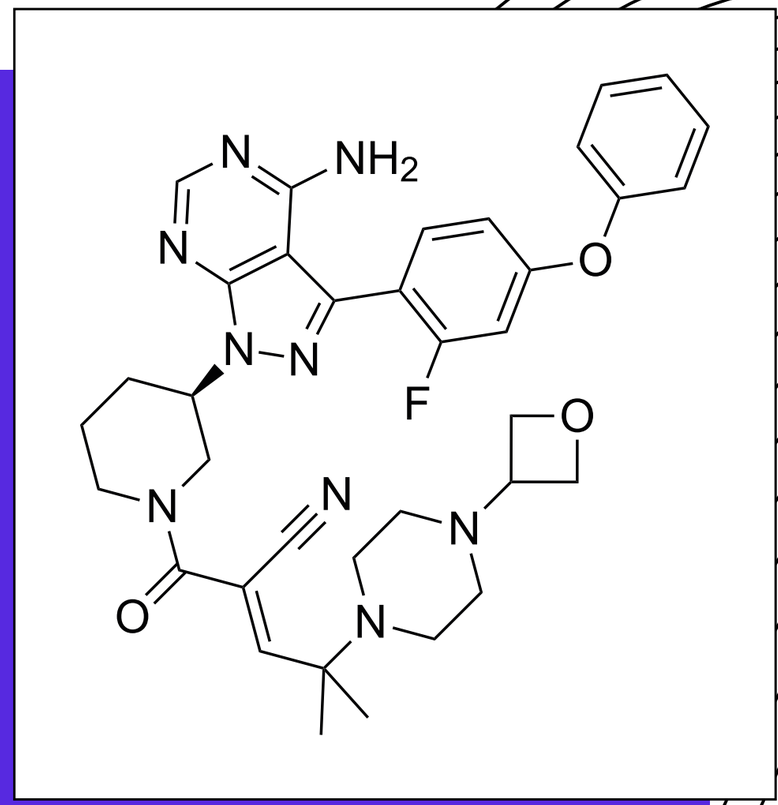
Small Molecules of the Month

March 2022

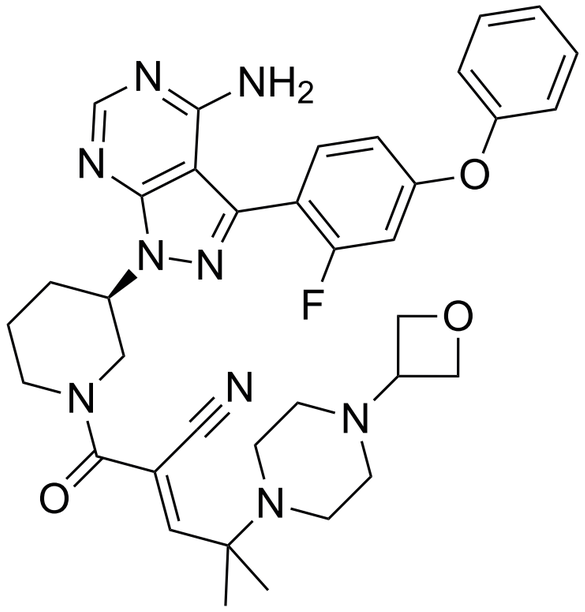


drug hunter

01	BTK	Sanofi
02	Pf 3D7	Novartis (NITD)
03	STING	Merck
04	KIF18A	Amgen
05	PPARγ	AstraZeneca
06	PK	Forma Therapeutics
07	AAK1	BMS
08	SMN2	Novartis (NIBR)
09	PRC2	Novartis (NIBR)
10	COX-2	Nanjing Heron Pharmaceutical

rilzabrutinib (PRN1008)

BTK



oral reversible covalent BTK inhibitor

in Ph. III for immune thrombocytopenia

from structure-based drug design + opt.

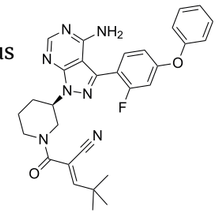
J. Med. Chem.

Principia Biopharma (Sanofi), So. San Francisco, CA

Context. PRN1008 (rilzabrutinib, Sanofi) is an oral, reversible covalent Bruton's tyrosine kinase (BTK) inhibitor in a Ph. III trial for immune thrombocytopenia. A simultaneously published inhibitor, PRN473, is in development as a topical treatment for atopic dermatitis. Following target discovery in 1993, and subsequent approval of the first BTK inhibitor 20 years later, research on these agents have intensified over the last decade, with numerous compounds currently either being marketed or in clinical trials for both oncology and immunology indications (e.g. ibrutinib, acalabrutinib, zanubrutinib). At the moment, there are no approved reversible BTK inhibitors, though non-covalent fenebrutinib (NCT05119569) is in late development for multiple sclerosis. Next-generation BTK inhibitors have focused on improving safety, as ibrutinib can lead to the development of cardiac arrhythmias, bleeding, infection, diarrhea, arthralgias, and hypertension in the patients, which have been acceptable risks in oncology but less so in other areas.

Reviewer Comments. Nominator and reviewer Bryan McKibben says, "The structures of both were previously disclosed at the 255th National ACS meeting in 2018. BTK inhibition is a crowded field with numerous compounds currently being marketed or in clinical trials for both oncology and immunology indications. PRN1008 and PRN473 are covalent reversible inhibitors and are hypothesized to have differentiated safety and efficacy profile from other irreversible and reversible BTK inhibitors. Initial oral clinical candidate, PRN473, was modestly absorbed in rat but well absorbed in dogs. In humans, PRN473 was poorly absorbed and therefore was later repurposed as a topic agent for atopic dermatitis (Phase IIa). The authors optimized whole blood cell activity, solubility, permeability and clearance to identify PRN1008 which is in Phase III for immune thrombocytopenia (ITP)." PRN1008 was previously in a Ph. III trial for human pemphigus vulgaris (PV), but this was terminated by Sanofi after the Phase III PEGASUS trial (NCT03762265) failed to meet its primary or key secondary endpoints.

Additional Clinical Development. Following results from a clinical trial which demonstrated the safety and tolerability of PRN1008 in healthy participants, the molecule is being evaluated in several Phase I/II and Phase III clinical trials for various autoimmune diseases. These studies include the Phase I/II NCT03395210 study involving adult patients with immune thrombocytopenia, the Phase II NCT04520451 study involving patients with active IgG4-related disease, the Phase II RILECSU study in patients with chronic spontaneous urticaria, the Phase II NCT05002777 study involving patients with warm autoimmune hemolytic anemia, and the Phase III LUNA 3 study in adult and adolescent patients with persistent or chronic immune thrombocytopenia. Two proof-of-concept PRN1008 studies involving patients with moderate-to-severe asthma (NCT05104892) and moderate-to-severe atopic dermatitis (NCT05018806) are also currently underway.



