

18. Wu J, Zong Y, Fei X, Chen X, Huang O, He J, *et al.* Presence of CHD1L over expression is associated with aggressive tumor biology and is a novel prognostic biomarker for patient survival in human breast cancer. PLoS One **2014**;9(8):e98673.

19. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell **1996**;87(2):159-70.

20. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature **2012**;487(7407):330-7.

21 Shitashige M, Hirohashi S, Yamada T. Wnt signaling inside the nucleus. Cancer Sci **2008**;99(4):631-7.

22. Sánchez-Tilló E, de Barrios O, Siles L, Cuatrecasas M, Castells A, Postigo A. β-catenin/TCF4 complex induces the epithelial-to-mesenchymal transition (EMT)-activator ZEB1 to regulate tumor invasiveness. Proc Natl Acad Sci USA **2011**;108(48):19204-9.

23. Zhou Q, Abraham AD, Li L, Babalmorad A, Bagby, Arcaroli JJ, et al. Topoisomerase IIα mediates TCF-dependent epithelial-mesenchymal transition in colon cancer. Oncogene **2016**;35(38):4990-9.

24. Abraham AD, Esquer H, Zhou Q, Tomlinson N, Hamill BD, Abbott JM, et al. Drug design targeting t-cell factor-driven epithelial-mesenchymal transition as a therapeutic strategy for colorectal cancer. J Med Chem **2019**;62(22):10182-203.

25. Chaffer CL, San Juan BP, Lim E, Weinberg RA. EMT, cell plasticity and metastasis. Cancer Metast Rev **2016**;35(4):645-54.

26. Lu B, Green BA, Farr JM, Lopes FC, Van Raay TJ. Wnt drug discovery: weaving through the screens, patents and clinical trials.Cancers **2016**;8(9).

27. Marisa L, de Reynies A, Duval A, Selves J, Gaub MP, Vescovo L, *et al.* Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. PLoS Med **2013**;10(5):e1001453.

28. Scott AJ, Song EK, Bagby S, Purkey A, McCarter M, Gajdos C, et al. Evaluation of the efficacy of dasatinib, a Src/Abl inhibitor, in colorectal cancer cell lines and explant mouse model. PLoS One **2017**;12(11):e0187173.

29. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol **2012**;9(6):338-50.

30. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernandez-Mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science **2018**;359(6378):920-6.

31. Drost J, Clevers H. Organoids in cancer research. Nat Rev Cancer **2018**;18;407-18.

32. Ahel D, Horejsi Z, Wiechens N, Polo SE, Garcia-Wilson E, Ahel I, et al. Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. Science **2009**;325(5945):1240-3.

33. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, *et al.* Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta catenin or APC. Science **1997**;275(5307):1787-90.

34. Idogawa M, Yamada T, Honda K, Sato S, Imai K, Hirohashi S. Poly(ADP-Ribose) polymerase-1 is a component of the oncogenic T-cell factor-4/β-catenin complex. Gastroenterology 924 **2005**;128(7):1919-36.

35. Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells in vitro. Nat Protoc **2006**;1(5):2315-9.

36. Ryan DP, Owen-Hughes T. Snf2-family proteins: chromatin remodellers for any occasion. Curr Opin Chem Biol **2011**;15(5):649-56.

37. Flaus A, Owen-Hughes T. Mechanisms for ATP-dependent chromatin remodelling: the means to the end. FEBS J **2011**;278(19):3579-95.

38. Lehmann LC, Hewitt G, Aibara S, Leitner A, Marklund E, Maslen SL, et al. Mechanistic Insights into Autoinhibition of the Oncogenic Chromatin Remodeler ALC1. Mol Cell **2017**;68(5):847-59.

39. Gottschalk AJ, Timinszky G, Kong SE, Jin J, Cai Y, Swanson SK, et al. Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. Proc Natl Acad Sci U S A **2009**;106(33):13770-4.

40. McDonald PC, Dedhar S. The role of epithelial-mesenchymal transition in cancer metastasis. In: Regad T, Sayers TJ, Rees RC, editors. Principles of stem cell biology and cancer future applications and therapeutics. 1st ed. West Sussex, UK: John Wiley & Sons, Ltd; **2015**. p.101-21.

41. Yun JA, Kim SH, Hong HK, Yun SH, Kim HC, Chun HK, et al. Loss of E-cadherin expression is associated with a poor prognosis in stage iii colorectal cancer. Oncology **2014**;86(5-6):318-28.

42. Richardson F, Young GD, Sennello R, Wolf J, Argast GM, Mercado P, et al. The evaluation of E-Cadherin and vimentin as biomarkers of clinical outcomes among patients with non-small cell lung cancer treated with erlotinib as second- or third-line therapy. Anticancer Res **2012**;32(2):537-52.

43. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, *et al.* Delineation of prognostic biomarkers in prostate cancer. Nature **2001**;412(6849):822-6.

44. Kashiwagi S, Yashiro M, Takashima T, Nomura S, Noda S, Kawajiri H, *et al.* Significance of E-cadherin expression in triple-negative breast cancer. Br J Cancer **2010**;103(2):249-55.

45. Toiyama Y, Yasuda H, Saigusa S, Tanaka K, Inoue Y, Goel A, *et al.* Increased expression of Slug and Vimentin as novel predictive biomarkers for lymph node metastasis and poor prognosis in colorectal 960 cancer. Carcinogenesis **2013**;34(11):2548-57.

46. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest **2009**;119(6):1429-37.

47. Sun J, Zhang L, Zhao H, Qiu X, Chen W, Wang D, *et al.* CHD1L regulates cell cycle, apoptosis, and migration in glioma. Cell Mol Neurobiol **2016**;36(4):565-767.

48. Lu M, Marsters S, Ye X, Luis E, Gonzalez L, Ashkenazi A. E-cadherin couples death receptors to the cytoskeleton to regulate apoptosis. Mol Cell **2014**;54(6):987-98.

49. Steinhusen U, Weiske J, Badock V, Tauber R, Bommert K, Huber O. Cleavage and shedding of E-cadherin after induction of apoptosis. J Biol Chem **2001**;276(7):4972-80.

50. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep **2017**;7(1):1-13.

51. Mu Q. J. et al. Chromodomain Helicase/ATPase DNA-Binding Protein 1-Like Gene (CHD1L) Expression and Implications for Invasion and Metastasis of Breast Cancer. PLoS One **2015**;10(11), e0143030.

52. Su FR., Ding JH, Bo L, Liu XG. Chromodomain helicase/ATPase DNA binding protein 1-like protein expression predicts poor prognosis in nasopharyngeal carcinoma. Exp Ther Med **2014**; 8, 1745-1750.

53. He, WP et al. CHD1L protein is overexpressed in human ovarian carcinomas and is a novel predictive biomarker for patients survival. BMC Cancer **2012**;12: 437 (8 pages).

54. Chen L. et al. CHD1L promotes hepatocellular carcinoma progression and metastasis in mice and is associated with these processes in human patients. J Clin Invest **2010**;120: 1178-1191.

55. Ji X. et al. CHD1L promotes tumor progression and predicts survival in colorectal carcinoma. J. Surg. Res. **2013**;185: 84-91.

56. Clevers H. Wnt/β-catenin signaling in development and disease. Cell **2006**;127: 469-480.

57. Korinek V. et al. Constitutive transcriptional activation by a β-catenin-TCF complex in APC-/- colon carcinoma. Science **1997**;275:1784-1787.

58. Hu Z. et al. Quantitative evidence for early metastatic seeding in colorectal cancer. Nat Genet **2019**;51:1113-1122.

59. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science **2011**; 33: 1559-1564.

60. Jemal A. et al. Global cancer statistics. CA Cancer J. Clin. **2011**;61: 69-90.

61. Fakih MG. Metastatic colorectal cancer: current state and future directions. J Clin Oncol **2015**; 33:1809-1824.

62. akis.

63. Polakis, P. Drugging Wnt signalling in cancer. EMBO J **2012**; 31, 2737-2746.

64. Li L. et al. An improved high yield total synthesis and cytotoxicity study of the marine alkaloid neoamphimedine: an ATP-competitive inhibitor of topoisomerase II alpha and potent anticancer agent. Mar Drugs **2014a**;12: 4833-4850.

65. Li L. et al. Design of an amide N-glycoside derivative of beta-glucogallin: a stable, potent, and specific inhibitor of aldose reductase. J Med Chem **2014b**;57: 71-77.

66. LaBarbera DV, Bugni TS, Ireland CM. The total synthesis of neoamphimedine. J Org Chem **2007**;72: 8501-8505.

67. Kumar D et al. Synthesis, antimalarial activity, heme binding and docking studies of 4-aminoquinoline–pyrimidine based molecular hybrids. RSC Adv **2014**; 4: 63655-63669.

68. Odingo J et al. Synthesis and evaluation of the 2,4-diaminoquinazoline series as anti-tubercular agents. Bioorg Med Chem **2014**; 22, 6965-6979.

69. Scheel C, Weinberg RA. Cancer stem cells and epithelial–mesenchymal transition: Concepts and molecular links. Semin Cancer Biol. **2012**; 2: 396-403.

70. Baell JB, Nissink JW. Seven year itch: pan-assay interference compounds (pains) in 2017- utility and limitations. ACS Chem Biol **2018**; 13: 36-44.

71. Baell JB, Holloway GA. New Substructure filters for removal of pan assay interference compounds (pains) from screening libraries and for their exclusion in bioassays. J Med Chem 2010; 53 (7): 2719–2740.

72. Abbott JM et al., First-in-Class Inhibitors of Oncogenic CHD1L with Preclinical Activity against Colorectal Cancer, Mol Cancer Therapeutics. June **2020**,19:1598-1612.

73. Di Veroli GY et al. Combenefit: an interactive platform for the analysis and visualization of drug combinations Bioinformatics, 32(18), **2016**, 2866.

74. Ivashkevich, A. et al. Use of the gamma-H2AX assay to monitor DNA damage and repair in translational cancer research. Cancer Lett, **2012**, 327:123-133.

75. Ji, J. et al. Phosphorylated fraction of H2AX as a measurement for DNA damage in cancer cells and potential applications of a novel assay. PLoS One **2017**, 12, e0171582.

76. Zhou et al. Polymerase Theta Inhibition Kills Homologous Recombination Deficient Tumors, May 2020 preprint DOI: 10.1101/2020.05.23.111658 available from the website bioRxiv.

77. Wahlberg E. et al. Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors, Nature Biotech, **2012**, 30(3):283-289.

78. D'Andrea AD, Mechanisms of PARP inhibitor sensitivity and resistance, DNA Repair, **2018**, 71:172-176.

79. Rouleau M et al. PARP inhibition: PARP1 and beyond. Nature Review, **2010**, 10:293-301.

80. Yi M et al. Advances and perspectives of PARP inhibitors. Ex Hematol Oncol, 2019, 8:29 (12 pages).

81. Hevener KE et al. Recent developments in topoisomerase-targeted cancer chemotherapy, Acta Pharma Sin B, **2018**, 8(6):844-861.

82. Lipinski, CA et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Del. Rev., **2001**, 46, 3-26.

83. Ghose, AK et al., A Knowledge-Based Approach in Designing Combinatorial or a Chemistry Libraries for Drug Discovery, J. Combin. Chem., **1999**, 1, 55-68.

84. Wutts, P.G. and Greene, T. Green's Protecting Groups in Organic Synthesis (Fourth Edition), **2007**, John Wiley & Sons, N.Y.

85. Jacques, et al., Enantiomers, Racemates and Resolutions. Wiley Interscience, New York, **1981**.

86. Wilen, S. H., et al., Strategies in optical resolutions. Tetrahedron, **1977**, 33:2725.

87. Eliel, E. L. Stereochemistry of Carbon Compounds. McGraw-Hill, N.Y., **1962**.

88. Wilen, S. H. Tables of Resolving Agents and Optical Resolutions. In E. L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, Ind., **1972**, p. 268.

