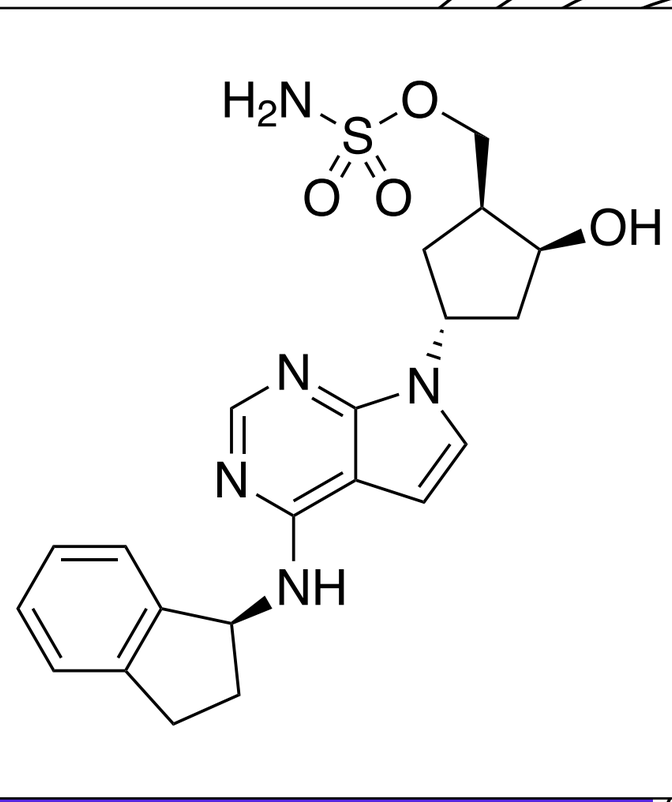
Small Molecules of the Month

May 2022

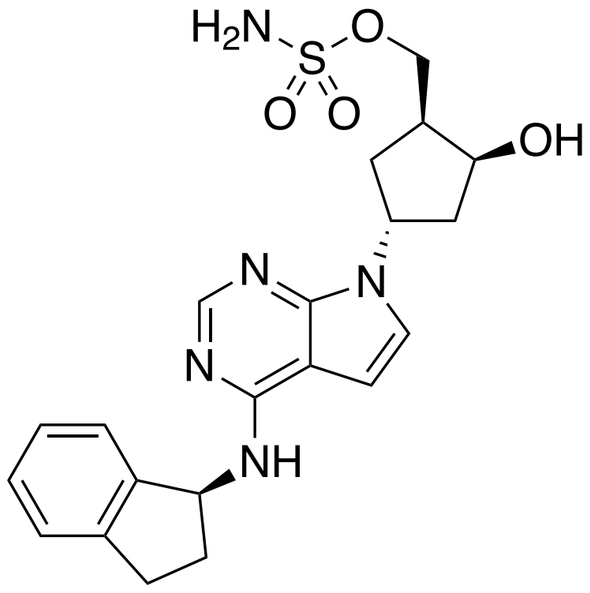


drug hunter

01	NEDD8-activating enzyme	Takeda
02	α5-GABA$_A$	Gedeon Richter
03	PI3Kα,β,δ	Novartis
04	FtsZ	TAXIS Pharmaceuticals
05	RXFP3	RTI
06	11β-HSD1	Incyte
07	p38α/β MAPK	Chiesi Farmaceutici
08	MAGL	Roche
09	Mycobact. ATP-Synthase	WuXi AppTec
10	Glucokinase	Hua Medicine

Pevonedistat

NEDD8-activating enzyme (NAE)



intravenous NEDD8-activating enzyme inhibitor

Ph. III candidate for oncology

from HTS and optimization

Drug Metab. Dispos.

Takeda, Lexington, MA

paper DOI: https://doi.org/10.1124/dmd.122.000842

drug hunter

Context. Pevonedistat (Takeda) is a first-in-class intravenous or subcutaneous NEDD8-activating enzyme (NAE) inhibitor being developed for leukemia and advanced solid tumors. NAE is a pivotal regulator of the NEDD8 conjugation pathway which regulates the activity of the Cullin-RING ligases (CRLs), key proteins involved in the ubiquitin-proteasome system (UPS), controlling important physiological processes such as cell-cycle and signal transduction. Neddylation of CRLs is an attractive target pathway as CRLs are often abnormally activated in cancers. Pevonedistat, which was first reported in a 2009 publication, is the most advanced NAE inhibitor in clinical development. The only other NAE inhibitor to ever enter clinical development was Otsuka's TAS4464, which was put on hold after a Ph. I trial was terminated following observation of cases of drug-induced liver injury that met the criteria for Hy's Law. Pevonedistat in combination with standard chemotherapy treatment was granted FDA Breakthrough Therapy Designation in 2020 for treatment of patients with higher-risk myelodysplastic syndromes (HR-MDS), further highlighting the potential for the first-in-class drug in oncology. The pivotal Ph. III PANTHER trial evaluating pevonedistat in combination with standard chemotherapy treatment azacitidine (Vidaza) failed to meet its primary event-free survival endpoint, and pevonedistat no longer appears in Takeda Oncology's pipeline. Despite disappointing results, the molecule had a fascinating mechanism, represented an important safety proof-of-concept, and now provides valuable information for future efforts in this space. The latest article shares valuable human metabolism and distribution data on the molecule, using radiolabeled drug - surprisingly, metabolism primarily takes place via CYP-mediated hydroxylation of the indane.