PRN473

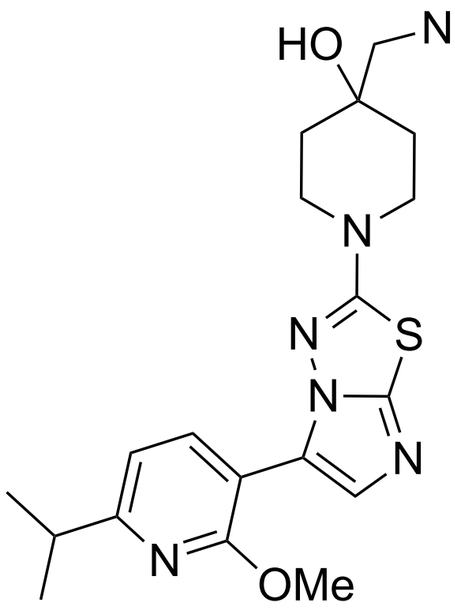
Mechanism of Action. Covalent BTK inhibitors target a noncatalytic cysteine 481 near the ATP binding pocket of BTK (PDB: 7L5P), a moiety available in only ten human kinases which explains the high selectivity attributed to these inhibitors. Unlike currently approved covalent BTK inhibitors which are irreversible and only indicated for certain hematologic malignancies, PRN1008 is a reversible covalent BTK inhibitor that is being evaluated as treatment for autoimmune disorders. Whereas irreversible covalent BTK inhibitors have a high potential for off-target activity due to their ability to irreversibly bind endogenous thiols, reversible covalent inhibitors have been shown to dissociate rapidly from common thiols while still maintaining their inhibitory effect on targets. Whether this translates into a clinical safety difference is unclear.

Hit-Finding Strategy. In a study by the Taunton group at UCSF, a series of chemically tuned electrophilic compounds were shown to bind reversibly to noncatalytic cysteines in a covalent manner such that binding times were dictated by the various stabilizing interactions in the binding site. In a subsequent study, an inverted orientation of the cysteine-reactive cyanoacrylamide electrophile was used to identify potent and selective BTK inhibitors that targeted noncatalytic cysteines in the protein in a covalent and reversible manner. The chemical matter for PRN1008 derived from this structure-based design strategy, focusing on the identification of compounds with longer binding site residence times. Initial screening tests were done using BTK enzyme assays, while kinetic competition assays were used to assess differences in inhibitor residence times; recombinant, full-length BTK was used in the assays. Interestingly, no association was observed between the residual times at the binding site and the IC50 values.

Preclinical Pharmacology. In vivo testing was done using the collagen-induced arthritis (CIA) animal model which has been used to evaluate the ability BTK inhibitors to modulate inflammatory responses. Ankle diameter in female Lewis rats treated at 3, 10, and 30 mg/kg was significantly reduced in all treatment groups vs vehicle controls, with the inhibition being dose dependent. Maximal efficacy was observed with the 30 mg/kg dose. Occupancy studies were conducted by measuring binding of a labeled covalent probe inhibitor to free BTK in rat splenocytes; whereas the concentrations PRN1008 were relatively low at 6 hr and further dropped significantly at 12 hr for all doses tested, occupancy was largely maintained at 12 hr. High occupancy (>80%) at the 30 mg/kg dose was associated with the greatest reduction in ankle diameter; these data and other pharmacokinetic/pharmacodynamic modeling data suggests that optimal activity of the molecule may be dictated by high and sustained occupancy at the BTK binding site.

INE963

Plasmodium falciparum 3D7



oral blood stage antimalarial

in Ph. I (healthy volunteers)

from 1.5M cmpd phenotypic screen + opt.

J. Med. Chem.

Novartis (NIBR), Emeryville, CA

Context. INE963 (Novartis Institute for Tropical Diseases) is an oral, single dose, fast-acting blood-stage antimalarial candidate. The molecule was identified using a phenotypic high-throughput screening approach which has been successfully used to identify new antimalarial chemotypes. Phenotypic screens can be preferable to target-based screens in antimalarial drug discovery because the emergence of antimalarial drug resistance to current front-line compounds means identification of first-in-class molecules is imperative. Although a mechanism of action has yet to be identified for the molecule, its activity against multidrug-resistant P. falciparum cell lines and demonstration of high barrier to resistance are suggestive of a first-in-class molecule.

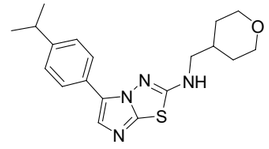
Reviewer Comments. Three attributes set INE963 apart from existing antimalarials according to nominator and reviewer Dennis Koester: its ability to quickly reduce parasitemia, its high barrier to resistance, and its long human half-life and potential for single dose cure of uncomplicated malaria.

"Speed of kill: INE963 has the ability to reduce parasite burden extremely fast. Speed of kill was tested in a Pf viability assay and after 24 h no viable parasites were found (5 log reduction of parasitemia). This is extremely important for a blood stage antimalarial to quickly alleviate symptoms by reducing parasite burden and also decreases the chances for resistance.

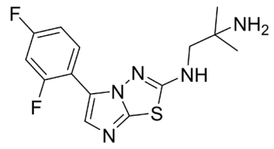
Irresistible: To date no INE963-resistant mutants have been identified, although ramping studies have been performed. INE963 is also active against lab Pf and Pv strains as well as several field isolates as well as drug resistant strains.

Long human half-life and potential for single dose cure: The holy grail of malaria therapy is the cure of the disease with a single dose. Due to it's long half-life and it's high potency INE963 has the potential to achieve single dose cure in the clinic."

Hit-Finding Strategy and Lead Optimization. A 5-aryl-2-amino-imidazothiadiazole early lead compound (compound 2; EC_{50} = 0.27 μM) was identified in a high-throughput phenotypic screen of 1.5M compounds using a blood stage P. falciparum growth inhibition assay. The late lead compound 3 with better potency (EC_{50} = 0.025 μM) was identified during optimization. Key issues for optimization included improving chemical stability due to degradation via a tautomerization mechanism, selectivity over human kinases (kinase **PDB:7SQM**), and eliminating a hERG Qpatch signal.



"compound 2" (EC_{50} = 0.27 μM)



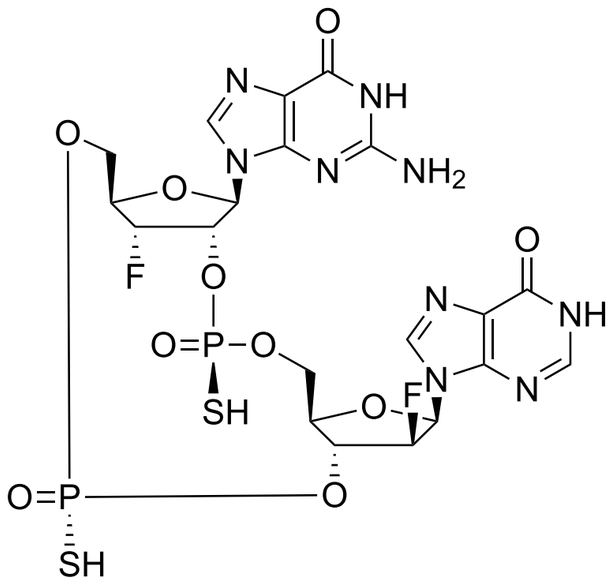
"compound 3" (EC_{50} = 0.25 μM)

Preclinical Pharmacology. The molecule is potent against Pf 3D7 blood stages (3.0-6.0 nM) as well as clinical isolates from other regions (0.01-7 nM). Studies in more than 15 P. falciparum drug-resistant cell lines also showed strong activity (0.5-15 nM); notably, no drug-resistant mutants have been generated by NITD scientists in selection studies despite the use of multiple protocols with various P. falciparum blood stage cultures in vitro. In vitro studies based on kinase biochemical assays also revealed strong selectivity vs. human cells. Animal studies were conducted in a humanized severe combined immunodeficient mouse model harboring Pf 3D7 parasites in the erythrocytic asexual stages; the effect of the molecule on blood parasitemia was evaluated by flow cytometry and microscopic analysis. Oral administration of 4 doses at 30 mg/kg showed significantly reduced parasitemia levels (>99.9%) at day 5 in comparison with untreated control mice, while animals who received the treatment demonstrated complete clearance of the parasite by day 60 following treatment. Similar findings were made with single doses of the treatment at >15 mg/kg, suggesting that the molecule has the potential for single-dose cure of malaria.