89. Wheate, N.J., et al. The status of platinum anticancer drugs in the clinic and in clinical trials. Dalton Transactions, **2010**, 39(35):8097-8340.

90. Papamichael, D. The Use of Thymidylate Synthase Inhibitors in the Treatment of Advanced Colorectal Cancer: Current Status, **2009**, Stem Cells, 18(3):166-175.

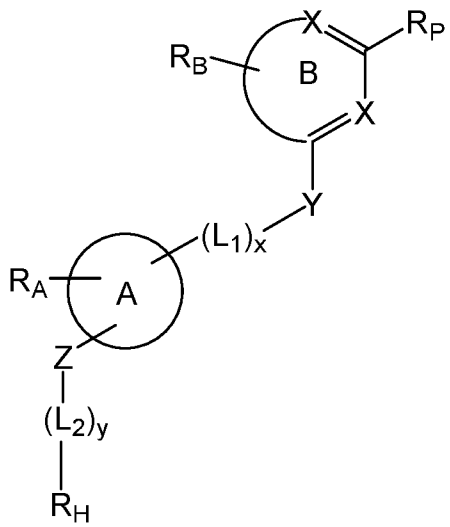
91. Lehman, N. Future potential of thymidylate synthase inhibitors in cancer therapy, **2002**, Expert Opin Investig Drugs, 11(12):1775-1787.

92. Esquer, H. et al. Isolating and targeting the real-time plasticity and malignant properties of epithelial-mesenchymal transition in cancer, Oncogene, March 19, 2021, doi.org/10.1038/s41388-021-01728-2 and Supplementary Information available from the publisher at doi.org/10.1038/s41388-021-01728-2.

Claims

1. A method for treatment of TCF-transcription driven cancers which comprises administering to a patient in need thereof an amount of a CHD1L inhibitor which is effective for CHD1L inhibition or inhibition of aberrant TCF transcription.

2. The method of claim 1 wherein the CHD1L inhibitor is a compound, or salt or solvate of formula I:



or salts, or solvates thereof,

where:

the B ring is a heteroaryl ring or ring system having one, two or three 5- or 6-member rings, any two or three of which can be fused rings, where the rings are carbocyclic, heterocyclic, aryl or heteroaryl rings and at least one of the rings is heteroaryl;

in the B ring, each X is independently selected from N or CH and at least one X is N;

R_P is a primary or secondary amine group [$–N(R_2)(R_3)$] or is a $–(M)_x$-P group, where P is $–N(R_2)(R_3)$ or an aryl or heteroaryl group, where x is 0 or 1 to indicate the absence or present of M and M is an optionally substituted linker $–(CH_2)_n–$ or $–N(R)(CH_2)_n–$, where each n is independently an integer from 1-6 (inclusive);

Y is a divalent atom or group selected from the group consisting of –O–, –S–, $–N(R_1)–$,

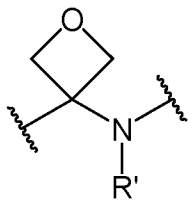
$–CON(R_1)–$, $–N(R_1)CO–$, $—SO_2N(R_1)–$, or $–N(R_1)SO_2–$;

L_1 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where x is 0 or 1 to indicate the absence or presence of L_1;

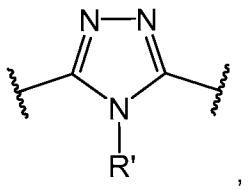
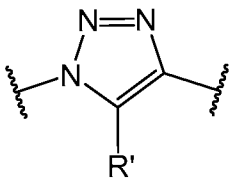
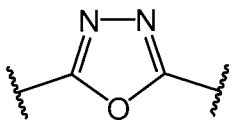
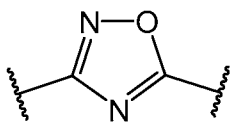
the A ring is a carbocyclic or heterocyclic ring or ring system having one, two or three rings, two or three of which can be fused, each ring having 3-10 carbon atoms and optionally 1-6 heteroatoms and wherein each ring is optionally saturated, unsaturated or aromatic;

Z is a divalent group containing at least one nitrogen substituted with a R' group,

where in embodiments, Z is a divalent group selected from –N(R')–, –CON(R')–, –N(R')CO–, –CSN(R')–, –N(R')CS–, –N(R')CON(R')–, $–SO_2N(R')–$, $–N(R')SO_2–$, $–CH(CF_3)N(R')–$, $–N(R')CH(CF_3)–$, $–N(R')CH_2CON(R')CH_2–$, $–N(R')COCH_2N(R')CH_2–$,


,

or the divalent Z group comprises a 5- or 6-member heterocyclic ring having at least one nitrogen ring member, for example,


,

,

, or

;

L_2 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where z is 0 or 1 to indicate the absence or presence of L_1;

R is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

each R' is independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_1 is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_2 and R_3 are independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted or

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_A and R_B represent hydrogens or 1-10 non-hydrogen substituents on the indicated A and B ring or ring systems, respectively, wherein R_A and R_B substituents are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or disubstituted amino (-NR_CR_D), alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, acyl, haloalkyl, –$COOR_C$, –$OCOR_C$, –$CONR_CR_D$, -$OCONR_CR_D$, -NR_CCOR_D, -SR_C, -SOR_C, -SO_2R_C,and –$SO_2NR_CR_D$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted;

each R_C and R_D is selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted with one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, heterocyclyl, aryl-substituted alkyl, or heterocyclyl-substituted alkyl; and

R_H is an optionally substituted aryl or heteroaryl group;

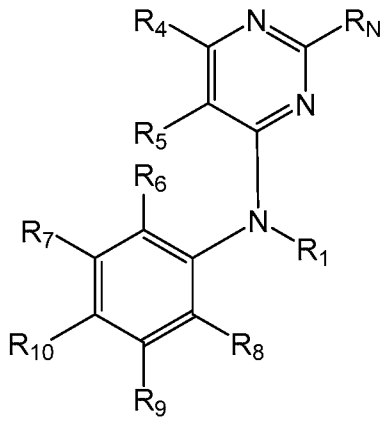
wherein optional substitution includes, substitution with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, –$COOR_E$, –$OCOR_E$, –$CONR_ER_F$, -$OCONR_ER_D$, -NR_ECOR_F, -SR_E, -SOR_E, -SO_2R_E, and –$SO_2NR_ER_F$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted and

each R_E and R_F is selected from hydrogen, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, each of which groups is optionally substituted with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4

alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, and C1-C6 acyl.

3. The method of claim 1, wherein the CHD1L inhibitors are not PARP inhibitors, inhibitors of topoisomerase, nor inhibitors of β-catenin.

4. The method of claim 1, wherein the CHD1L inhibitors are compounds, salts or solvates of formula XX:



and salts or solvates thereof, where:

R_{10} is –NRy-CO-(L_2)y-R_{12} or –CO-NRy--(L_2)y-R_{12}, where y is 0 or 1 to indicate the absence of presence of L_2 which is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two, carbons of the linker are optionally replaced with O, NH, NRy or S, where Ry is hydrogen or a 1-3 carbon alkyl, and R_{12} is an aryl group, cycloalkyl group, heterocyclic group, or heteroaryl group, each of which is optionally substituted;

R_N is an amino moiety –N(R_2)(R_3) wherein R2 and R3 are hydrogen or optionally substituted alkyl groups or R2 and R3 together with the nitrogen to which they are attached form a 5-10-member heterocyclic ring which is saturated or partially unsaturated; and

R_1, R_6-R_9 represent hydrogen or optional substituents.

5. A method of preventing tumor growth, invasion and/or metastasis in CHD1L-driven, EMT-driven or TCF-transcription driven cancers by administering to a patient in need thereof of an amount of a CHD1L inhibitor which is effective for CHD1L inhibition or inhibition of aberrant TCF transcription.

6. The method of claim 5, wherein the CHD1L inhibitor is a compound, salt or solvate of any one of formula I or XX.

7. The method of claim 5, wherein the CHD1L inhibitors are not PARP inhibitors, inhibitors of topoisomerase, nor inhibitors of β-catenin.

8. A method for treatment of CRC, including mCRC, which comprises administering to a patient in need thereof an amount of a CHD1L inhibitor which is effective for inhibition of aberrant TCF transcription.

9. The method of claim 8, wherein the CHD1L inhibitor is a compound, salt or solvate of formula I or XX.

10. The method of claim 8, wherein the CHD1L inhibitors are not PARP inhibitors, inhibitors of topoisomerase, nor inhibitors of β-catenin.

11. A method of treatment of drug-resistant cancer which comprises administering to a patient in need thereof of an amount of a CHD1L inhibitor effective for CHD1L inhibition or inhibition of aberrant TCF transcription in combination with a known treatment to which the cancer has become resistant.

12. The method of claim 11, wherein the CHD1L inhibitor is a compound, salt or solvate of formula I or XX.

13. The method of claim 11, wherein the CHD1L inhibitors are not PARP inhibitors, inhibitors of topoisomerase, nor inhibitors of β-catenin.

14. The method of claim 11, wherein the treatment to which the cancer has become resistant is conventional chemotherapy.

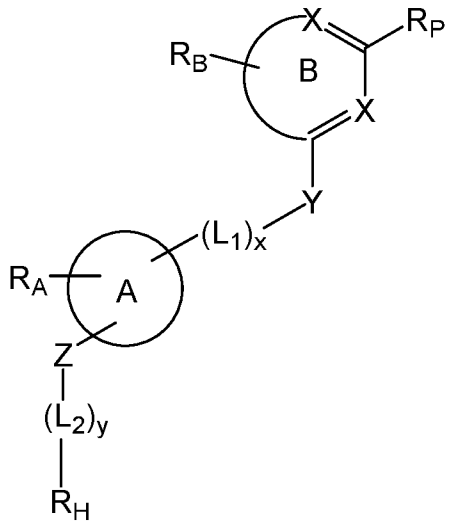
15. A combination method for treatment of cancer which comprises administration of a CHD1L inhibitor in combination with a PARP inhibitor, a topoisomerase inhibitor, a platinum-based anti-neoplastic agent or a thymidylate synthase inhibitor.

16. A method for identifying a CHD1L inhibitor, which inhibits CHD1L dependent TCF transcription which comprises determining if a selected compound inhibits a CHD1L ATPase.

17. A method for identifying a compound useful for treatment of cancer which comprises determining if a compound inhibits a CHD1L ATPase.

18. A pharmaceutical composition for treatment of cancer which comprises one or more compounds, salts or solvates of formula I or XX.