Target. In the UPS, ubiquitin-activating E1 enzymes, ubiquitin-conjugation E2 enzymes, and ubiquitin E3 ligases mediate the polyubiquitination of target intracellular proteins, resulting in their degradation. CRLs are the largest class of ubiquitin E3 ligases. Proteins degraded by CRLs are involved in important physiological functions such as cell-cycle progression, DNA damage, stress responses, and signal transduction. To activate a CRL, binding of ubiquitin-like proteins called NEDD8 is required through a neddylation process which requires an E1 NAE. Due to the genetically validated involvement of CRLs in key biological processes, pharmacological validation thanks to pevonedistat (MLN4924) and accumulating evidence revealing the overexpression of NEDD8 in some human diseases such as cancers, targeting neddylation continues to be explored for cancer drug discovery.

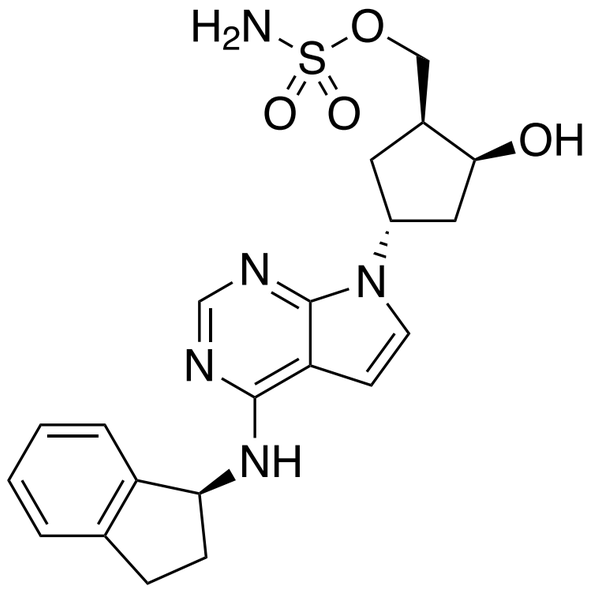
Mechanism of Action. Interestingly, pevonedistat acts by binding covalently to NEDD8, the substrate of NAE, in the presence of enzymatically active NAE to form a pevonedistat-NEDD8 adduct which binds to NAE and precludes the E1 conjugation of NEDD8 to CRL. Overall, this leads to the accumulation of CRL-dependent substrates and subsequent induction of a host of cell death pathways in tumor cells due to impaired activity of the ligase.

Hit-Finding Strategy. The discovery of pevonedistat began with *N6*-benzyl adenosine, which was identified as an NAE inhibitor via a high throughput screening effort.

Lead Optimization. An iterative medicinal chemistry effort from *N6*-benzyl adenosine ultimately identified pevonedistat as a potent inhibitor of NAE (IC_{50} of 4 nM). These efforts produced a structure related to adenosine 5'-monophosphate (AMP), with several key differences: the ribose moiety was replaced with a carbocycle, devoid of the 2'-hydroxyl and had flipped stereochemistry at C4'; the adenine base was replaced with a deazapurine equipped with an aminoindane at N6; and the phosphate was replaced with a sulphamate.

Pevonedistat

NEDD8-activating enzyme (NAE)



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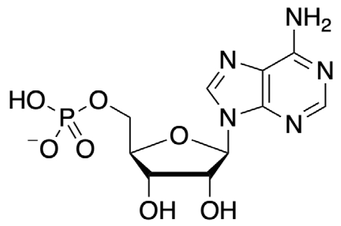
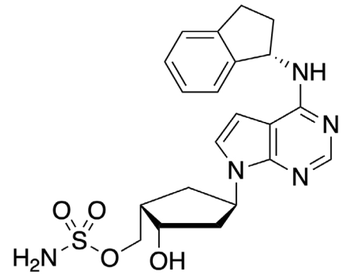


AMP



pevonedistat

Binding mode: Brownell and colleagues from Millennium Pharmaceuticals identified the binding mode through x-ray crystallographic studies (**PDB: 3GZN**), which involves a covalent bond via N-acylation of the sulfamate.

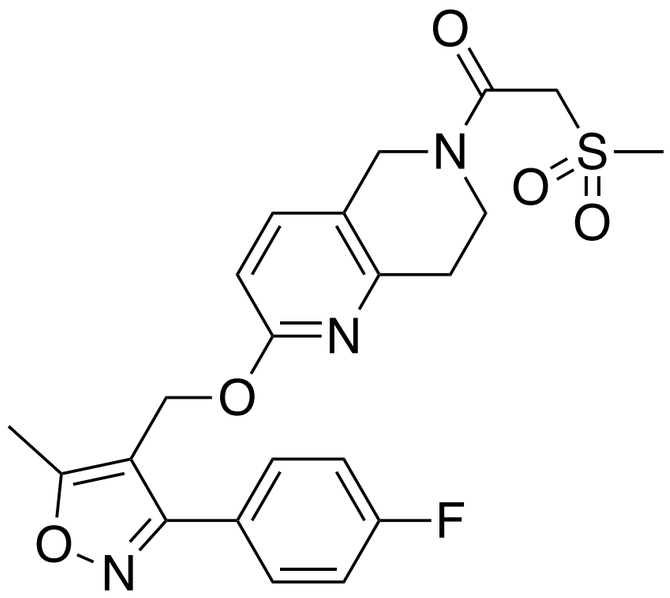
Preclinical Pharmacology. In vivo inhibition of NAE by pevonedistat was assessed by analyzing NEDD8–cullin, NRF2 and CDT1 protein levels in HCT-116 tumor-bearing mice following subcutaneous dosing of the drug at 60 mg/kg. A dose- and time-dependent decrease of NEDD8–cullin levels, and a dose- and time-dependent accumulation of the steady-state levels of NRF2 and CDT1 were observed. In mice bearing HCT-116 tumor xenografts, subcutaneous pevonedistat dosed at 30 and 60 mg/kg QD or BID led to significant dose-dependent inhibition of tumor growth, with comparable efficacy being observed for the 30 mg/kg BID and 60 mg/kg QD doses. Similar observations were made in H522 lung tumor and Calu-6 lung carcinoma xenograft mice. In a subsequent study, the anti-tumor effects of pevonedistat were evaluated in HL-60 human leukemia xenograft mice; a dose-dependent decrease in tumor burden was seen and all animals dosed at 60 and 90 mg/kg had stable regressions.