Clinical Development. A Phase 1 placebo-controlled, single ascending and multiple oral dose study in health participants is currently underway (NCT04896632), with the goal to assess the safety, tolerability, and pharmacokinetics of the treatment. The study comprises two parts; Part A is a single ascending dose study in 4 planned cohorts containing 8 participants each and a food effect cohort with 10 participants, whereas Part B is a multiple dose study with 1 planned cohort containing 9 participants. In Part B, participants will be randomized to either a single dose of INE963 or placebo, or a 3-day dose of INE963 or placebo, with a dose taken every 24 hr. Safety, tolerability and pharmacokinetics assessment will be done over a 16-day period for participants who receive a single dose and over an 18-day period for those who receive multiple doses. The primary endpoint will be adverse events, serious adverse events, and ECG as assessed by the investigator. Secondary endpoints will include various pharmacokinetics parameters.

ulevostinag (MK-1454)

STING



intratumoral STING agonist

in Ph. II for met. HNSCC

cyclic dinucleotide derivative

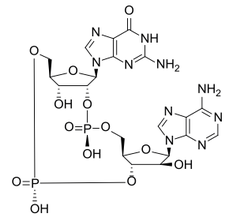
J. Med. Chem.

Merck, Kenilworth, NJ

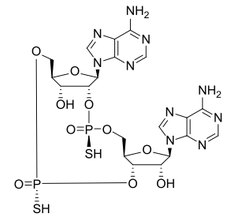
Context. MK-1454 (Merck) is an intratumoral stimulator of interferon (IFN) genes (STING) agonist that is being developed for treatment of advanced solid tumors and lymphomas. The molecule follows Aduro's ADU-S100, which was terminated after Ph. II due to a lack of substantial activity. Another chemotype of STING agonist, Eisai's E7766, has also been highlighted. Despite having significantly transformed the cancer treatment landscape, the effectiveness of immunotherapy is still challenged by the inadequate pre-existence of anti-tumor immunity ("cold tumors"). With increasing data suggesting that the therapeutic efficacy of immunotherapy depends largely on type I IFN signaling, interest increased in targeting targets such as STING that can induce strong innate immune responses in tumors and can be combined with immunotherapy to enhance tumor killing. MK-1454 is currently being combined clinically with pembrolizumab, a key PD-1 checkpoint immunotherapy marketed by Merck and approved for several cancer indications. The molecule was nominated by Christian Kuttruff.

Target. The 2′,3′-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS)-STING pathway plays a key role in sensing cytosolic cyclic dinucleotides and subsequently inducing expression of type I interferon. The signaling pathway is characterized by synthesis of cGAMP which binds to the endoplasmic reticulum-transmembrane adaptor protein STING, leading to a conformational change and translocation to the perinuclear compartment that ultimately results in transcription of innate immunity genes. MK-1454 is a cGAMP analogue and demonstrates a comparable binding mode and kinetic binding profile as endogenous cGAMP.

Binding Mode. A crystal structure of MK-1454 bound to STING (PDB ID 7MHC) shows it binds similarly to cGAMP. **cGAMP**:



ADU-S100:



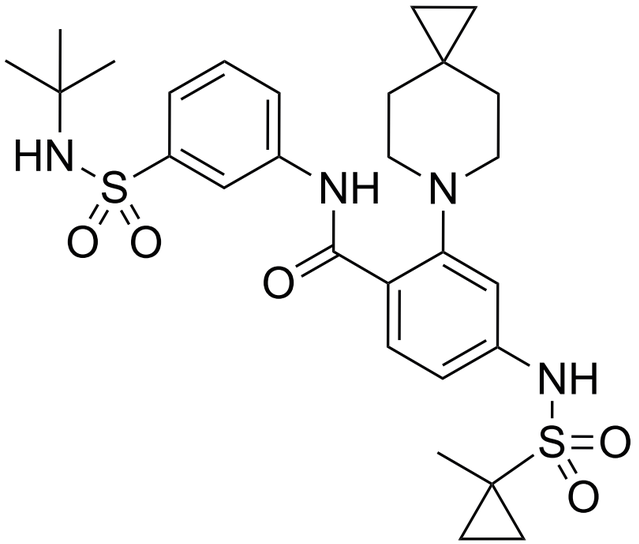
Synthesis. Merck simultaneously published an impressive biocatalytic synthesis in Nature and chemical synthesis of the molecule in JACS. Along with the biocatalytic synthesis of islatravir, this case study highlights the increasing utility of biocatalytic methods in drug discovery.

Preclinical Pharmacology. Preclinical pharmacology was comparable to that reported for previous STING agonists. In the MC38 syngeneic (immune competent) tumor model, animals were treated intratumorally with 50 µL of MK-1454, 2′,3′-cGAMP, or vehicle. Complete responses (CR) were seen in 9 of 10 mice treated with MK-1454, while only 2 or 3 CRs were observed with 2′,3′-cGAMP. The molecule was also shown to elicit tumor-specific immune responses in injected tumors as well as inhibit tumor growth at untreated, distal sites. Subsequent studies combined MK-1454 with an anti-programmed cell death protein 1 (PD-1) antibody and observed significant tumor growth inhibition vs. either MK-1454 or anti-PD-1 monotherapy.

Clinical Development. A Phase II study, NCT04220866, is underway and involves evaluation of pembrolizumab with or without MK-1454 as first-line therapy for metastatic or unresectable, recurrent head and neck squamous cell carcinoma. The primary endpoint is objective response rate (ORR); key secondary endpoints include progression-free survival (PFS), overall response (OS), duration of response (DOR). A Phase 1 study (NCT03010176) evaluating the safety and tolerability of MK-1454 alone or combined with the PD-1 inhibitor pembrolizumab in various advanced or metastatic solid tumors or lymphomas is currently underway, with preliminary data demonstrating an acceptable safety profile and promising efficacy of the combination treatment.

compound 24

KIF18A



oral kinesin motor protein inhibitor

relative AMG 650 in Ph. I for cancer

from ~600k compounds HTS + opt

J. Med. Chem.

Amgen, Thousand Oaks, CA

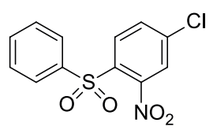
Context. "Compound 24" (Amgen) is a kinesin-like protein 18A (KIF18A) inhibitor being developed for a subset of cancers with chromosomally unstable tumor cells. Their first-in-class, oral clinical candidate, AMG 650, was recently disclosed at ACS Spring 2022 and is in Ph. I for cancer. Both antimitotic drugs targeting microtubules and those targeting essential mitotic kinases have been studied extensively, although the high toxicities associated with these agents and associated moderate efficacy have dampened interests in them over the years. Common events associated with MT inhibitors are dose-limiting neurotoxicities, myelosuppressive, and gastrointestinal effects (almost all approved MT inhibitors have caused one or more of these side-effects in at least 50% of treated patients). These limitations spurred researchers to search for alternative mechanisms of action and mitotic kinesins were identified as potentially viable drug targets, whose inhibition may have a greater therapeutic window by disrupting mitosis without more broadly impacting microtubules. The first lower-quality KIF18A chemical probe was BTB-1, an irreversible, ATP-competitive compound with a 1.7 μM IC_{50}, whose optimization failed to deliver improvements, prompting the search for better molecules.