19. A compound, salt or solvate of formula I:



where:

the B ring is a heteroaryl ring or ring system having one, two or three 5- or 6-member rings, any two or three of which can be fused rings, where the rings are carbocyclic, heterocyclic, aryl or heteroaryl rings and at least one of the rings is heteroaryl;

in the B ring, each X is independently selected from N or CH and at least one X is N;

R_P is a primary or secondary amine group [$–N(R_2)(R_3)$] or is a $–(M)_x$-P group, where P is $–N(R_2)(R_3)$ or an aryl or heteroaryl group, where x is 0 or 1 to indicate the absence or present of M and M is an optionally substituted linker $–(CH_2)_n–$ or $–N(R)(CH_2)_n–$, where each n is independently an integer from 1-6 (inclusive);

Y is a divalent atom or group selected from the group consisting of –O–, –S–, $–N(R_1)–$,

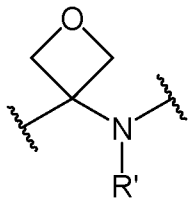
$–CON(R_1)–$, $–N(R_1)CO–$, $—SO_2N(R_1)–$, or $–N(R_1)SO_2–$;

L_1 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where x is 0 or 1 to indicate the absence or presence of L_1;

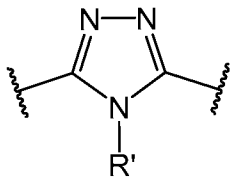
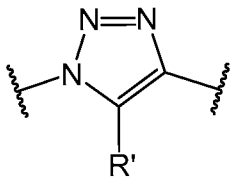
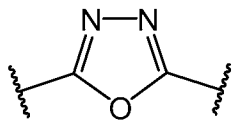
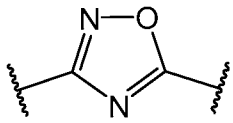
the A ring is a carbocyclic or heterocyclic ring or ring system having one, two or three rings, two or three of which can be fused, each ring having 3-10 carbon atoms and optionally 1-6 heteroatoms and wherein each ring is optionally saturated, unsaturated or aromatic;

Z is a divalent group containing at least one nitrogen substituted with a R' group,

where in embodiments, Z is a divalent group selected from –N(R')–, –CON(R')–, –N(R')CO–, –CSN(R')–, –N(R')CS–, –N(R')CON(R')–, –SO_2N(R')–, –N(R')SO_2–, –CH(CF_3)N(R')–, –N(R')CH(CF_3)–, –N(R')CH_2CON(R')CH_2–, –N(R')$COCH_2$N(R')CH_2–,


,

or the divalent Z group comprises a 5- or 6-member heterocyclic ring having at least one nitrogen ring member, for example,


,

,

, or

;

L_2 is an optional 1-4 carbon linker that is optionally substituted and is saturated or contains a double bond (which can be cis or trans), where z is 0 or 1 to indicate the absence or presence of L_1;

R is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

each R' is independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_1 is selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted;

R_2 and R_3 are independently selected from the group consisting of hydrogen, an aliphatic group, a carbocyclyl group, an aryl group, a heterocyclyl group and a heteroaryl group, each of which groups is optionally substituted or

R_2 and R_3 together with the N to which they are attached form an optionally substituted 5- to 10-member heterocyclic ring which is a saturated, partially unsaturated or aromatic ring;

R_A and R_B represent hydrogens or 1-10 non-hydrogen substituents on the indicated A and B ring or ring systems, respectively, wherein R_A and R_B substituents are independently selected from hydrogen, halogen, hydroxyl, cyano, nitro, amino, mono- or disubstituted amino (-NR_CR_D), alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, acyl, haloalkyl, –$COOR_C$, –$OCOR_C$, –$CONR_CR_D$, -$OCONR_CR_D$, -NR_CCOR_D, -SR_C, -SOR_C, -SO_2R_C,and –$SO_2NR_CR_D$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted;

each R_C and R_D is selected from hydrogen, alkyl, alkenyl, cycloalkyl, cycloalkenyl, heterocyclyl, aryl, or heteroaryl, each of which groups is optionally substituted with one or more halogen, alkyl, alkenyl, haloalkyl, alkoxy, aryl, heteroaryl, heterocyclyl, aryl-substituted alkyl, or heterocyclyl-substituted alkyl; and

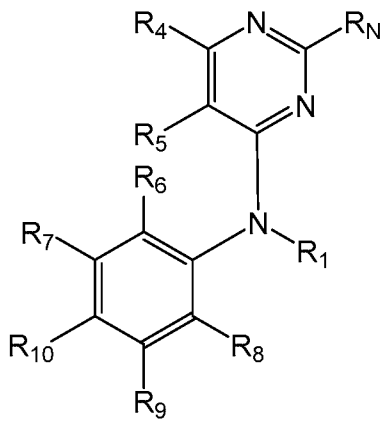
R_H is an optionally substituted aryl or heteroaryl group;

wherein optional substitution includes, substitution with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, –$COOR_E$, –$OCOR_E$, –$CONR_ER_F$, -$OCONR_ER_D$, -NR_ECOR_F, -SR_E, -SOR_E, -SO_2R_E, and –$SO_2NR_ER_F$, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl, heterocyclyl, alkoxy, and acyl, are optionally substituted and

each R_E and R_F is selected from hydrogen, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, C1-C6 acyl, each of which groups is optionally substituted with one or more halogen, nitro, cyano, amino, mono- or di-C1-C3 alkyl substituted amino, C1-C3 alkyl, C2-C4 alkenyl, C3-C6 cycloalkyl, C3-C6-cycloalkenyl, C1-C3 haloalkyl, C6-C12 aryl, C5-C12 heteroaryl, C3-C12 heterocyclyl. C1-C3 alkoxy, and C1-C6 acyl;

with the exception that the compound is not one of compounds **1-9**.

20. A compound of formula XX or a salt or solvate thereof:



and salts or solvates thereof, where:

R_{10} is –NRy-CO-(L_2)y-R_{12} or –CO-NRy--(L_2)y-R_{12}, where y is 0 or 1 to indicate the absence of presence of L_2 which is an optional 1-6 carbon atom linker group which linker is optionally substituted and wherein one or two, carbons of the linker are optionally replaced with O, NH, NRy or S, where Ry is hydrogen or a 1-3 carbon alkyl, and R_{12} is an aryl group, cycloalkyl group, heterocyclic group, or heteroaryl group, each of which is optionally substituted;

R_N is an amino moiety –N(R_2)(R_3) wherein R2 and R3 are hydrogen or optionally substituted alkyl groups or R2 and R3 together with the nitrogen to which they are attached form a 5-10-member heterocyclic ring which is saturated or partially unsaturated; and

R_1, R_6-R_9 represent hydrogen or optional substituents, with the exception that the compound is not compounds **1-9**.

21. A compound selected from compounds **6.5**, **6.11 – 6.29** whose structures are in Scheme 1 or a salt or solvate thereof

22. A compound selected from compounds **6.5, 6.11, 6.16, 6.18, 6.20** and **6.21** whose structures are given in Scheme 1 or a salt or solvate thereof.

23. A pharmaceutical composition comprising a compound selected from compounds **6.5**, **6.11 – 6.29** or salts or solvates thereof and a pharmaceutically acceptable carrier.

24 The use of a CHD1L inhibitor in the manufacture of a medicament for the treatment of cancer, particularly for treatment of CRC and mCRC.

25. A CHD1L inhibitor for use in the treatment of cancer, particularly for treatment of CRC and mCRC.

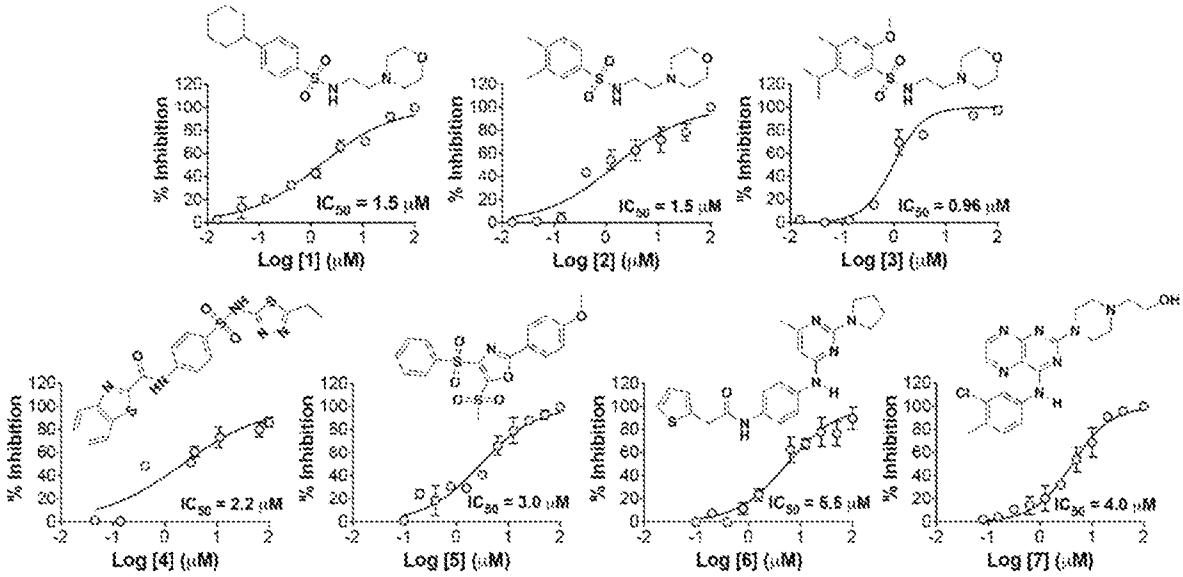

% Inhibition
IC50 = 1.5 μM
Log [1] (μM)
IC50 = 1.5 μM
Log [2] (μM)
IC50 = 0.96 μM
Log [3] (μM)
IC50 = 2.2 μM
Log [4] (μM)
IC50 = 3.0 μM
Log [5] (μM)
IC50 = 6.6 μM
Log [6] (μM)
IC50 = 4.0 μM
Log [7] (μM)

FIG. 1A

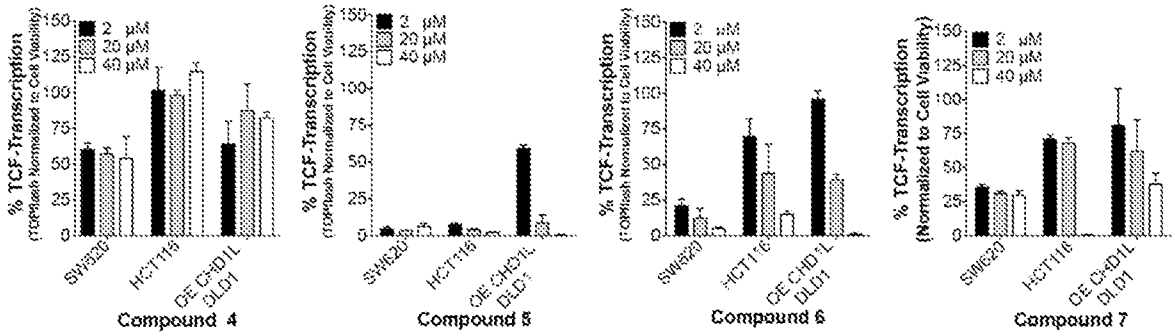

% TCF-Transcription
(TOPflash Normalized to Cell Viability)
2 μM
20 μM
40 μM
SW620
HCT116
CE CHD1L
DLD1
Compound 4
Compound 5
Compound 6
Compound 7