Clinical Development. Pevonedistat completed several phase 1 and 2 studies and now was being evaluated in two phase 3 clinical trials. The PEVOLAM Ph. 3 trial (NCT04090736) is an open-label clinical trial to compare pevonedistat in combination with azacytidine versus azacytidine alone in patients with acute myeloid leukemia not eligible for intensive chemotherapy. The PANTHER Ph. 3 trial (NCT03268954) aims to determine whether the combination of pevonedistat and azacitidine improves the survival of patients with AML, HR-MDS, and CMML when compared to azacitidine alone. In both studies, pevonedistat is being dosed at 20 mg/m2 intravenous on days 1, 3, and 5 in a 28-day cycle regimen plus azacitidine. A Takeda 2021 update stated the PANTHER trial failed to achieve statistical significance at its primary endpoint of event-free survival. In the intervention arm, the median survival was 17.7 months versus 15.7 months in the azacitidine alone arm. The median overall survival was 20.3 versus 16.8 months, whereas the response rates were 28% and 32% respectively. More recently it also failed to show an improvement in complete responses relative to azacytidine, which had a higher CR rate (32%) relative to historical data likely due to the longer duration of treatment used in this study. 19 active trials with pevonedistat remain sponsored by Takeda, NCI, and MD Anderson, though the molecule is no longer listed in Takeda Oncology's pipeline.

Patents. Takeda's first two patents describing the invention of pevonedistat and other E1 activating enzyme inhibitors to treat cell proliferation disorders, cancers (as a single agent or in combination with chemotherapy), inflammatory, neurodegenerative disorders; and inflammation associated with infection and cachexia were granted in 2011 and 2015 (US7951810B2/US8980850B2).

Compound 20

α5-GABA$_A$



oral α5-GABAA receptor NAM

oral efficacy in CIAS rodent models

scaffold hopping from literature starting point

J. Med. Chem.

Gedeon Richter, Budapest, HU

paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00414

drug hunter

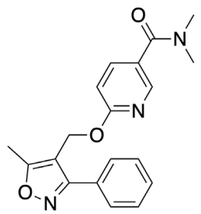
Context. "Compound 20" (Gedeon Richter) is an oral α5-GABA$_A$ receptor negative allosteric modulator (NAM) being developed for cognitive disorders. GABA receptor NAMs are known to exert effects opposite to the anxiolytic and anticonvulsant effects of positive allosteric modulators. However, NAM agents targeting α5-GABA$_A$ receptors, which have been implicated in learning and memory, have been heavily pursued as a potential treatment for cognitive impairment. Despite promising preclinical efficacy and safety data, NAMs have continually failed in clinical development due to poor efficacy and/or tolerability. Notably, early NAM agents developed in the mid-to-late 2000s, such as Merck's L-655,708 and α5IA (and its follow-up compound MRK-016), which was the first NAM to enter clinical development, have all failed in Ph. I/II studies. Further, the development of Roche's basmisanil (RG1662; RO5186582) also appears to be halted after a key trial in Down's syndrome did not meet its primary efficacy endpoint leading to the termination of another planned study. While compound 20 is not yet in development, and the clinical path for the drug class has so far proven challenging, Gideon Richter shares interesting preclinical data on the molecule (e.g. in vitro hERG signal but guinea pig and dog CV NOAEL >70 mg/kg). The molecule is also an interesting case study for designing around a toxic metabolite issue from a literature lead while generating unique IP. The molecule was identified by reviewer Bryan McKibben.

Target. α5-GABA$_A$ receptors, a subset of the ligand-gated GABA$_A$ receptors, which regulate inhibitory neurotransmission in the brain, are mostly expressed in the hippocampus and cortex of the mammalian brain. α5-GABA$_A$-mediated hippocampal hyperactivity is observed in the aging brain and in patients with schizophrenia and neuropsychiatric disorders with cognitive deficits. Further, antagonization of α5-GABA$_A$ receptor activity either through gene deletion or the use of selective inhibitors in animal models has been associated with enhanced gamma frequency oscillations in the hippocampus. Therefore, these receptors have been heavily explored as ideal targets for cognitive disorders.

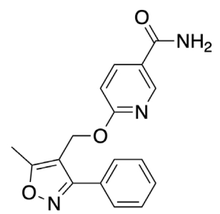
Mechanism of Action. GABA$_A$ receptor modulators bind to different locations on the receptor to elicit different effects. Ligands and agents that activate the receptor typically exert anxiolytic, anticonvulsant, sedative, and muscle relaxation properties, while those that inhibit receptor function exert opposite effects such as convulsion and anxiogenesis. Allosteric modulators of the receptor bind to the benzodiazepine (BZD) binding site. In an electrophysiology study, the BZD site in α5-GABA$_A$ receptors was observed to be more distinct from that of other GABA$_A$ receptor subtypes which allows for selective targeting of receptors bearing the α5-subunit.

Hit-Finding Strategy. A series of fused [6 + 6] bicyclic moieties were designed and synthesized based on an initial candidate disclosed by Roche, that had metabolic liabilities stemming from a dimethylamide.

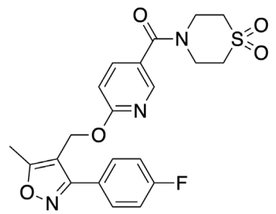
Lead Optimization. During the clinical trials of the Roche candidate starting point, safety problems were observed due to the formation of a toxic amide metabolite. Alterations to the basic scaffold by Roche generated the Phase 2 clinical candidate basmisanil. Gideon Richter was able to generate novel IP while still circumventing toxic metabolite formation by a strategy of ring opening/ring closure and relocation of the carbonyl group. Subsequent modulation of the physicochemical properties resulted in novel 1,6-naphthyridines, including compound 20, with improved profiles, combining good potency, selectivity, ADME, and safety properties.