Target. KIF18A is an ATP-dependent kinesin motor protein that binds tubulin and is involved in chromosome congression, regulating chromosome positioning and spindle length during cell division. KIF18A was first recognized as an microtubule depolymerase based on observations that KIF18A-depleted cell lines harbored aberrantly long mitotic spindles with disrupted chromosome organization. Additional work showed that KIF18A knockdown in cancer cell lines but not normal cell lines induced mitotic vulnerability and led to apoptosis. The protein is overexpressed in a subset of human cancers and has been shown to be required specifically by chromosomally unstable tumors for maintenance of bipolar spindle integrity and proliferation, potentially making it a PARP-like synthetic lethality opportunity to treat certain cancers.

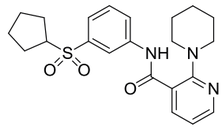
Mechanism of Action. Although a co-crystal structure of KIF18A with this inhibitor class is unavailable, structural predictions support experimental evidence for a binding site that locks the interaction between α-tubulin to KIF18A, preventing it from moving across microtubules, leading to mitotic delays and apoptosis.

Hit-Finding Strategy. The starting point, "compound 3," was discovered through a high-throughput screen of ~600K compounds from the Amgen library, and 800 of these were further analyzed for their effect on cell phenotype. Compounds were screened for inhibition of KIF18A MT-ATPase motor activity using a kinesis motor assay while selectivity against the essential mitotic kinesins Eg5 and CENP-E were assessed. A chromosomally unstable TP53-mutated TNBC cell line (MDA-MB-157) with demonstrated sensitivity to KIF18A knockdown by siRNA was used for the screening.

BTB-1
(IC_{50} = 1.7 μM)



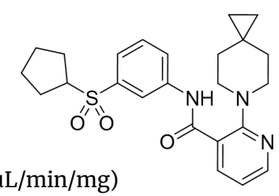
"compound 3"
(IC_{50} = 6.2 μM)



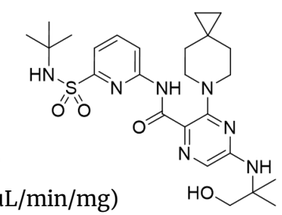
"compound 24"
(IC_{50} = 0.061 μM)

Lead Optimization. Initial substitution of the piperidine C4 with a spirocyclopropyl group showed >10-fold IC50 improvement with better lipophilicity (compound 6) but was rapidly metabolized. This was addressed by adding a tert-butyl amide plus substitutions at the pyridine ring (compound 22), significantly improving both metabolic stability and potency. Solubility was increased by adding a sulfonamide moiety as well as reformulating using a tween-based IP formulation.

"compound 6"
(IC_{50} = 0.41 μM, CL_{int} > 399 μL/min/mg)



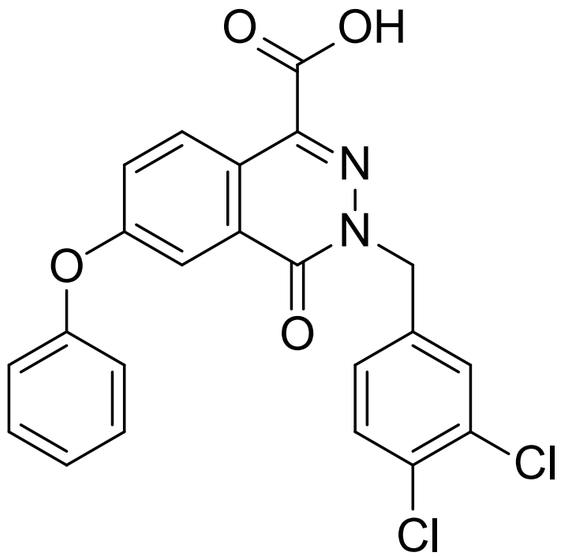
"compound 22"
(IC_{50} = 0.096 μM, CL_{int} = 64 μL/min/mg)



Preclinical Pharmacology. A tumor pharmacodynamic model of mitotic arrest was used to evaluate the late lead compounds, with accumulation of pH3 being used as biomarker and a pharmacodynamic readout of KIF18A inhibition. IP administration of compound 24 at 100 mg/kg in a mouse tumor model resulted in a significantly greater plasma concentration after 24 hrs compared to compound 22 (below the limit of detection) with comparable drug concentrations in tumors as the positive control (PLK-1 inhibitor) BI-2536 (~1 μM). Mice treated with the late lead compounds showed significant increases in pH3 positive cells vs. vehicle alone. Further compound optimization led to the development of "compound 23" and "compound 24", both of which demonstrated a significant and sustained increase in pH3 positive cells at a single dose of 100 mg/kg (intraperitoneal).

compound 10

PPARγ



oral PPARg phosphorylation inhibitor,
partial agonist
efficacy in diabetic mouse model
from structure-based virtual screen
ACS Med. Chem. Lett.
AstraZeneca, Gothenburg, SE

Context. "Compound 10" (AstraZeneca) is an oral CDK5-mediated PPARγ phosphorylation inhibitor and partial PPARγ agonist being developed as an antidiabetic drug. Although widely used PPARγ agonists such as rosiglitazone and pioglitazone are effective antidiabetics, they are limited by their side effects which have been attributed to PPARγ agonism. AstraZeneca researchers therefore sought compounds with only negligible PPARγ agonism but demonstrating potent binding and subsequent inhibition of CDK5-mediated Ser273 phosphorylation PPARγ which is thought to mediate the positive antidiabetic effects of PPARγ agonists. Preclinical data revealed that the molecule only has moderate plasma glucose and insulin lowering effects, making further development and improvement necessary if the compound is to have a future in antidiabetic therapy. The molecule was nominated by Dr. Callie Bryan.

Reviewer Comments. Callie says, "For the AZ compound 10, what I find interesting is that it circumvents the safety issues commonly seen with PPARg agonism while still being a potent binder and modulator. In flipping the script, they started from a structure-based virtual screen and developed small molecules capable of inhibiting the S273 phosphorylation step with oral bioavailability. This led to compound 10 advancing into an ob/ob diabetic mouse model (the cutest mouse model but that is an aside). While the conclusion was only modest improvement in insulin sensitivity, I agree with the authors that it does provide a starting point for further development."

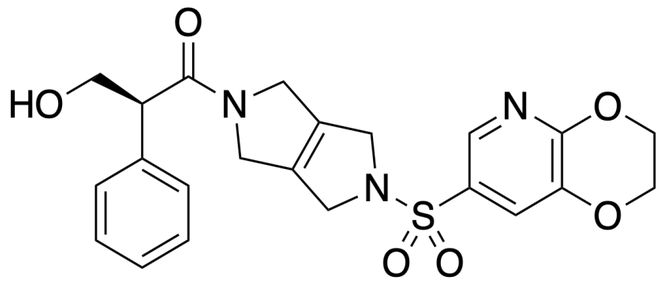
Hit-Finding Strategy. A structure-based virtual screening approach (FRED and Glide SP using AZ compound collection) based on a previously described protocol that identified potent PPARγ partial agonists was used. Compounds were selected based on their binding to a so-called AF-2 region which is regarded as a key part of the coactivator-binding site, with partial agonists expected to cause a diminished conformational stabilization of the region unlike full agonists that are expected to completely interact and stabilize the region. A total of 2943 compounds were identified, of which 201 (hit rate = 7%) were identified as active following testing for activity in both a human PPARγ time-resolved fluorescence energy transfer binding assay and a PPARγ reporter gene agonist assay. Further computational DMPK studies using in-house models led to identification of "compound 10" which bound strongly to PPARγ (24 nM) but exhibited little to no PPARγ agonism at concentrations <10 μM; the compound also exhibited significantly lower PPARγ agonist potency (EC_{50} > 50 μM).