FIG. 1B

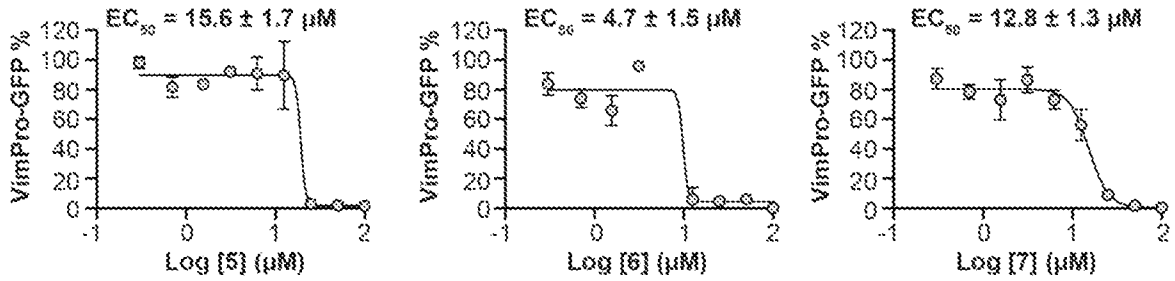


FIG. 2A

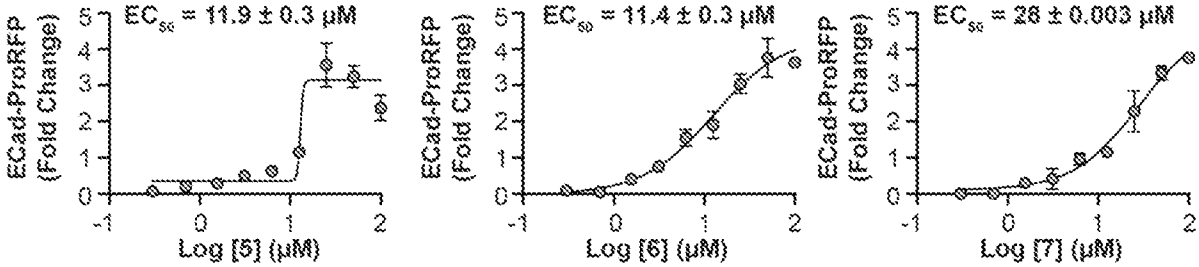


FIG. 2B

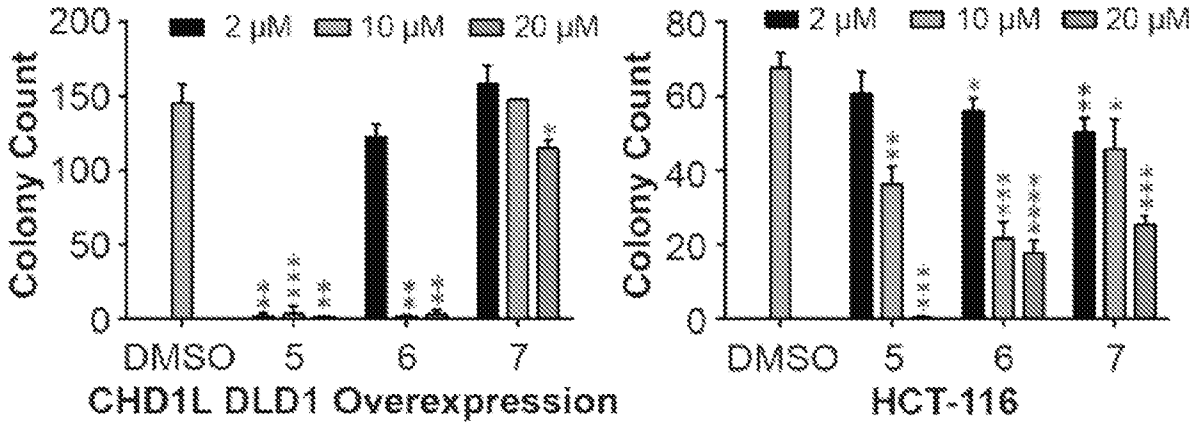


FIG. 2C

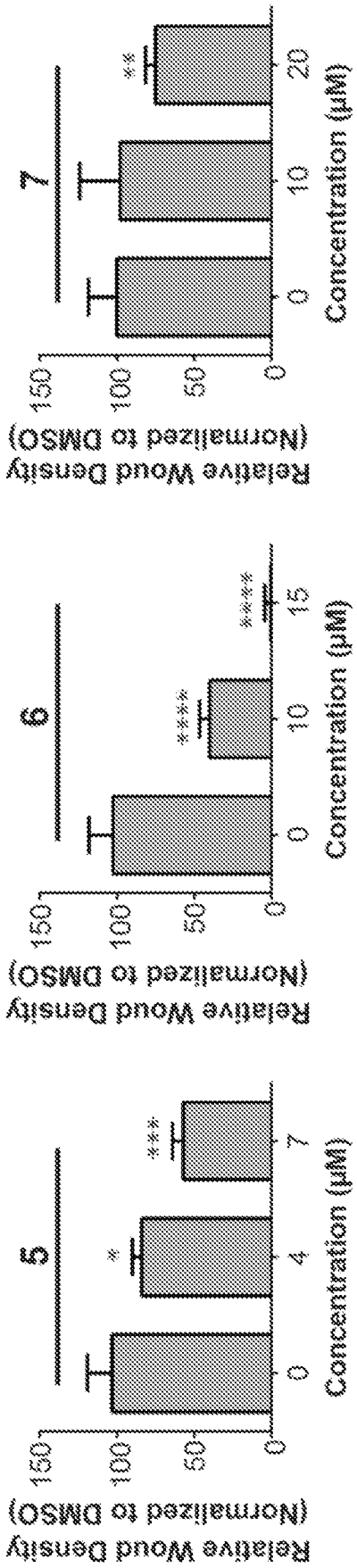

5
6
7
Relative Wound Density
(Normalized to DMSO)
Concentration (μM)
0
50
100
150
0
4
7
10
15
20
*
***
****
**

FIG. 2D

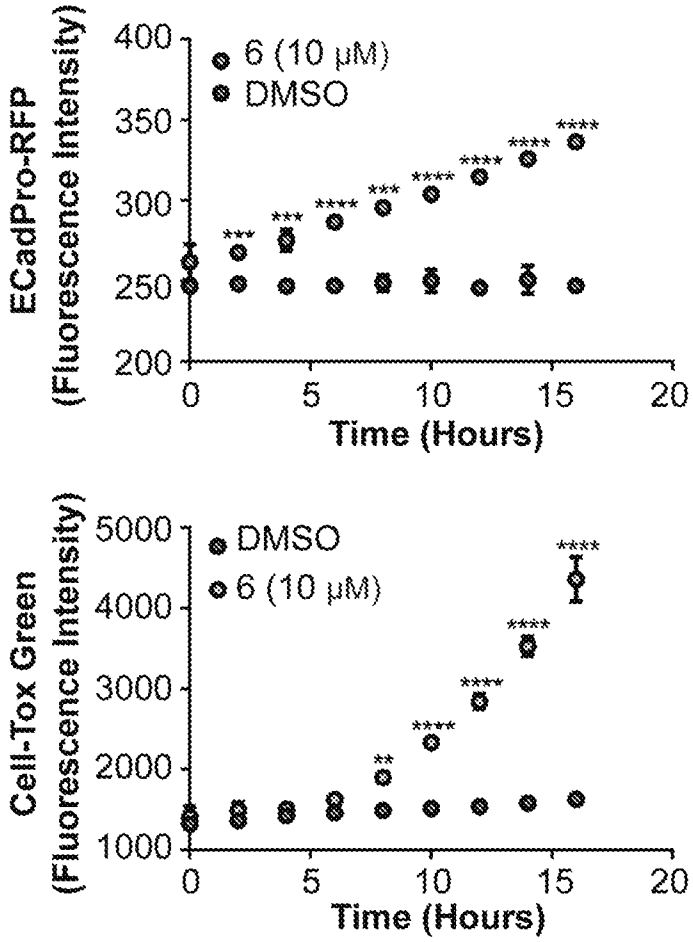

ECadPro-RFP
(Fluorescence Intensity)
400
350
300
250
200
6 (10 μM)
DMSO
0
5
10
15
20
Time (Hours)
Cell-Tox Green
(Fluorescence Intensity)
5000
4000
3000
2000
1000
DMSO
6 (10 μM)
0
5
10
15
20
Time (Hours)

FIG. 3A

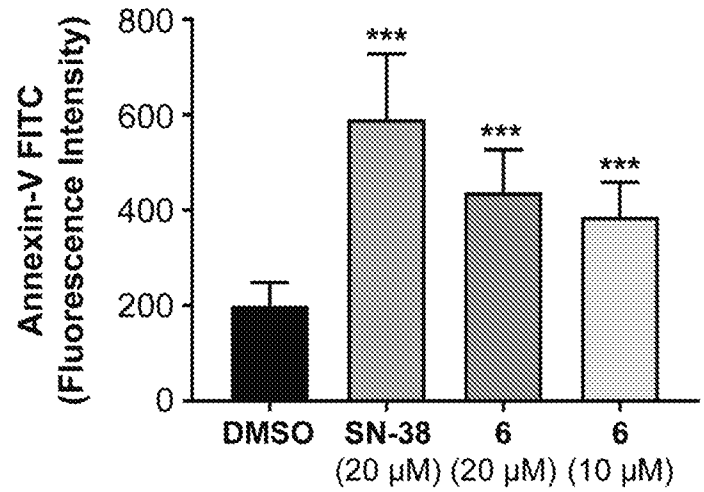

Annexin-V FITC
(Fluorescence Intensity)
800
600
400
200
0
DMSO
SN-38
(20 μM)
6
(20 μM)
6
(10 μM)

FIG. 3B

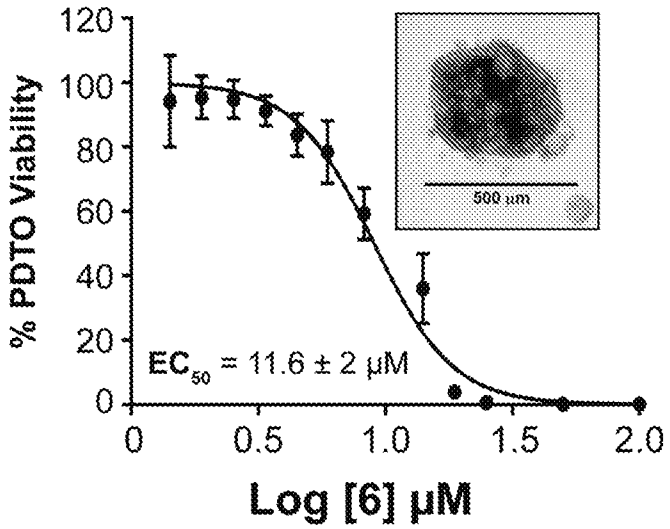

% PDTO Viability
120
100
80
60
40
20
0
EC50 = 11.6 ± 2 µM
0
0.5
1.0
1.5
2.0
Log [6] µM
500 µm

FIG. 3C

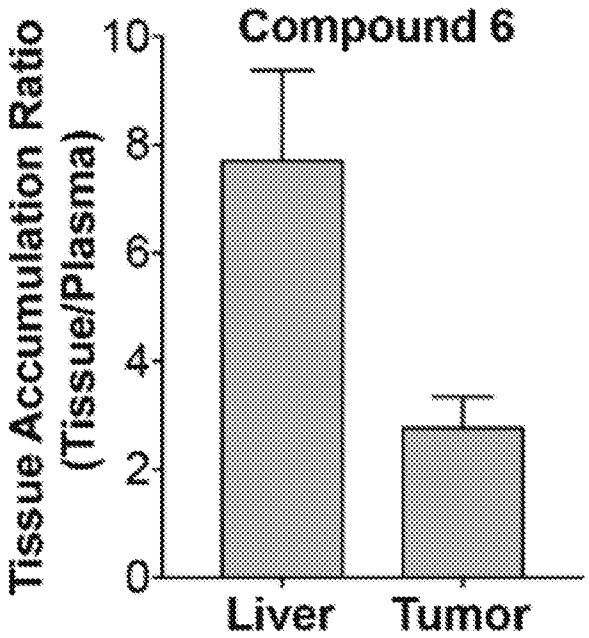

Compound 6
Tissue Accumulation Ratio
(Tissue/Plasma)
10
8
6
4
2
0
Liver
Tumor

FIG. 4

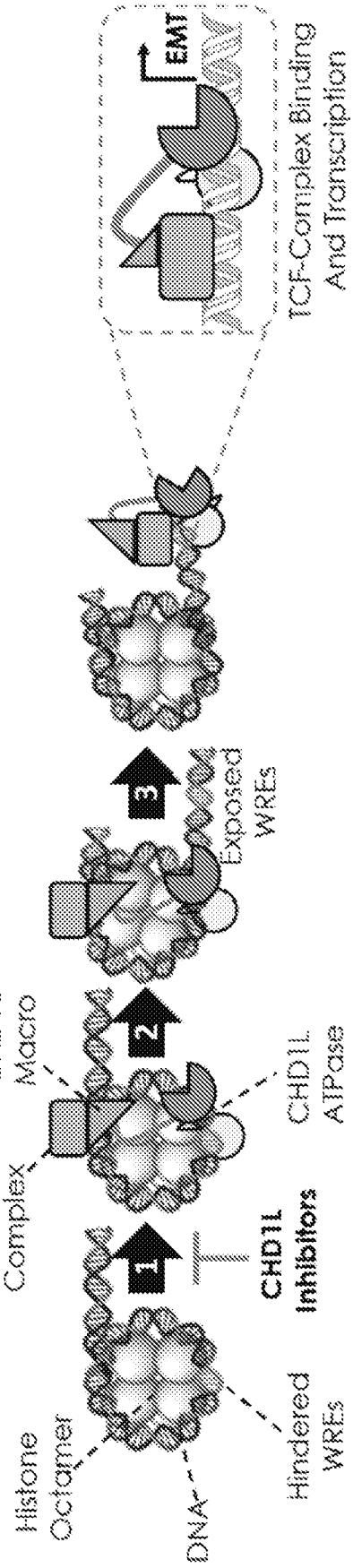

EMT
TCF-Complex Binding
And Transcription
Exposed
WREs
CHD1L
Macro
CHD1L
ATPase
TCF
Complex
CHD1L
Inhibitors
Histone
Octamer
Hindered
WREs
DNA
1
2
3