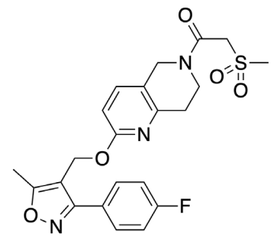
Roche Candidate



Toxic Metabolite



Basmisanil



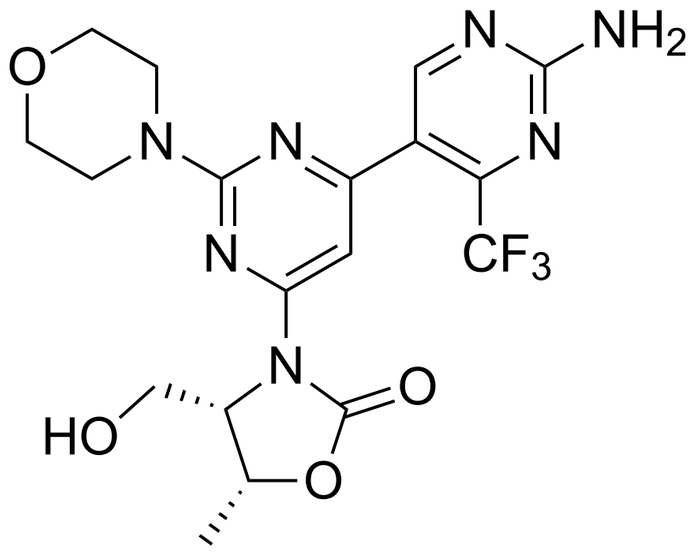
compound 20

Preclinical Pharmacology. In vivo efficacy of the compound was evaluated using a novel object recognition assay involving rodents induced with subchronic doses of phencyclidine (PCP) to exhibit schizophrenic symptoms and cognitive impairment. Subchronic administration of PCP resulted in increased expression of α5-GABA$_A$ receptors and a subsequent increase in the activity of GABAergic neurons. Rats were dosed with compound 20 at 1, 3, and 10 mg/kg; a dose-dependent reversal of PCP-induced cognitive deficit being observed, with significant reversal being seen with the 10 mg/kg dose.

Patent. In 2019, Gedeon Richter filed a patent US20210386718A1 describing the invention of α5-GABA$_A$ negative allosteric modulators to treat disorders related to α5-GABA$_A$, including stroke, cognitive impairment, schizophrenia, dementia-related conditions, or diseases related to impaired social cognition.

NVP-CLR457

pan-class IA PI3K



oral pan-class IA PI3K inhibitor

Ph. I in advanced solid tumors

opt. from previous clinical candidate

J. Med. Chem.

Novartis (NIBR), Basel, CH

paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00267

drug hunter

Context. NVP-CLR457 (Novartis Institutes for BioMedical Research) is an oral, non-CNS-penetrating pan-class IA phosphoinositide 3-kinase (PI3K) inhibitor. Due to the key involvement of PI3K in the famed PI3K-Akt-mTOR pathway, whose deregulation has been observed in several cancers, it is no surprise that the last decade has seen over 600 medicinal chemistry-based publications and patents focusing on inhibitors targeting at least one of the 4 class I PI3K isoforms (α, β, δ, and γ). Consequently, over 30 anti-PI3K clinical candidates have been developed during this time, culminating in the approval of 5 isoform-selective agents: idelalisib (PI3Kδ), copanlisib (PI3Kα/δ), duvelisib (PI3Kδ/γ), alpelisib (PI3Kα), and the recently withdrawn umbralisib (PI3Kδ, CK1ε). This is a great teaching example for medicinal chemistry as the authors of the recent paper explain their thought process for lead optimization in depth. The molecule was nominated by reviewer Julien Lefranc, who helped note several key points here.

Mechanism of Action. As a pan-class IA PI3K inhibitor, NVP-CLR457 inhibits PI3Kα, β, and δ. Though the focus of the ultimately approved agents and emerging molecules like mPI3Kα degrader GDC-0077 has been on isoform or mutant selectivity, hindsight is 20/20. At the outset, it was (and still is) not clear what properties are needed for an effective PI3K-targeting drug in different settings, and research groups had many different hypotheses that were not disclosed until after the trials. For example, initially it was thought by many that pan-class I agents could potentially demonstrate enhanced efficacy and act on diverse tumor types, before drug tolerability became fully appreciated as a major barrier. One of the earliest clinical candidates in this space, Novartis's pan-PI3Ki, buparlisib (NVP-BKM120; currently AN2025, following acquisition by China's Adlai Nortye) continues to be evaluated in several clinical studies.

Rationale. In developing NVP-CLR457, Novartis scientists were hoping to improve on three properties of buparlisib that were suspected to account for some of the tolerability and efficacy issues encountered: 1) off-target microtubule stabilization; 2) CNS-penetration (which was thought to be responsible for the treatment-related mood disorders observed with the drug); and 3) its selective strong inhibition of PI3Kα relative to PI3Kβ and PI3Kδ. Nonetheless, a Ph. I clinical trial evaluating NVP-CLR457 was terminated due to safety, tolerability, and limited activity, likely ending the clinical journey for the compound, which has been removed from Novartis' pipeline.