Binding Mode. X-ray crystal structure of the PPARγ LBD in complex with compound 10 **(PDB:7QB1)**. The X-ray and docked PPARγ-compound 10 structures have nearly identical configurations.

Preclinical Pharmacology. ob/ob diabetic mice were used for in vivo animal studies. Two treatment groups were dosed at either 10 or 100 μmol/kg/day with either "compound 10" or a positive control (rosiglitazone); vehicle and lean controls were also included. Unlike mice dosed with rosiglitazone that showed normalized terminal fasting plasma glucose and insulin levels comparable to those of lean controls as expected, mice dosed with "compound 10" only demonstrated modest reductions in plasma glucose and insulin. The compound showed >100% oral bioavailability (109%) and moderate half-life (5 h) in mouse.

etavopivat

Pyruvate kinase



oral pyruvate kinase activator

in Ph. II/III for genetic anemias

200 or 400 mg QD, follows etavopivat

J. Pharmacol. Exp. Ther.

Forma Therapeutics, Watertown, MA

Context. Etavopivat (Forma Therapeutics) is an oral erythrocyte pyruvate kinase activator being developed for sickle cell disease and other hemoglobinopathies, previously reported in 2019. Hydroxyurea had been the only approved drug for sickle cell disease until the "sickle cell drug boon" began in 2017 starting with approval of l-glutamine and subsequent approvals of crizanlizumab-tmca and voxelotor in 2019. Therefore, approval of the etavopivat will provide additional treatment options for the rare blood disorder. Early data from clinical trials are promising and it's no surprise that the FDA has granted the drug Fast Track, Rare Pediatric Disease and Orphan Drug designations. Another pyruvate kinase activator, the first-in-class drug mitapivat, was approved in February for treatment of hemolytic anemia in adults with pyruvate kinase deficiency and was recently featured on our website; whether the indication for mitapivat will be expanded further for sickle cell disease remains to be seen.

Target. Erythrocyte pyruvate kinase is an isoform of the key glycolytic enzyme pyruvate kinase that is expressed in red blood cells and plays an important role in erythrocyte metabolism. Like its isoform, erythrocyte pyruvate kinase catalyzes the last and rate-limiting step of glycolysis: conversion of phosphoenol-pyruvate to pyruvate while generating ATP from ADP. It has been shown in studies that patients with pyruvate kinase deficiency have anemia resulting from decreased erythrocyte pyruvate kinase activity. Further, erythrocyte pyruvate kinase activation was shown to improve erythrocyte survival and increase hemoglobin levels.

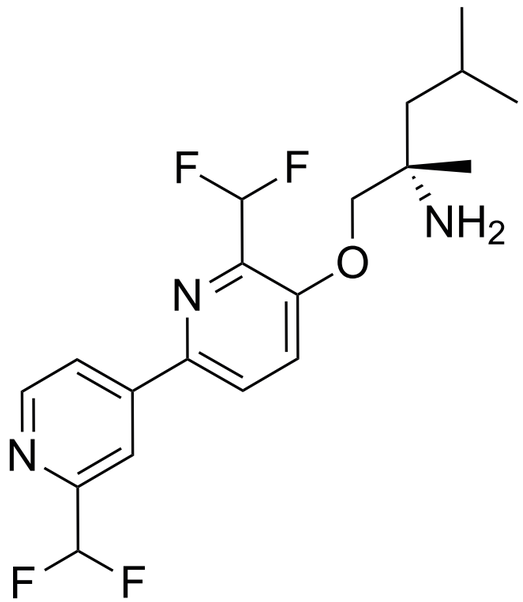
Mechanism of Action. Stimulation of erythrocyte pyruvate kinase activity is proposed to lower red blood cell sickling by decreasing production of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes which results in increased hemoglobin oxygen affinity, ultimately reducing hemoglobin polymerization and sickling. Additionally, activation of erythrocyte pyruvate kinase increases ATP produced via glycolytic flux, helping to preserve membrane integrity and RBC deformability. More details can be found in our discussion of mitapivat here.

Preclinical Pharmacology. The molecule's discovery story has not yet been disclosed. It has a PKR AC_{50} value of less than 1 μM (US10675274B2). Single (50 mg/kg) and multiple-dose (3, 8, or 22 mg/kg) experiments were conducted in male cynomolgus monkeys. The drug was found to significantly decrease 2,3-DPG levels for both animals treated with either dosing scheme. Significant increases in ATP levels were observed for monkeys that received multiple (5-day) doses at 8 and 22 mg/kg. Ex vivo experiments were conducted using whole blood from donors with sickle cell disease demonstrated an improved oxygen-carrying capacity of hemoglobin under conditions of decreased oxygen pressure. These findings corroborated those of a previous study in Berkeley SCA mice where reduced sickling and membrane damage was seen with etavopivat (FT-4202) treatment.

Clinical Development. A Phase 2/3 study (HIBISCUS; NCT04624659) is currently underway involving patients with sickle cell disease who will receive a double-blind dose of either 200 mg or 400 mg, double-blind placebo dose, or select open label dose of etavopivat, all taken once daily. The primary endpoints are hemoglobin response rate and annualized vaso-occlusive crisis. Another Phase 2 study (NCT04987489) is currently investigating the safety and efficacy of the drug in patients with thalassemia. A Phase 1 study (NCT03815695) previously demonstrated the safety and tolerability of etavopivat in healthy human participants and as well as activity of a single dose of the molecule in patients with sickle cell disease.

BMS-986176/LX-9211

AAK1



oral CNS-penetrant AAK1 kinase inhibitor

in Ph. II for neuropathic pain

from high-throughput screen + opt.

J. Med. Chem.

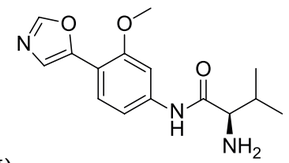
Bristol-Myers Squibb, Wallingford, CT

Context. BMS-986176/LX-9211 (BMS) is an oral, CNS-penetrable AAK1 kinase inhibitor being developed for treatment of neuropathic pain. There are six main classes of pain medications, NSAIDs, opioids, ion channel modulators, serotonergic, monoaminergic, and GABAnergic drugs that are broadly used for pain in general, but neuropathic pain due to nerve damage or dysfunction is much harder to treat. Less than half of therapies achieve >50% pain reduction, while adverse effects are commonly associated with these drugs. BMS-986176/LX-9211 is a highly CNS-penetrant molecule with the potential to address a significant unmet need for patients with neuropathic pain. The drug was granted an FDA Fast Track designation in 2020. It is a rare example of a brain-penetrant kinase inhibitor outside of cancer (others being for serious diseases like Parkinson's; LRRK2, DLK, RIPK1), and is especially interesting in that it is being explored for pain, an indication with a traditionally high safety bar. Another AAK inhibitor from BMS was previously highlighted.

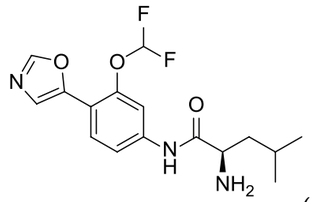
Target. AAK1 is an Ark1/Prk1 serine/threonine kinase family member abundantly expressed in the nervous system and involved in endocytosis regulation. Acute and persistent pain behavior assays using 3097 mouse knockout lines identified the adapter protein-2 associated kinase 1 (AAK1) protein as a novel therapeutic target for neuropathic pain. A null allele of AAK1 resulted in a normal response in acute pain but significantly reduced response to persistent pain, while mice lacking the gene failed to develop tactile allodynia following spinal nerve ligation.