FIG. 5

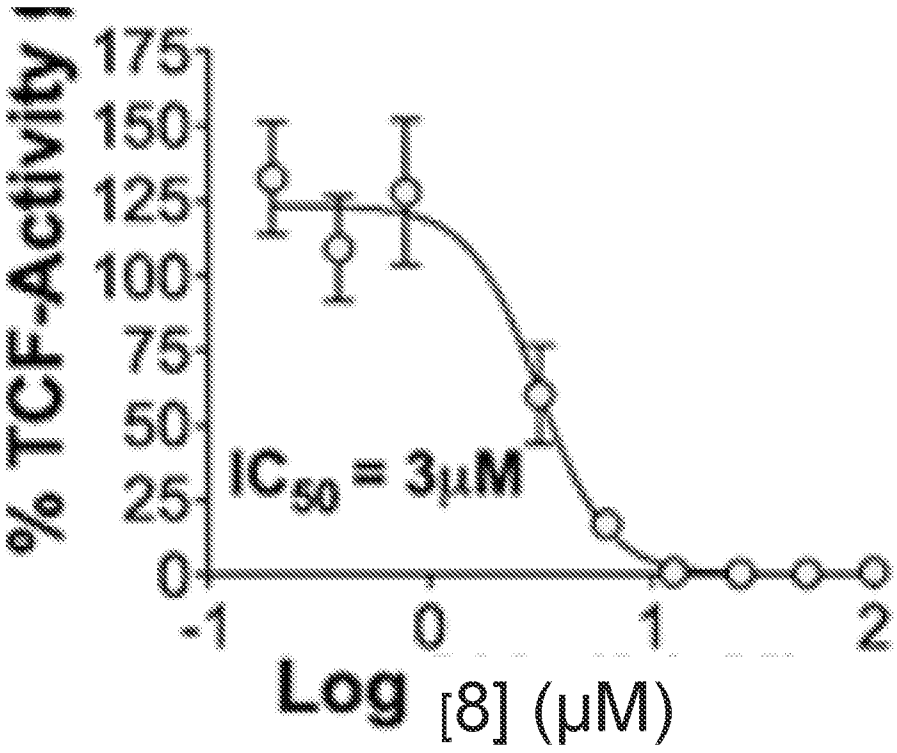

% TCF-Activity I
175
150
125
100
75
50
25
0
IC50 = 3μM
-1
0
1
2
Log [8] (μM)

FIG. 6A

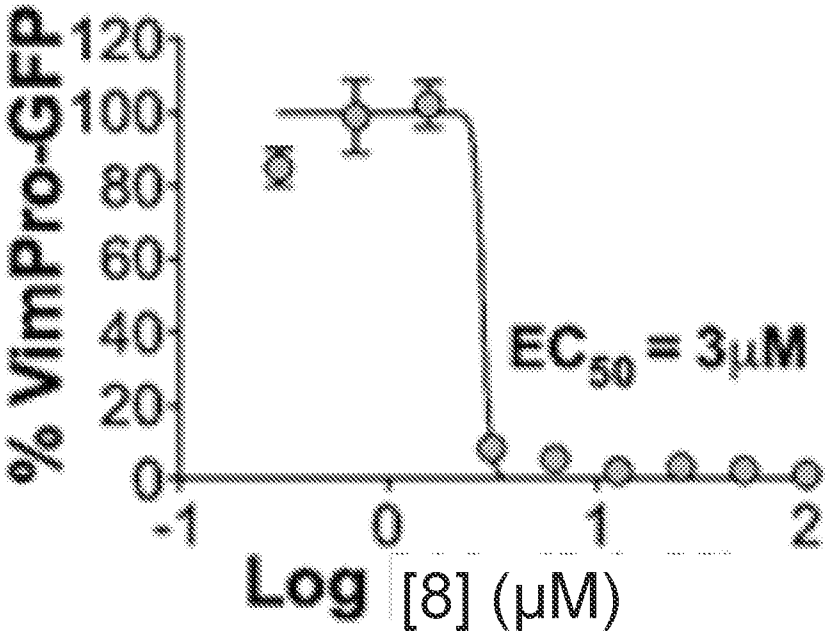

% VimPro-GFP
120
100
80
60
40
20
0
EC50 = 3μM
-1
0
1
2
Log [8] (μM)

FIG. 6B

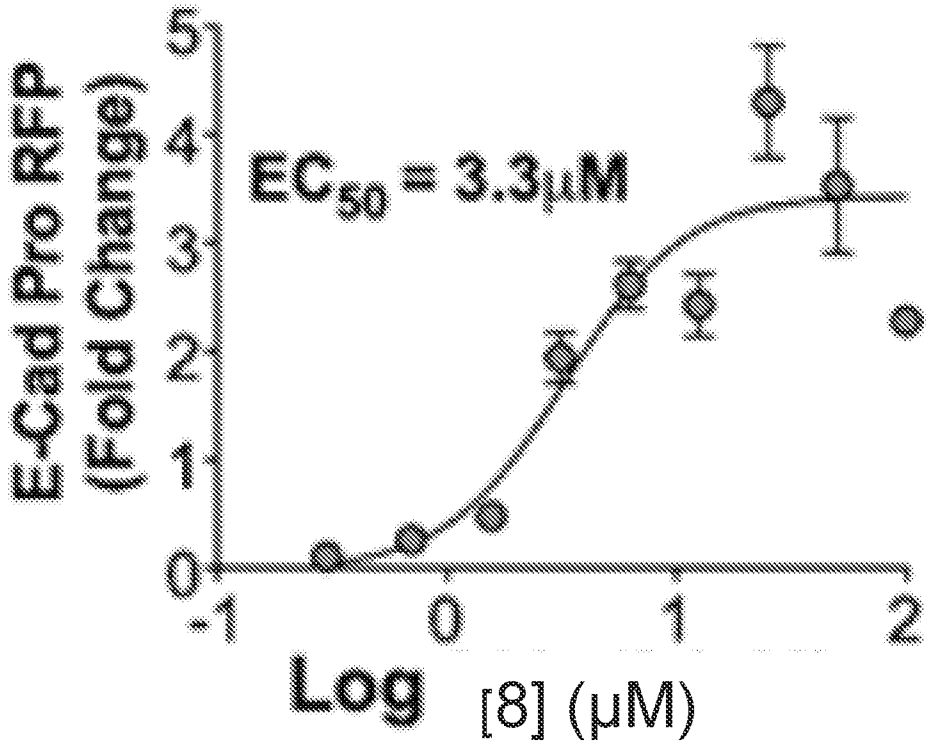

E-Cad Pro RFP
(Fold Change)
EC50 = 3.3μM
Log [8] (μM)
0
1
2
3
4
5
-1
0
1
2

FIG. 6C

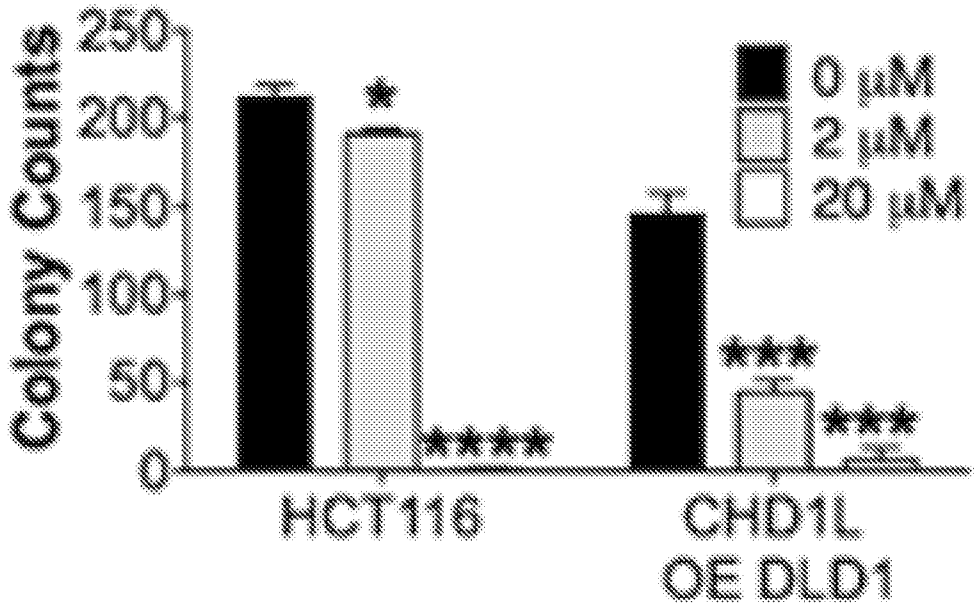

Colony Counts
250
200
150
100
50
0
0 μM
2 μM
20 μM
HCT116
CHD1L
OE DLD1

FIG. 6D

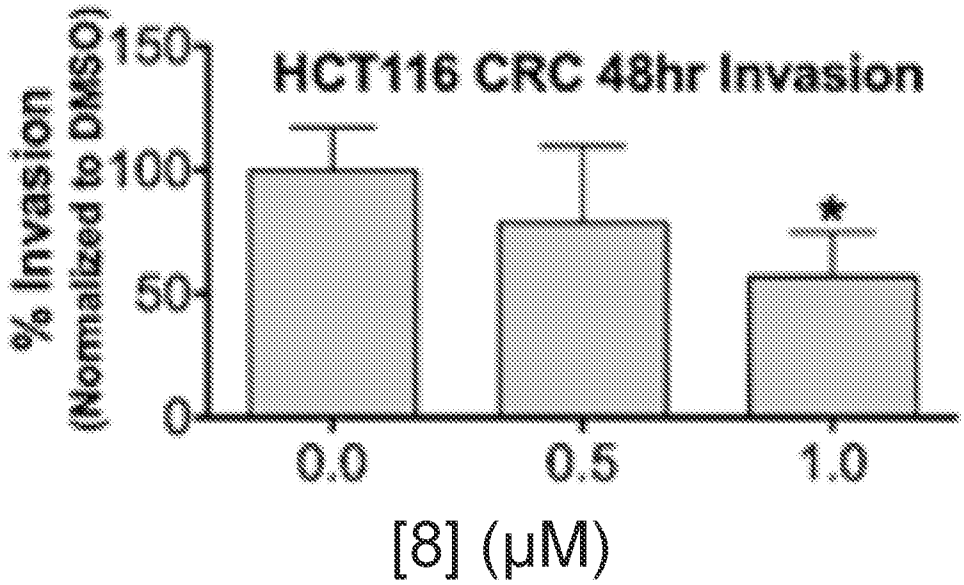

HCT116 CRC 48hr Invasion
% Invasion
(Normalized to DMSO)
150
100
50
0
0.0
0.5
1.0
*
[8] (μM)