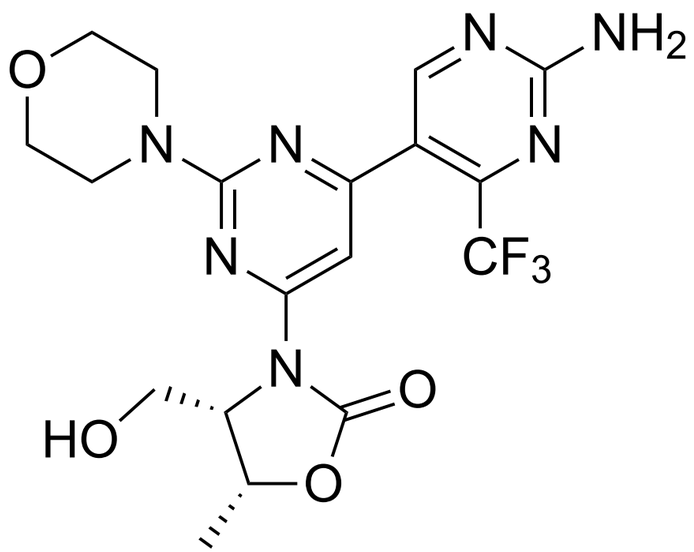
Target. The class I family of PI3Ks comprises four lipid kinase members that can be further categorized as either class IA comprising the α, β, and δ isoforms, and class IB containing the γ isoform. Class I PI3Ks catalyze the phosphorylation of the phospholipid phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate, with the latter being a key signaling molecule that mediates several molecular pathways, including the PI3K-Akt-mTOR pathway which is pivotal for cell growth, proliferation, survival, and metabolism. Aberrations in this pathway have been implicated in the pathogenesis of several human cancers.

Hit-Finding Strategy. Pool deconvolution and screening of a solid phase combinatorial library of 2,4,6-trisubstituted pyrimidines against PI3K led to the identification of compound 1, which had notable potency (0.031 μM). Initial attempts to improve pharmacokinetic properties resulted in a clinical candidate NVP-BKM120 (buparlisib), however, a cytotoxic off-target effect was observed, resulting in interference with tubulin dynamics leading to cell cycle arrest. A phenotypic screen, which measured cell cycle arrest by scoring for the percentage of cells with condensed DNA, was used to identify SAR that could differentiate tubulin binding and maintain the targeted pan-PI3K potency range.

Lead Optimization. Initial efforts began with modification of all regions within buparlisib to understand how to minimize effects on tubulin. Adding a second nitrogen to the pyridine ring allowed for hydrogen bonding with Lys802, and exchange of the C6-morpholine with a more polar substituent allowed for subsequent optimization to *minimize* CNS penetration. Furthermore, increasing the number of H-bonding groups along with the increased polarity improved the drug's susceptibility to efflux by transporters. The SAR observed during these optimizations suggested that *N*-bonded cyclic *N*-acyl analogs at the C6-position had the highest probability of lowering microtubule binding and minimizing CNS penetration, with cyclic carbamates giving the best results. Further optimization of the carbamate moiety led to an oxazolidinone with a 4-methyl substituent, which has favorable van der Waals interactions with the methyl group of Thr833 and the side chain of Met900 of PI3Kδ. This favorable van der Waals interaction was then exploited by introducing a hydroxymethyl group at C4, which added a hydrogen bond to the threonine. Finally, an additional methyl group was added at C5, which both increased chemical stability and added another favorable van der Waals interaction, leading to NVP-CLR457.

NVP-CLR457

pan-class IA PI3K



oral pan-class IA PI3K inhibitor

Ph. I in advanced solid tumors

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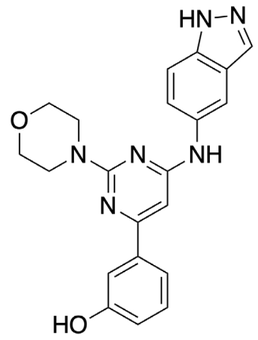
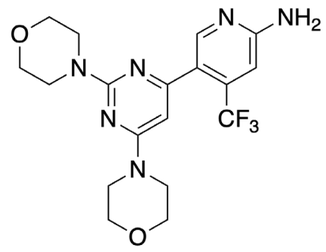
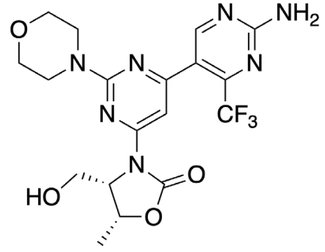


Compound 1



Buparlisib



NVP-CLR457

Preclinical Pharmacology. In vivo studies were done in Rat1-myr-p110α xenograft mice dosed at 3, 10, and 20 mg/kg QD. Dose-dependent inhibition of tumor growth was seen with all doses, with significant differences vs. vehicle seen with the 10, and 20 mg/kg doses.
In a follow-up study involving dosing xenograft mice at 20 and 40 mg/kg BID, >80% tumor growth inhibition was observed with both doses after a 9-day dosing schedule, although the 20 mg/kg dose was the well-tolerated dose with respect to weight-loss and blood sugar levels. Additional animal studies in mice probed the effect of the pan-class I PI3K inhibitor on glucose homeostasis due to the key involvement of PI3Kα in the insulin receptor substrate (IRS)-PI3K pathway; at 3, 10, and 20 mg/kg, blood glucose levels were comparable to that of the vehicle-treated control group. Plasma levels of the drug correlated with tumor S473P-Akt inhibition (IC_{50} = 200 nM). Analysis of S473P-Akt levels in the brain also showed very limited inhibition of Akt signaling, demonstrating the low CNS-penetrating effects of the compound.