Mechanism of Action. AAK1 is involved in the phosphorylation of the μ2 subunit of the associated protein-2 (AP2) complex which mediates binding of μ2 to sorting motifs on cargo receptors that contain tyrosine residues. Ultimately, AAKI-mediate phosphorylation of μ2 is associated with enhancement of internalization efficiency of the protein. The activity of AAKI inhibitors is thought to be mechanistically linked (behaviorally and electrophysiologically) to α2 adrenergic signaling pathway which is known to be antinociceptive in humans.

Hit-Finding Strategy. The starting point, "compound 5" was identified through a previous high-throughput screening campaign to identify AAK1 inhibitors. Target compounds were tested using enzymatic, radioactive displacement as well as a AAK1 cellular assays.



"compound 5"
(AAK1 IC_{50} = 133 nM)



"compound 6"
(AAK1 IC_{50} = 12 nM)

BMS-986176
(AAK1 IC_{50} = 2.0 nM, cell IC_{50} = 2.0 nM)

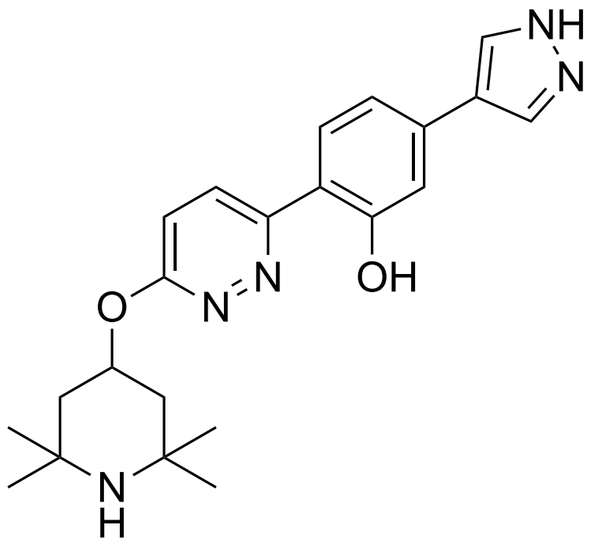
Lead Optimization. Minor modifications from compound 5 on alkyl substituents led to a >10x increase in potency and an in vivo tool compound (compound 6). Some CV issues in rat telemetry and rabbit EP studies were observed, and an ex vivo aortic ring assay was used to filter molecules. Several heterocycle head groups were tested to replace the oxazole but pyridine was uniquely active. Related pyridines showed liver and CV tox. despite otherwise favorable properties, leading the to selection of BMS-986176.

Preclinical Pharmacology. In vivo studies with "compound 34" (LX-9211) was done in the CCI-induced model in rats. Treatment with the compound at a dose of 1 mg/kg significantly reduced thermal hyperalgesia with 97% reversal of the pain response achieved. Additional studies in a streptozotocin (STZ)-induced diabetic peripheral neuropathic pain rat model was done, with mechanical allodynia as the endpoint; treatment with the compound at 1 mg/kg reduced mechanical allodynia with >60% inhibition of pain response achieved. Evaluation of rats treated with BMS-986176/LX-9211 in the accelerating rotarod assay showed that the compound does not impair rotarod performance. The compound was also highly CNS-penetrant, leading BMS researchers to speculate on the existence of active transporter-assisted influx into the CNS, although this was not investigated further.

Clinical Development. Phase I studies in healthy human participants demonstrated that the drug was safe and well tolerated. Two Phase II studies are currently underway: the RELIEF-DPN 1 trial (NCT04455633) in patients with diabetic peripheral neuropathic pain and the RELIEF-PHN1 (NCT04662281) trial involving patients with postherpetic neuralgia. In both trials the primary endpoint is the change from baseline in Average Daily Pain Score (ADPS). The Ph. II studies use an initial loading dose of 100 mg or 200 mg PO, followed by once daily doses of 10 mg or 20 mg.

branaplam

SMN2



oral, CNS-penetrant splicing modulator w/ HTT activity

in Ph. II for Huntington's (prev. SMA)

further characterization of SMA candidate

Nat. Commun.

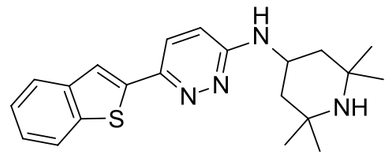
Novartis, Cambridge, MA

Context. Branaplam (Novartis Institutes for Biomedical Research) is an oral brain-penetrant splicing modulator, originally developed as a small molecule splicing modulator for spinal muscular atrophy (SMA). It turns out to also lower huntingtin levels and is being developed (with Fast Track Designation) for patients with Huntington's Disease which is driven by mutant huntingtin prion. It is expected that treatments that reduce levels of the toxic mutant huntingtin protein (mHTT) in the brain will slow progression of the disease. Current treatments only target symptoms of the disease, while the more effective therapeutic modalities such as antisense oligonucleotides and RNAis which focus on lowering mHTT levels require delivery techniques that are not ideal in clinical settings. These challenges make an agent such as branaplam that can be administered orally and has shown strong preclinical brain penetrating and mHTT lowering properties the potential holy grail the field has been waiting for. Another HTT lowering small molecule, HTT-D3, which also modulates splicing and was featured in our December 2021 Molecules of the Month, is similarly currently in clinical trials. One open question that remains regarding these HTT lowering agents that may be addressed in the clinical trials is the risk-benefit profile that comes with reducing either variant HTT or both variant and wild-type HTT long term, though the tolerability of long-term partial lowering of normal HTT appears to have been demonstrated in SMA children treated with branaplam.

Mechanism of Action. Branaplam promotes pseudoexon inclusion in the primary transcript of mHTT, lowering mHTT protein levels in HD patient cells and in SMA patient blood samples in SMA trial patients (NCT02268552). Development of branaplam for SMA was based on its modulation of splicing of the survival motor neuron 2 (SMN2) gene which is typically poorly spliced in SMA, restoring the SMN2 protein to normal levels. Specifically, the drug stabilizes the transient interaction between pre-mRNA of SMN2 and the U1 snRNP complex which boosts SMN2 exon7 inclusion. This class of splicing modulators is known to affect splicing of other genes more broadly (and initially was a selectivity/safety concern). The identification of HTT splicing modulation as an "off target" mechanism turns out to be useful.

Hit-Finding. Branaplam (also named NVS-SM1 and LMI070) was first reported in 2014 in patents (WO2014028459) and then in a 2015 paper. An initial hit, compound 2, was identified by a high-throughput phenotypic screen of 1.4M compounds employing a NSC34 motor neuron cell line expressing an SMN2 minigene reporter.

compound 2. (SMN EC_{50} = 0.6 uM, cLogD = 2.7):

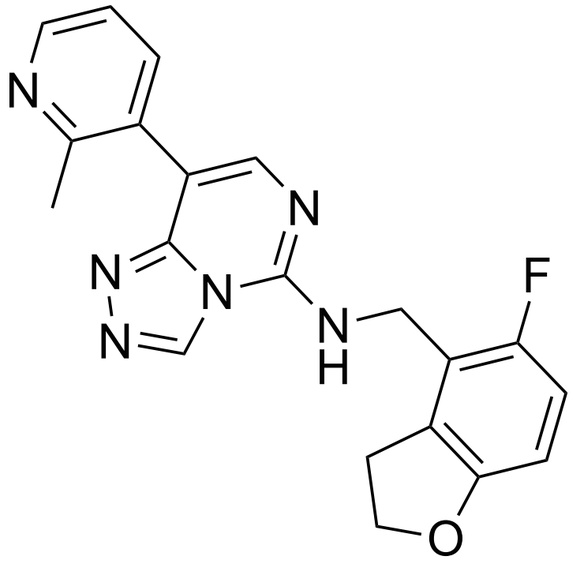


Lead Optimization. The main disadvantages of compound 2 observed were the high in vivo plasma clearance (113 mL/min/kg), modest bioavailability (F % = 18), low brain exposure, and potent hERG inhibitory activity. During optimization, the introduction of an ortho-hydroxy group to the pyridazine core significantly increased the potency, though this also introduced tautomers. Branaplan was discovered at the end of SAR studies.