FIG. 6E

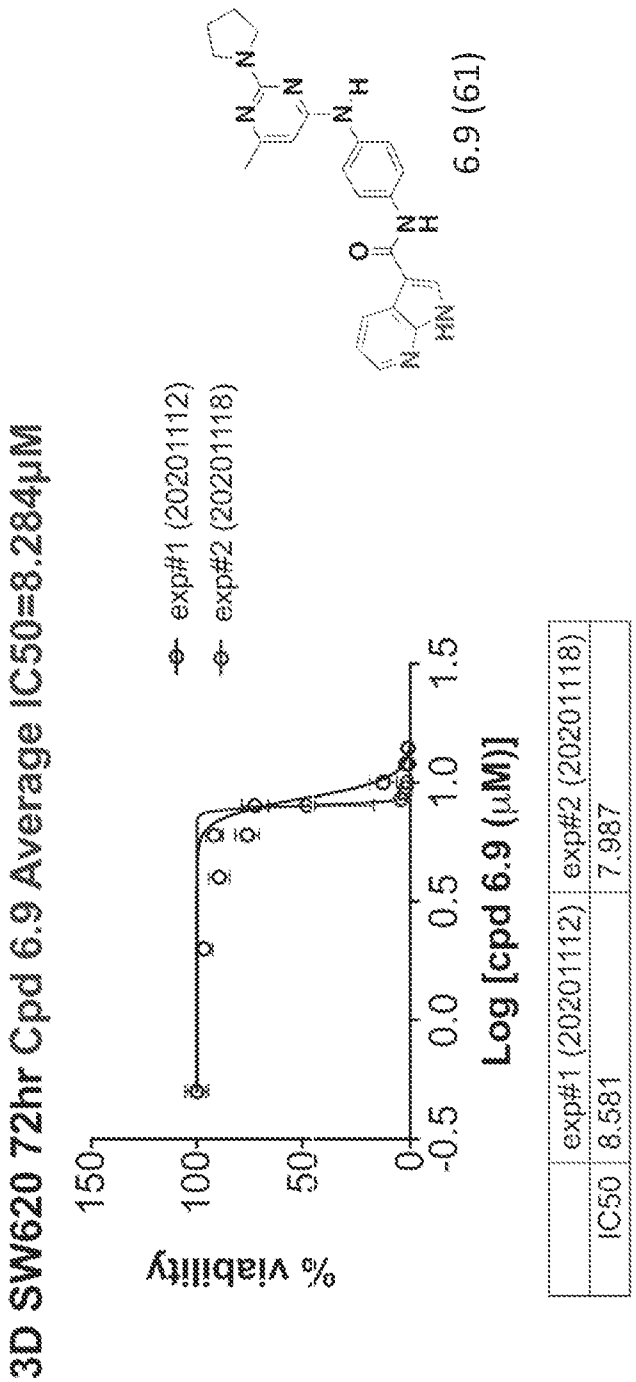
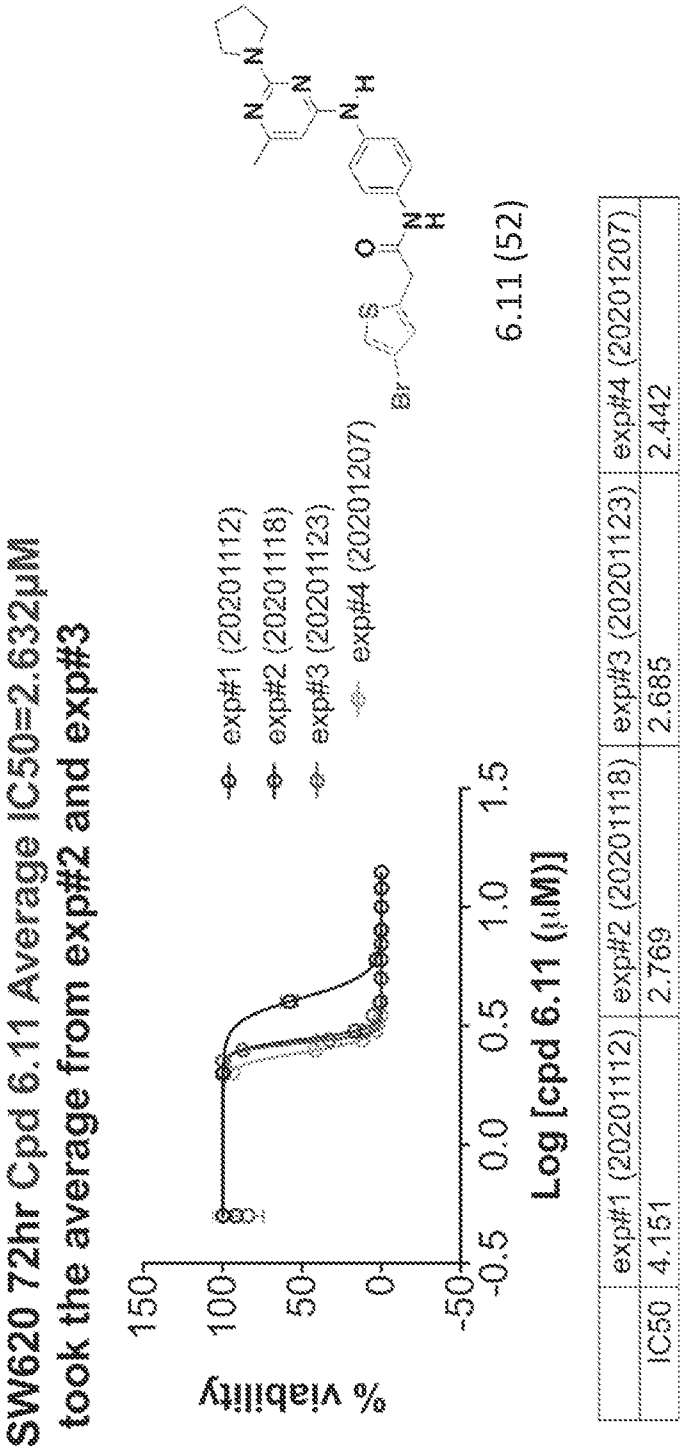

3D SW620 72hr Cpd 6.9 Average IC50=8.284μM
% viability
150
100
50
0
-0.5
0.0
0.5
1.0
1.5
Log [cpd 6.9 (μM)]
exp#1 (20201112)
exp#2 (20201118)
6.9 (61)
exp#1 (20201112) exp#2 (20201118)
IC50 8.581 7.987

FIG. 7A



	exp#1 (20201112)	exp#2 (20201118)	exp#3 (20201123)	exp#4 (20201207)
IC50	4.151	2.769	2.685	2.442

FIG. 7B

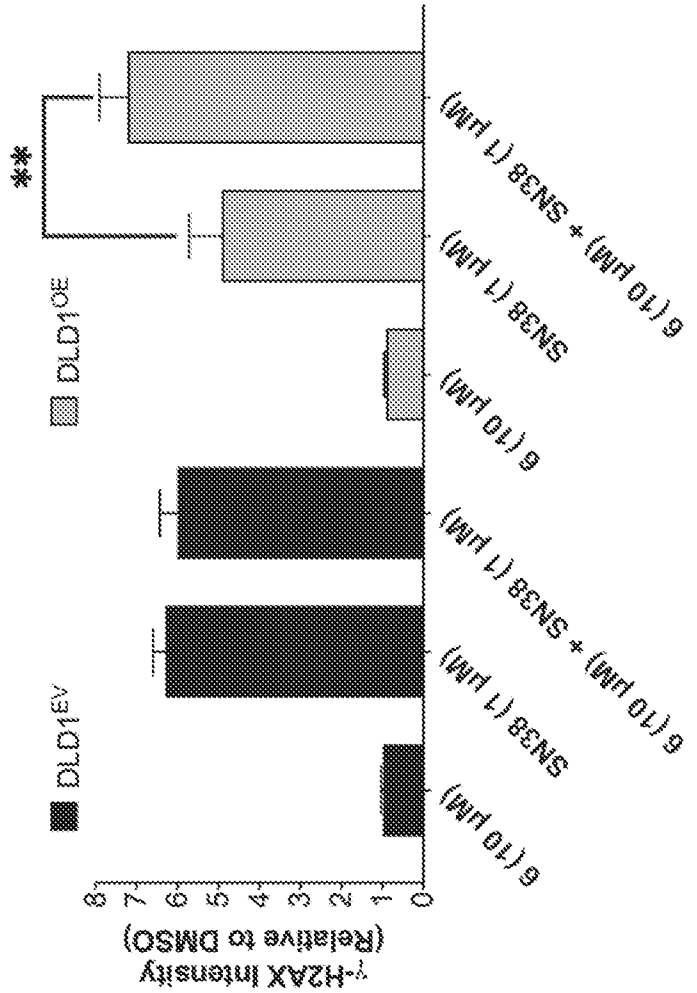

γ-H2AX Intensity
(Relative to DMSO)
0
1
2
3
4
5
6
7
8
DLD1EV
DLD1OE
**
6 (10 μM)
SN38 (1 μM)
6 (10 μM) + SN38 (1 μM)
6 (10 μM)
SN38 (1 μM)
6 (10 μM) + SN38 (1 μM)