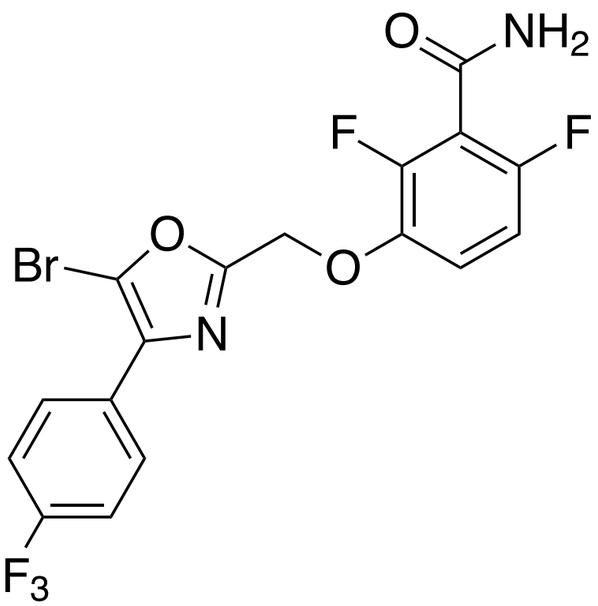
Binding mode: X-ray cocrystal structures of studied compounds in the 2-(*N*-morpholino)pyrimidine series revealed that the general binding mode within the ATP-binding site of the PI3Ks isoforms is conserved across the class I PI3K isoforms. The cocrystal structure of NVP-CLR457 bound within the ATP binding site of PI3Kα (**PDB: 7TZ7**) was disclosed. The affinity region is occupied by the aminopyrimidine, the morpholine moiety acts as the hinge-binder, whereas the oxazolidinone in the selectivity region exhibits hydrophobic interactions with Met772, conserved across all the class I PI3K isoforms.

Clinical Development. NVP-CLR457 was evaluated in a phase 1 study (NCT02189174) with 31 patients diagnosed with PI3K pathway-activated advanced solid tumors. The patients received increasing NVP-CLR457 doses according to a Bayesian escalation model based on the rate of dose-limiting toxicity (DLT) in the first 28-day cycle. The starting dose was 5 mg PO QD, and the dose escalated up to 300 mg PO QD until MTD determination. However, NVP-CLR457 was poorly tolerated, with gastrointestinal toxicities and other grade 3 and 4 adverse events leading to treatment interruption or discontinuation. Consequently, doses above 100 mg were not explored in the clinical study. At 100 mg PO QD, with drug exposures similar to those observed in xenograft models that achieved tumor regression, limited antitumor activity was observed.

Patent. No patents were found. Patents appear to have been abandoned. WO2017093905A1/US20180353515A1

TXA6101

FtsZ



gram-negative FtsZ inhibitor

in vitro efficacy against E. coli

literature starting point and opt

J. Antibiot.

TAXIS Pharmaceuticals, Monmouth Junction, NJ

paper DOI: https://doi.org/10.1038/s41429-022-00531-9

Context. TXA6101 (TAXIS Pharmaceuticals) is a filamentous temperature-sensitive mutant Z (FtsZ) protein inhibitor being developed for targeting gram-negative bacteria. Drug resistance continues to be a paramount challenge in antibiotic therapy and there is an ongoing need to explore and develop novel antibacterial drugs. FtsZ has long been considered an attractive antibiotic target due to its critical role in bacterial cell division coupled with its high conservation in prokaryotes, but low homology to eukaryotic tubulin. Various peptides, natural products, and synthetic small molecules have been shown to induce bacterial cell death through inhibition of FtsZ, the most notable of which was Prolysis Ltd's PC190723 which struggled to progress in part due to issues with solubility. The most advanced FtsZ inhibitor is another TAXIS agent TXA709, which was developed through optimization of PC190723, and is early Ph. I studies according to the company. With TXA6101, the company is hopeful for a similarly successful benzamide class compound that may uniquely target gram-negative bacteria. However, the compound was found to be a substrate for several efflux pumps in these bacteria which presents a significant hurdle needed to be overcome by TAXIS.

Target. FtsZ, a direct prokaryotic homolog of eukaryotic tubulin, is essential for cell division and viability in bacteria. During binary division and in the presence of GTP, the protein self-assembles and forms a ring-shaped protofilament, called the Z-ring. The Z-ring functions as a scaffold for the recruitment and organization of various cellular proteins involved in cell division including structural proteins, peptidoglycan biosynthesis, and degradation enzymes, and other proteins. FtsZ is a well-researched protein and is considered a suitable antibiotic drug target due to being essential for viability in bacteria, as well as being highly conserved and universal in bacteria, with low off-target and cytotoxic potential in humans.

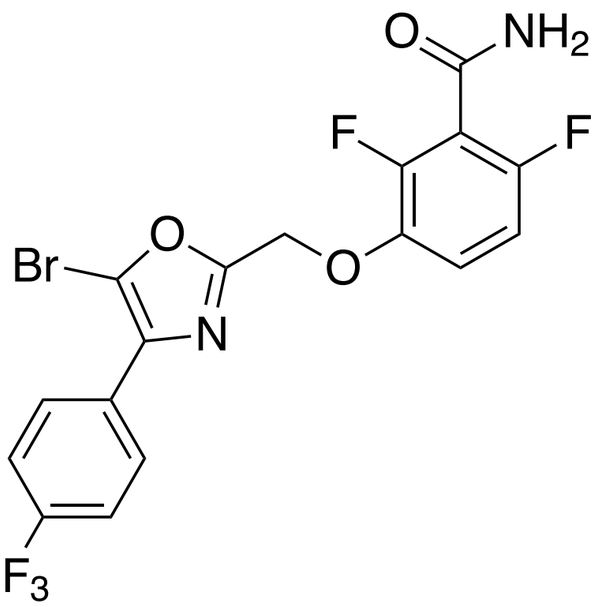
Mechanism of Action. Inhibitors of FtsZ act by either precluding its GTPase activity or modifying the assembly/disassembly dynamics of the Z-ring, destabilizing the structural integrity of the protein which ultimately leads to protein degradation and cell death. The actual mechanisms underlying the activity of TXA6101 has yet to be reported by TAXIS scientists.