Clinical Development. A Phase II randomized, double-blind, placebo-controlled study (VIBRANT-HD; NCT05111249) evaluating safety and efficacy of the drug in early manifest Huntington's Disease is currently ongoing. Patients will receive a 28, 56, 84, or 154 mg oral solution of the drug or matching placebo once weekly. The primary endpoints are the % reduction in mHTT in the CSF and the number of treatment emergent adverse events and serious adverse events. The drug continues to be developed for spinal muscular atrophy (SMA) based on its originally intended mechanism of action and is currently being tested in infants with SMA in Phase I/II trial. Its development for SMA was paused in 2016 due to signs of nerve injury, and resumed in 2017 with different doses.

MAK683

PRC2



oral, allosteric PRC2 inhibitor (EED)

in Ph. I/II for cancer (DLBCL); preliminary efficacy

from micromolar HTS hit

J. Med. Chem.

Novartis, Emeryville, CA

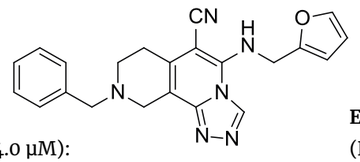
Context. MAK683 is an oral, allosteric and selective PRC2 inhibitor intended for PRC2-dependent cancers. PRC2 is an essential epigenetic regulator of gene expression and enhanced or diminished PRC2 function have been implicated in a number of cancers. Enhancer of zeste homolog 2 (EZH2), one of the four core components of PRC2 that directly trimethylates K27 of histone 3 (H3K27me3), has been the most extensively studied PRC2 subunit and a number of EZH2 mutations and post-translational modifications have reportedly been associated with disease progression. In addition, the approval of the first EZH2 inhibitor, Epizyme's tazemetostat, for advanced/metastatic epithelioid sarcoma and relapsed/refractory follicular lymphoma further validated EZH2 and other PRC2 components as viable drug targets. Resistance mechanisms to EZH2 inhibitors have been observed in cell lines, and the development of other PRC2 inhibitors are underway. Of the five in-development compounds targeting EED, MAK683 is the most clinically advanced. Preliminary clinical data suggest that the drug is tolerable and has signs of efficacy.

Target. The Polycomb Repressive Complex 2 (PRC2) is a polycomb group protein that modulates chromatin structure through repression of its target genes. PRC2 catalyzes the methylation of histone H3 lysine 27 (H3K27) to generate mono-, di-, or trimethylated forms of H3K27; trimethylated H3K27 (H3K27me3) subsequently mediates the suppression of polycomb target genes. PRC2 comprises a catalytic, SET-domain-containing histone methyltransferase (EZH1 or EZH2) as well as two core proteins required for full histone methyltransferase activity: embryonic ectoderm development protein (EED) and suppressor of zeste 12 (SUZ12). EED bridges H3K27me3 binding with EZH2 stimulation and is essential for basal PRC2 activity. It is one of the four core components, together with EZH1/2, SUZ12, and RBBP4/7, that make up the minimal core PRC2 particle. Allosteric activation of PRC2 is initiated by binding of the protein to H3K27me3 through an aromatic cage in EED. The role of PRC2 and polycomb protein deregulation in cancer progression has been extensively studied.

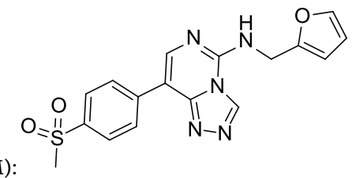
Mechanism of Action. Although other PRC2 inhibitors, including the recently approved tazemetostat, target EZH1 or EZH2 or both, NIBR researchers sought to target the H3K27me3 pocket in EED to inhibit PRC2's methyltransferase activity. This allosteric mechanism of PRC2 inhibition via EED is unlike competitive inhibition of the S-adenosylmethionine (SAM)-binding site of EZH2.

Starting Point. The starting point, "compound 7" (IC50 of 4.0 uM) was identified from a high-throughput screen of 1.4M compounds in the Novartis library and had potency in the single digit micromolar range. Guided by X-ray crystallography, compound EED226 (IC_{50} of 0.022 uM) was discovered by fragmentation and regrowth of compound 7. The key interactions in the binding of compound 7 include two hydrogen bonds and a set of π-π interactions. EED226 was previously shown to induce potent and sustained tumor regression in animal studies, but had preclinical toxicology findings in a 2-week rat study (dose-dependent cortical adrenal gland degeneration and inflammation) likely due to the reactive furan group. CYP-profiling suggested that CYP11A1 bioactivates the furan in adrenal mitochrondria, a mechanism which has previously been implicated in adrenal toxicity.

"compound 7"
(Biochem. IC50 = 4.0 µM):



EED226
(Biochem. IC_{50} = 0.022 µM, Cellular IC_{50} = 0.22 µM):



Lead Optimization. Replacement of the furan in EED226 was key but electron-rich systems were found to be preferred due to a cation-pi interaction with R367 at the bottom of the EED pocket. The furan moiety was ultimately replaced with a dihydrobenzofuran, mitigating adrenal toxicity, and introduction of a bulky surface-facing methylpyridine reduced CYP2D6 inhibition by >350-fold. Further optimization was made through the addition of a fluorine in dihydrobenzofuran, increasing selectivity against the off-targets PDE4D, α2C adrenergic receptor, and α2A adrenergic receptor.

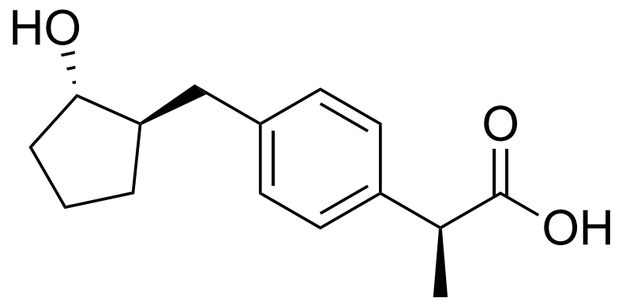
Binding Mode. X-ray co-structure of EED with MAK683 is available, PDB: 7QK4.

Preclinical Pharmacology. MAK683 is significantly more potent than EED226 (cell IC_{50} = 3 nM vs. 220 nM, GI_{50} = 8 nM vs. 80 nM). MAK683 was well tolerated in a repeat dose 2-week rat toxicology study at plasma exposure levels associated with complete tumor regression in Karpas 422 xenograft-bearing nude mice and rats and no evidence of rat adrenal gland toxicity was observed at these exposure levels. Global HK27me3 levels in tumor and additional tissues was the main pharmacodynamic marker. Complete tumor regression was seen at most of the different doses tested (0.3-1 mg/kg), while animals that showed complete regression remained tumor-free for >67 days. Mouse, rat, and dog at F % at 2 mg/kg was comparably high (77, 69, and 73% respectively) with higher clearance in mouse (38 mL/min/kg) compared to rat and dog (10 and 13 mL/min/kg).