FIG. 8B

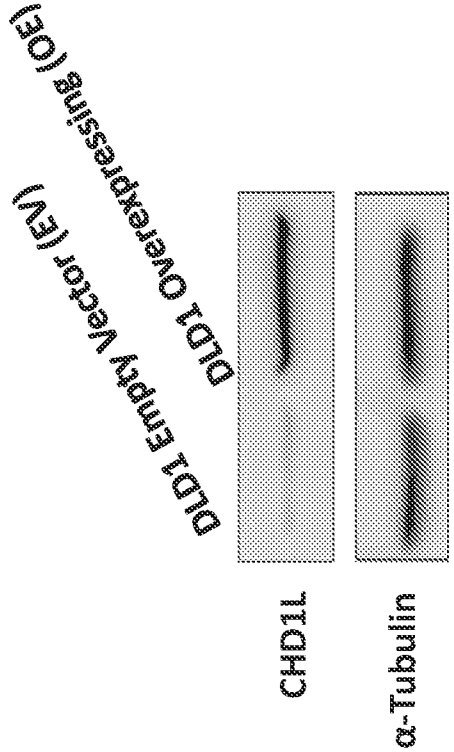
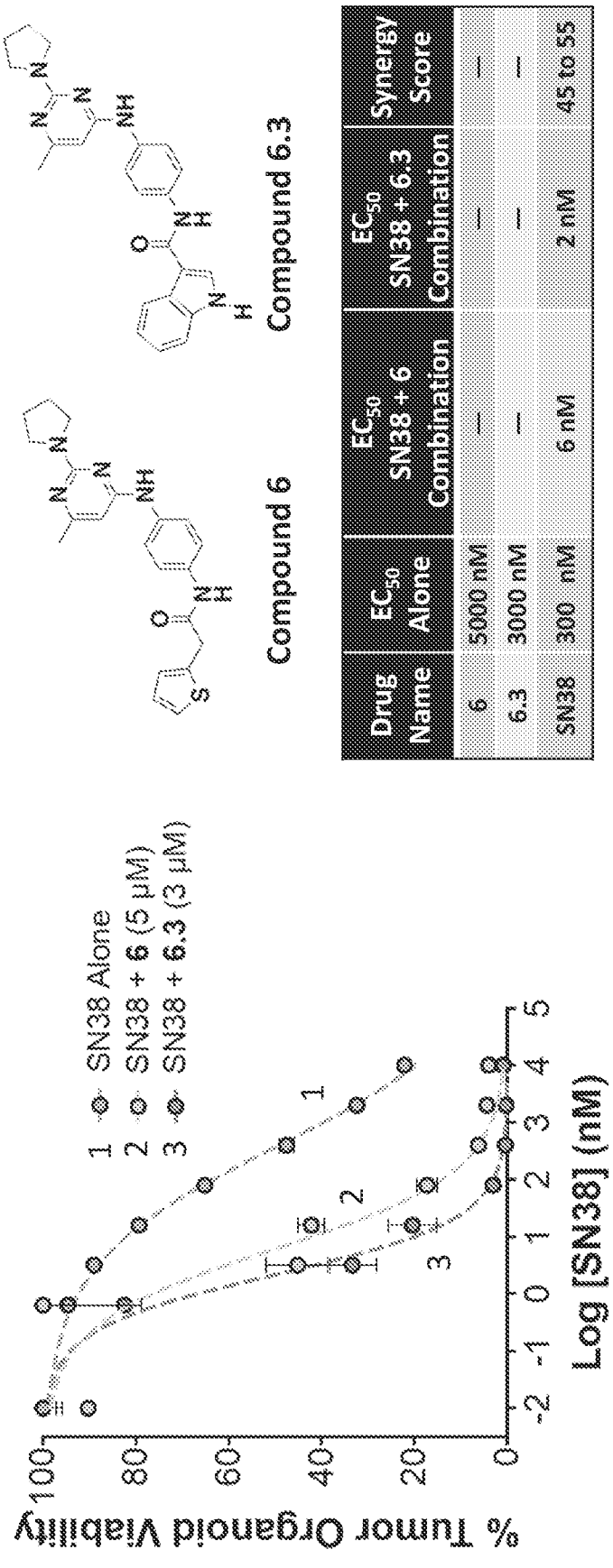
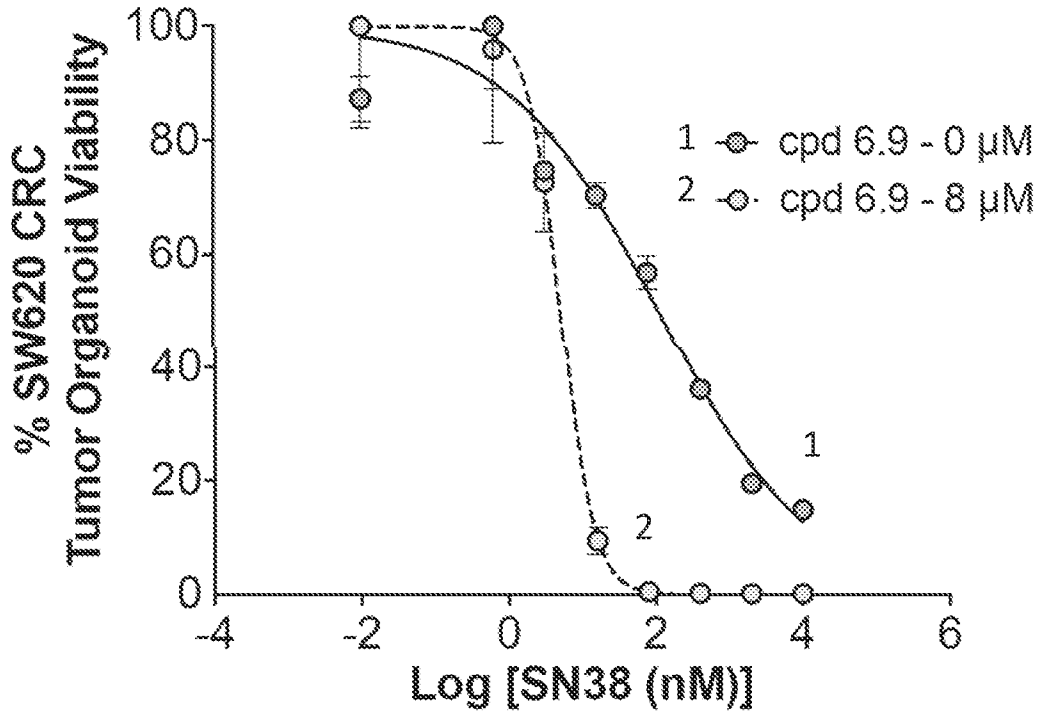

DLD1 Empty Vector (EV)
DLD1 Overexpressing (OE)
CHD1L
α-Tubulin

FIG. 8A



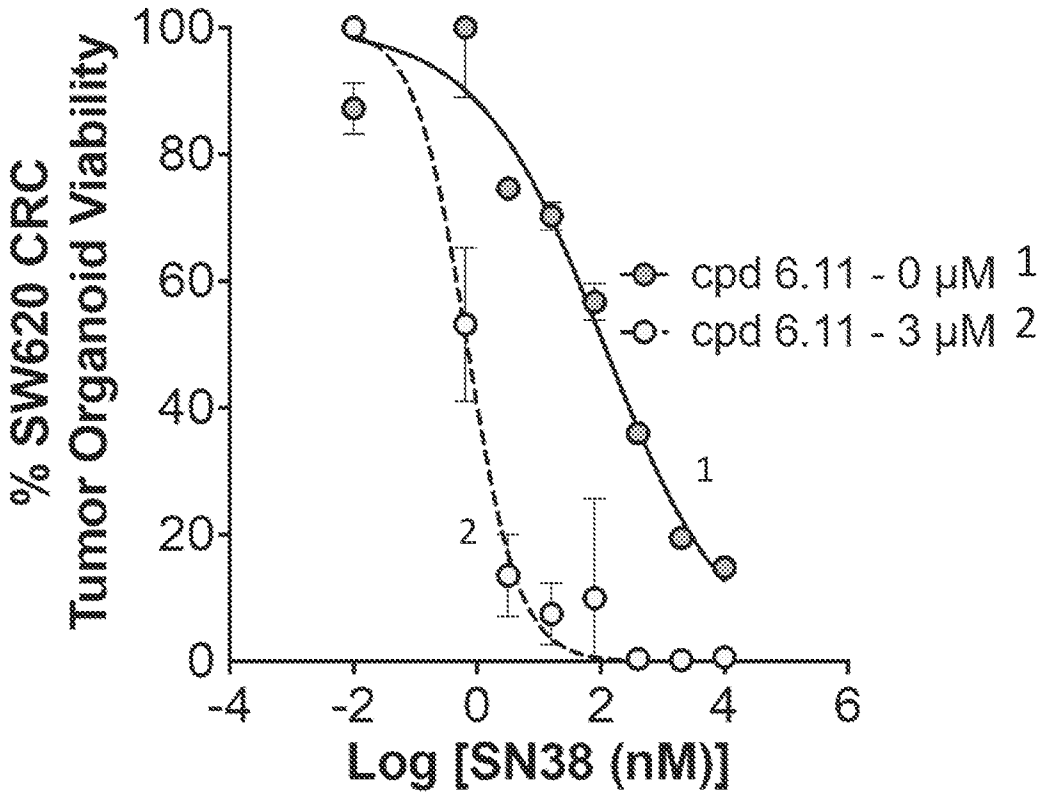
Drug Name	EC_{50} Alone	EC_{50} SN38 + 6 Combination	EC_{50} SN38 + 6.3 Combination	Synergy Score
6	5000 nM	—	—	—
6.3	3000 nM	—	—	—
SN38	300 nM	6 nM	2 nM	45 to 55

FIG. 9A



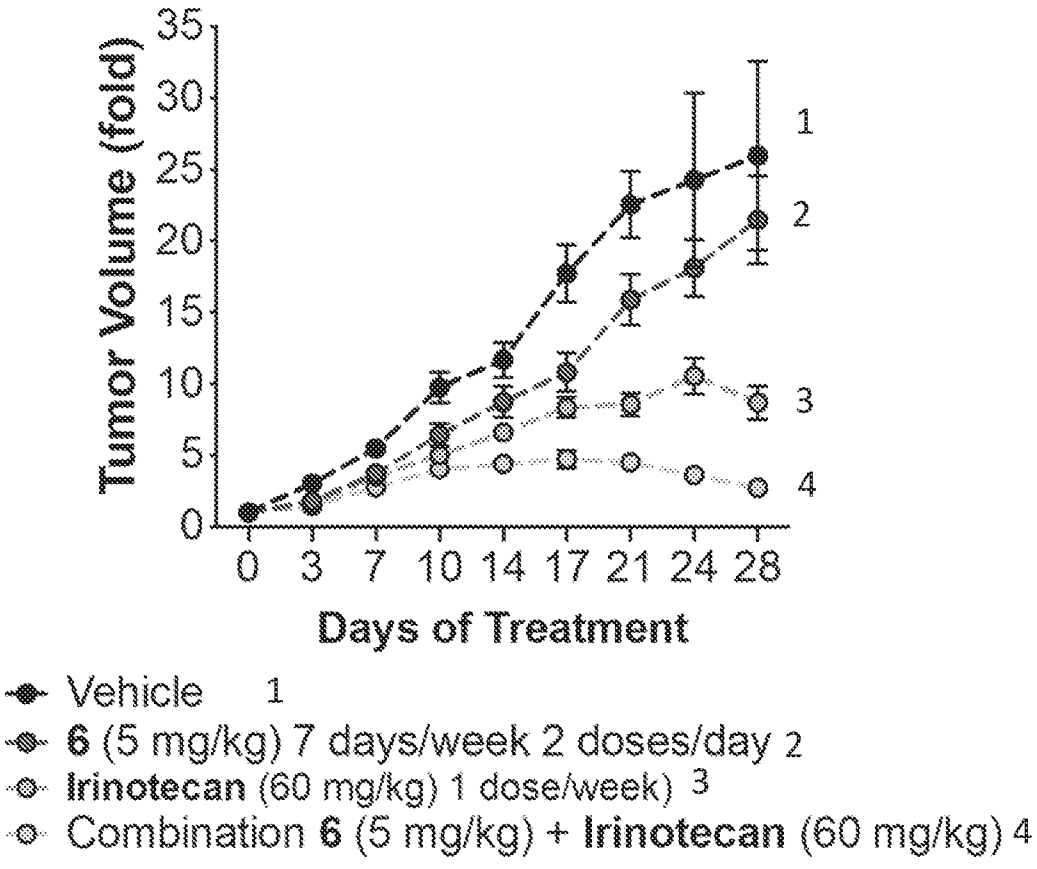
Drug Name	EC_{50} Alone	EC_{50} SN38 + 6.11 Combination	Synergy Score
6.11	3000 nM	—	—
SN38	112 nM	0.69 nM	≥33

FIG. 9B



Drug Name	EC_{50} Alone	EC_{50} SN38 + 6.11 Combination	Synergy Score
6.11	3000 nM	—	—
SN38	112 nM	0.69 nM	54