Hit-Finding Strategy. Scientists at Prolysis (now Biota) used an early report on the ability of 3-methoxybenzamide (3-MBA) to target FtsZ, and inhibit cell division in the Gram-positive bacterium *Bacillus subtilis* was used as a starting point to develop novel antibacterials effective against multi-drug-resistant bacteria. A fragment-based approach to determining structure-activity relationships of the benzamide and the effects of methoxy group extension led to the analysis of more than 500 analogs, using antibiotic activity and microscopic cell morphology to determine efficacy. PC190723, constructed by a combination of a difluoro-substituted benzamide and thiazolopyridine moiety, was identified as a lead.

Lead Optimization. Variations of the alkoxy fragment with the exchange of the thiazolo[5,4-b]pyridinyl core ring to five-membered heterocycle-substituted benzamides resulted in TXA6101, having a minimum inhibitory concentration (MIC) of 0.25 μg mL^{-1} against MRSA and MSSA. In addition, the oxazole-benzamide core ring enhanced metabolic stability, solubility, and favorable pharmacokinetic profiles.

TXA6101

FtsZ



gram-negative FtsZ inhibitor

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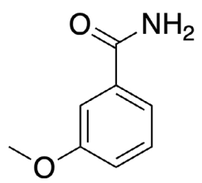
literature starting point and opt

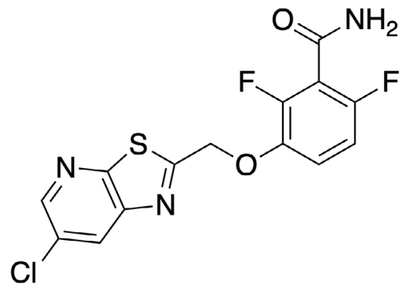
J. Antibiot.

TAXIS Pharmaceuticals, Monmouth Junction, NJ

paper DOI: https://doi.org/10.1038/s41429-022-00531-9



3-MBA



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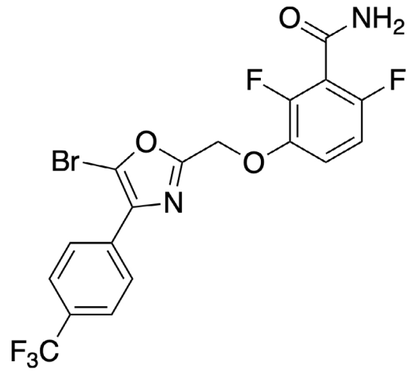


TXA6106

Binding Mode. Crystallographic structure analysis show TXA6101 binds to S. aureus FtsZ in a hydrophobic pocket (**PDB: 5XDU/5XDV**) inducing conformational rearrangements. Within this pocket, was observed the existence of additional space around the CF3 group of TXA6101 suggesting the possibility for further structural optimization of these inhibitors.

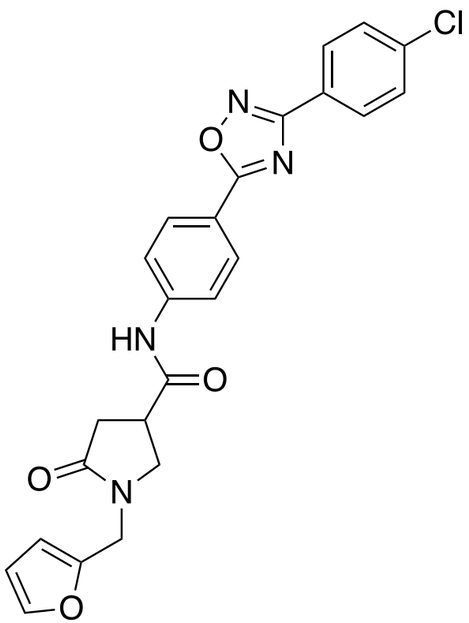
Preclinical Pharmacology. MIC and minimum bactericidal concentration (MBC) assays were used to evaluate the inhibitory action of TXA6101 against different bacterial strains. The molecule was potent against some selected efflux-compromised *E. coli* strains (MIC = 0.25-16 µg/mL); further tests confirmed that the molecule was a substrate for the *E. coli* AcrAB-TolC efflux pump but not the NorE pump. TXA6101 was also poorly active against wild-type Enterobacteriaceae strains, such as *E. coli*, and *K. pneumoniae* (MIC >128 16 µg/mL). Further testing revealed that this was attributable to efflux by the RND pumps in Enterobacteriaceae species. Similar poor activity was observed with *P. aeruginosa* and A. *baumannii*, key bacteria implicated in antibiotic drug resistance. Microscopic evaluation in *E. coli* demonstrated the ability of TXA6101 to induce morphology changes in the bacteria, indicative of inhibition of cell division, while spectrophotometric analysis confirmed the inhibition of FtsZ polymerization in a concentration-dependent manner.

Clinical Development. Preclinical.

Patent. TAXIS Pharmaceuticals holds the right to these compounds through the following patents US9511073B2/ WO2012142671A1 until 2032.

RLX-33

RXFP3



intraperitoneal Relaxin-3/RXFP3 antagonist

in vivo efficacy in induced food intake rats models

from 19000 compound HTS and opt.

J. Med. Chem.

Research Triangle Institute, Durham, NC

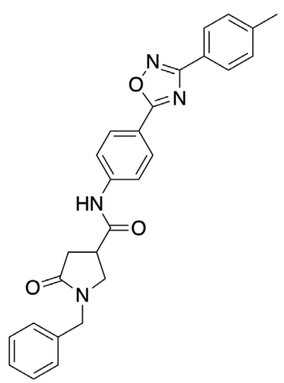
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00508

Context. RLX-33 (Research Triangle Institute) is a nonpeptide relaxin-3/RXFP3 system antagonist. Evidence over the last 2 decades, since the discovery of relaxin-3 in 2001, suggests that the protein, through its receptor RXFP3, regulates responses to stress, anxiety-related and motivated behaviors, circadian rhythms, learning and memory, and alcohol-seeking behavior, making the relaxin-3/RXFP3 system a desirable target for neuropsychiatric agents. Relaxin-3/RXFP3 system inhibitors described in the literature have all been peptide ligands, making RLX-33 the first nonpeptide antagonist to be reported. The small molecule has a key advantage over its peptide competitors in being more metabolically stable and amenable to oral administration. The preclinical properties of RLX-33 make it a promising lead from which a clinical candidate may emerge.