Clinical Development. The drug is currently being evaluated in a Phase I/II (NCT02900651) study involving patients with various advanced malignancies; the primary endpoints are incidence of dose-limiting toxicities (DLTs) as well as safety and tolerability. Preliminary data from the study suggests that the drug has acceptable safety profile and promising efficacy.

HR1405-01

COX



intravenous NSAID

faster T_{max} and lower dose vs. loxoprofen in Ph. I

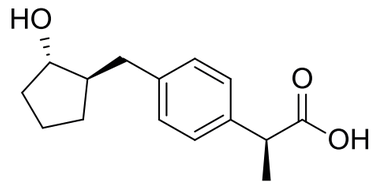
from loxoprofen metabolite

Eur. J. Med. Chem.

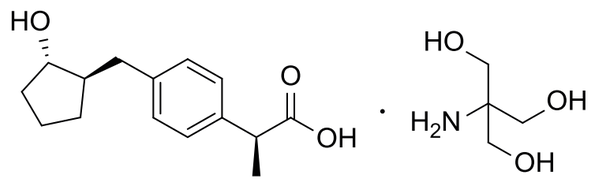
Nanjing Heron Pharmaceutical Co., Nanjing, CN

Context. HR1405-01 (Nanjing Heron Pharmaceutical) is an intravenous NSAID with inflammatory and analgesic activities. Oral NSAIDs (ibuprofen, naproxen..) are widely used, and it is interesting to see justification for a new intravenous NSAID. Intravenous ibuprofen was the first intravenous NSAID to be approved, following which a couple of other agents have also been approved for fever treatment. Intravenous NSAIDs can be desirable due to the potential to combine them with opioids to reduce risk of dependence. However, poor solubility and side effects associated with these drugs have limited their clinical use. Nominated by Bryan McKibben, HR1405-01 is an active metabolite of loxoprofen, an oral phenylpropionate NSAID approved in Brazil, Mexico, and Japan for the treatment of inflammatory diseases and postoperative analgesia. Preclinical data point to a potent molecule with high solubility and a desirable safety profile and it will be interesting to see how the drug does in clinical development.

Identification. The starting point, "compound 6" (loxoprofenol-SRS) was identified from an analysis of the metabolic profile of loxoprofen. Compound 6 has previously been reported to be selective for COX-2 (IC50 for COX-1: 1.47 mM; IC50 for COX-2: 0.026 mM). It is another interesting example of an active metabolite making a good drug starting point (another recent example we covered was remogliflozin).



compound 6
(loxoprofenol-SRS):



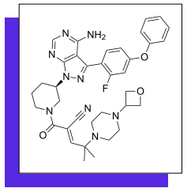
HR1405-01
(tromethamine salt):

Preclinical Pharmacology. Inflammation and analgesic assays showed strong analgesic activity for HR1405-01 and more modest anti-inflammatory activity. Pharmacokinetics studies demonstrated that the compound exhibited strong stereochemical stability in the plasma of animals tested. Plasma protein binding experiments with plasma for mice, Sprague-Dawley rats, Beagle dogs, Cynomolgus monkeys, and humans showed that humans had the strongest plasma protein binding (>95%). A vascular stimulation test was conducted in rabbits showing no inflammatory cell infiltration or necrosis of endothelial cells after treatment, suggesting good local tolerance for IV dosing. HR1405-01 is more potent and soluble (176 mg/mL) than ibuprofen injection or parecoxib injection, giving it a significantly better dose/solubility ratio than clinical IV NSAIDs. No co-solvent was needed for formulation due to the high solubility of HR-1405-01, which further reduces risks of side effects.

Clinical Pharmacology. The authors state that **HR1405-1** is in a Ph. I clinical trial and that initial data shows it has a faster peak T_{max} (30 min IV and 60 min oral) than loxoprofen.

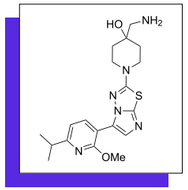
Small Molecules of the Month

March 2022
drughunter.com



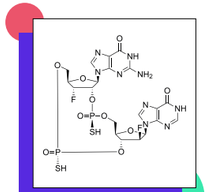
rilzabrutinib (PRN1008) | BTK

oral reversible covalent BTK inhibitor
in Ph. III for immune thrombocytopenia
from structure-based drug design + opt.
J. Med. Chem.
Principia Biopharma (Sanofi), So. San Francisco, CA



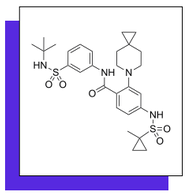
INE963 | Pf 3D7

oral blood stage antimalarial
in Ph. I (healthy volunteers)
from 1.5M cmpd phenotypic screen + opt.
J. Med. Chem.
Novartis (NIBR), Emeryville, CA



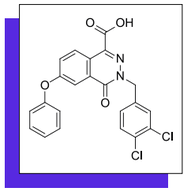
ulevostinag (MK-1454) | STING

intratumoral STING agonist
in Ph. II for met. HNSCC
cyclic dinucleotide derivative
J. Med. Chem.
Merck, Kenilworth, NJ



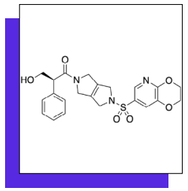
compound 24 | KIF18A

oral kinesin motor protein inhibitor
relative AMG 650 in Ph. I for cancer
from ~600k compounds HTS + opt
J. Med. Chem.
Amgen, Thousand Oaks, CA



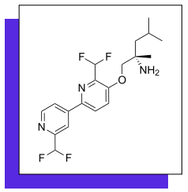
compound 10 | PPARγ

oral PPARg phosphorylation inhibitor, partial agonist
efficacy in diabetic mouse model
from structure-based virtual screen
ACS Med. Chem. Lett.
AstraZeneca, Gothenburg, SE



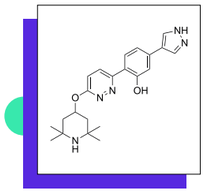
etavopivat | PKR

oral pyruvate kinase activator
in Ph. II/III for genetic anemias
200 or 400 mg QD, follows etavopivat
J Pharmacol Exp Ther.
Forma Therapeutics, Watertown, MA



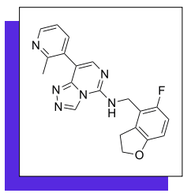
BMS-986176/LX-9211 | AAK1

oral CNS-penetrant AAK1 kinase inhibitor
in Ph. II for neuropathic pain
from high-throughput screen + opt.
J. Med. Chem.
Bristol-Myers Squibb, Wallingford, CT



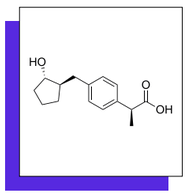
branaplam | SMN2

oral, CNS-penetrant splicing modulator w/ HTT activity
in Ph. II for Huntington's (prev. SMA)
further characterization of SMA candidate
Nat. Commun.
Novartis, Cambridge, MA



MAK683 | PRC2 (EED subunit)

oral, allosteric PRC2 inhibitor (EED)
in Ph. I/II for cancer (DLBCL); preliminary efficacy
from micromolar HTS hit
J. Med. Chem.
Novartis, Emeryville, CA



HR1405-01 | COX

intravenous NSAID
faster T_{max} and lower dose vs. loxoprofen in Ph. I
from loxoprofen metabolite
Eur. J. Med. Chem.
Nanjing Heron Pharmaceutical Co., Nanjing, CN

drug hunter

HIGHLIGHTS FROM DRUG DISCOVERY ARTICLES PUBLISHED ONLINE | MAR. 2022

discover together

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