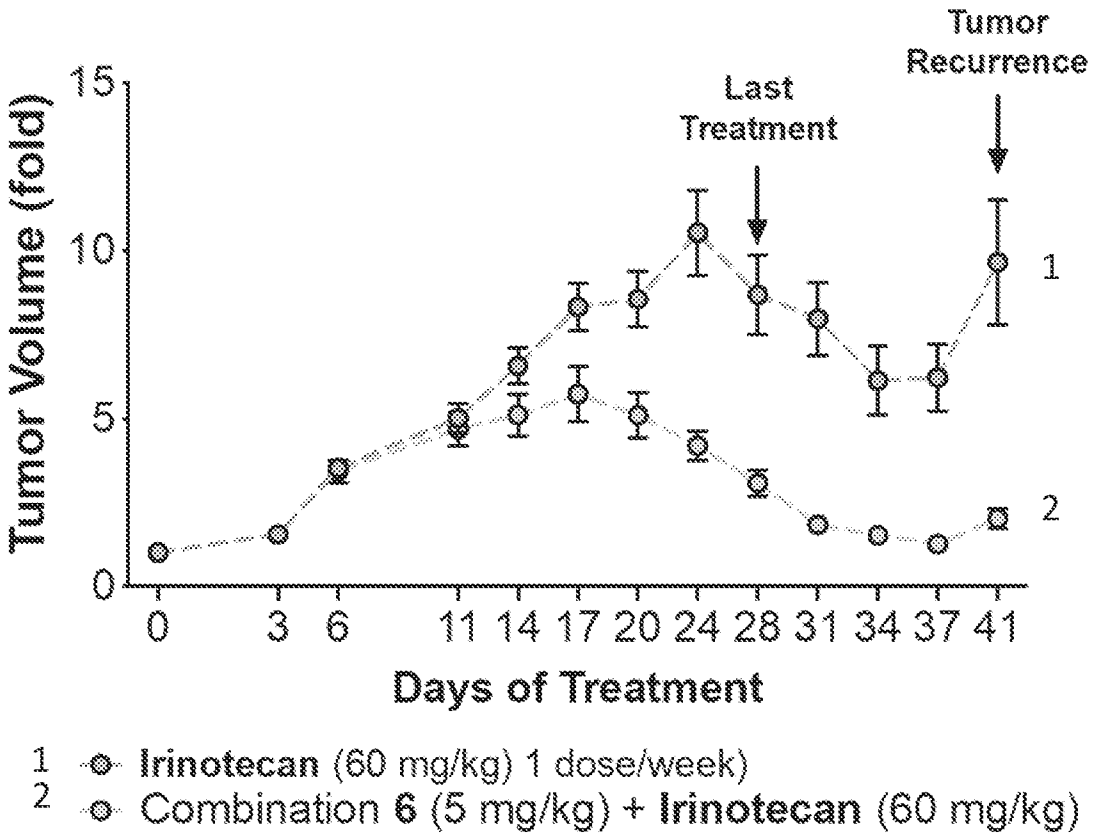
FIG. 9C



Animal Groups	Day 10	Day 14	Day 17	Day 21	Day 24	Day 28
Vehicle vs. cpd 6 5 mg/kg	ns	ns	****	****	*	ns
Vehicle vs. Irinotecan 60 mg/kg	**	**	****	****	****	****
Vehicle vs. Combination	***	****	****	****	****	****
cpd 6 5 mg/kg vs. Irinotecan 60 mg/kg	ns	ns	ns	****	****	****
cpd 6 5 mg/kg vs. Combination	ns	**	****	****	****	****
Irinotecan 60 mg/kg vs. Combination	ns	ns	ns	*	****	***

ns = not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

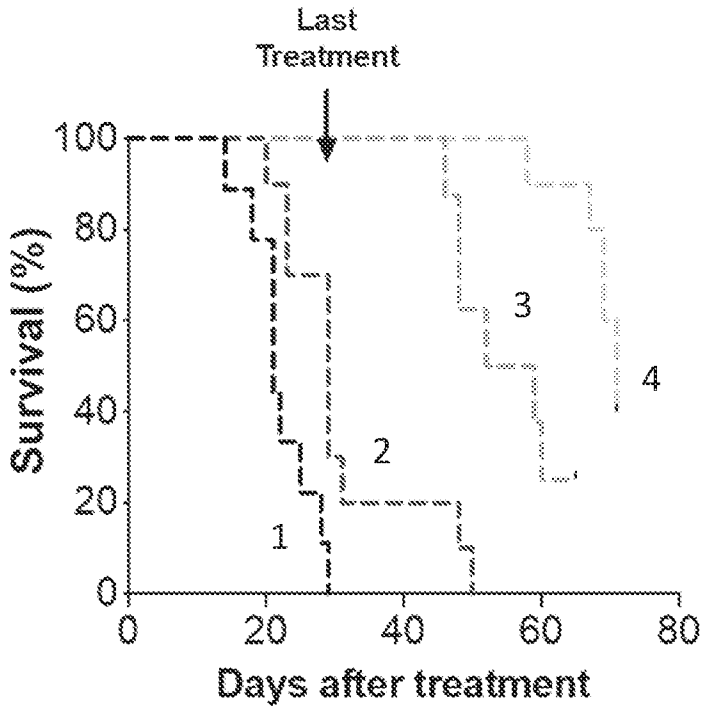
FIG. 10



Animal Groups	Day 31	Day 34	Day 37	Day 41
Irinotecan 60 mg/kg vs. Combination	****	***	****	****

***P<0.001, ****P<0.0001

FIG. 11

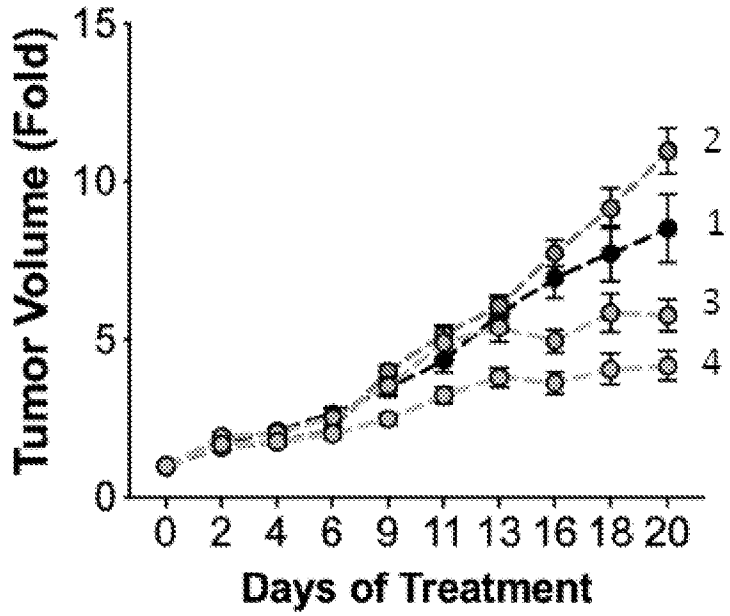


1 Vehicle
2 **6** (5 mg/kg) 7 days/week 2 doses/day
3 **Irinotecan** (60 mg/kg) 1 dose/week)
4 Combination **6** (5 mg/kg) + **Irinotecan** (60 mg/kg)

	Vehicle	5 mg/kg Cmpd 6	60 mg/kg Irinotecan	5 mg/kg + Irino
Median survival	21	29	55.5	71

Survival Gehan-Breslow-Wilcoxon test	to day 65	to day 71
Vehicle vs. cpd 6 5 mg/kg	**	**
Vehicle vs. Irinotecan 60 mg/kg	***	***
Vehicle vs. Combo	****	****
cpd 6 5 mg/kg vs. Irinotecan 60 mg/kg	**	**
cpd 6 5 mg/kg vs. Combo	****	****
Irinotecan 60 mg/kg vs. Combo	**	**

FIG. 12

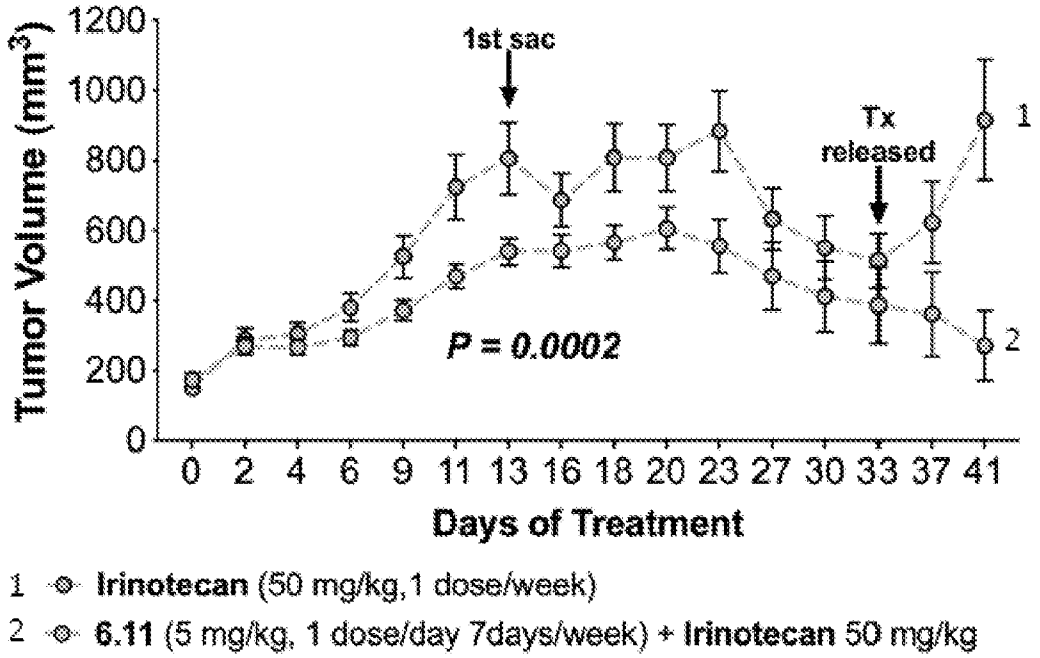


1 Vehicle
2 **6.11** 5 mg/kg
3 Irinotecan 50 mg/kg
4 Combination **6.11** 5 mg/kg + Irino 50 mg/kg

Animal Groups	Day 9	Day 11	Day 13	Day 16	Day 18	Day 20
Vehicle vs. cpd 6.11 5 mg/kg	ns	ns	ns	ns	ns	ns
Vehicle vs. Irinotecan 50 mg/kg	ns	ns	ns	ns	ns	ns
Vehicle vs. Combination	***	*	**	**	**	*
cpd 6.11 5 mg/kg vs. Irinotecan 50 mg/kg	ns	ns	ns	**	*	***
cpd 6.11 5 mg/kg vs. Combination	**	***	**	****	**	***
Irinotecan 50 mg/kg vs. Combination	*	*	ns	ns	ns	ns

ns = not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

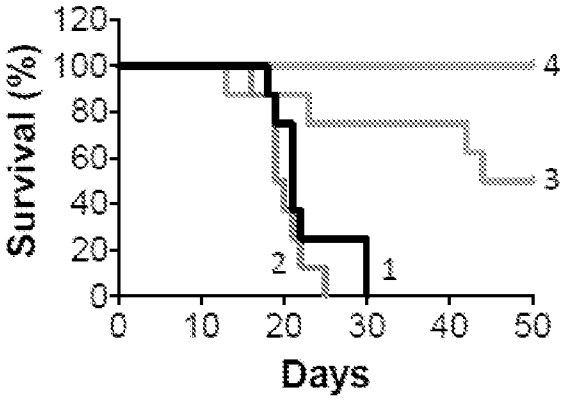
FIG. 13



1 **Irinotecan** (50 mg/kg,1 dose/week)

2 **6.11** (5 mg/kg, 1 dose/day 7days/week) + **Irinotecan** 50 mg/kg

FIG.14



Log-Rank Mantel-Cox Test

1 Vehicle
2 **6.11** 5 mg/kg
3 **Irinotecan** (50 mg/kg,1 dose/week)
4 **6.11** (5 mg/kg, 1 dose/day 7days/week) + **Irinotecan** 50 mg/kg

** * ****

*P=0.025, **P=0.005, ****P<0.0001

FIG. 15