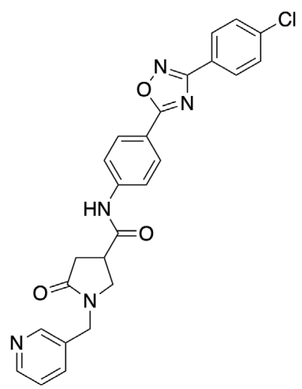
Target. Relaxin-3 is a 5 kDa neuropeptide predominantly expressed in the brain. The protein acts on the relaxin-family peptide 3 receptor (RXFP3) and the pathway has been putatively implicated in the regulation of behavioral alterations due to the high expression of RXFP3 in brain regions known to be key for feeding and metabolism, stress, and reward. Within the last 2 decades, the involvement of the relaxin-3/RXFP3 system in stress response, feeding and other motivated behaviors, learning and memory, circadian rhythm and arousal, and emotional and anxiety-like behavior has been well-described. RXFP3 agonists have been shown to be orexigenic following administration, while inhibitors have been associated with reduced feeding. In humans, polymorphisms in the relaxin-3/RXFP3 system were associated with metabolic syndrome in patients on antipsychotics. The relaxin-3/RXFP3 system has also been implicated in alcohol-seeking.

Mechanism of Action. RLX-33 was found by RTI scientists to exert its effects through a noncompetitive, allosteric mechanism. In cell-based assays, activation of RXFP3 by relaxin-3 was associated with reduced accumulation of intracellular cAMP and subsequent activation of the ERK1/2 pathway, similar to the action of most neuropeptides in these assays. Data are currently scant on the direct effect of RXFP3 activation on the physiological or neurochemical activity of target neurons.

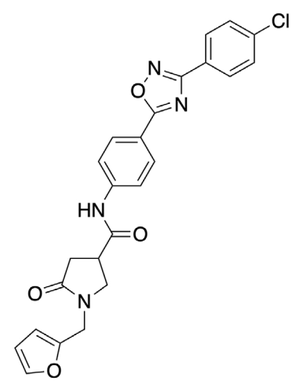
Hit-Finding Strategy. A high-throughput screening campaign was employed using an in-house library consisting of ~19,000 compounds. The library was screened at 20 μM final concentration using a previously disclosed CHO-hRXFP3 cell-based cAMP assay to identify antagonists against relaxin-3. The top 25 cherry-picked hits were confirmed in a cAMP concentration-response assay and a counter screen in CHO cells devoid of RXFP3, ultimately identifying three top hits, all belonging to a single structural family, illustrated by Compound 1.



Compound 1



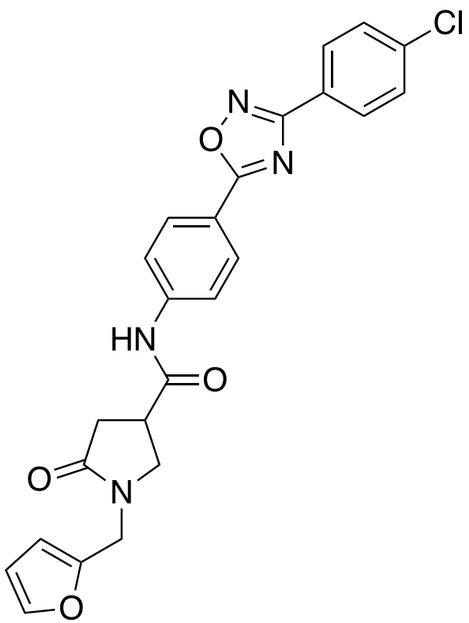
Compound 30



Compound 33 (RLX-33)

RLX-33

RXFP3



intraperitoneal Relaxin-3/RXFP3 antagonist
in vivo efficacy in induced food intake rats models
from 19000 compound HTS and opt.

J. Med. Chem.

Research Triangle Institute, Durham, NC

paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00508

Lead Optimization. Optimization was focused on two regions of Compound 1, the N-benzyl moiety on the pyrrolidinone (site A) and the *p*-tolyl moiety on the oxadiazole (site B). A series of 14 analogs with modifications at site A were initially investigated, following the determination that the lone stereogenic center in the molecule was of little consequence (cAMP IC_{50} = 7.80 μM for the (4*R*) enantiomer of Compound 1 and 6.98 μM for the (4*S*) enantiomer).

Activity was sensitive to site A modifications: removal of the methylene spacer completely eliminated activity, as did the removal of aromaticity (with isobutyl substitution). Increasing the spacer with a phenethyl moiety reduced potency (IC_{50} > 10 μM). Replacement of the phenyl ring with a heteroaromatic ring gave the best results, with the 3-pyridyl (IC_{50} =2.12 μM) and 2-furyl (4.72 μM) moieties the most promising. Next, holding the (pyridin-3-yl)methyl group at site A constant, a series of modifications at site B were made. Removal of the 4-substituent resulted in ~4-fold decrease in activity while removing aromaticity altogether or inserting a methylene spacer completely eliminated it. Ultimately, it was found that the addition of an electron-withdrawing group at the *para*-position gave the most potent compounds, with the 4-chloro substituent having the highest potency (Compound 30, 1.49 μM). The 4-chlorophenyl derivative at site B was then combined with the 2-furyl derivative at site A to give a compound with similar potency (Compound 33, 2.36 μM). No rationale was provided for why the furan-containing compound was chosen over the pyridyl-containing compound.

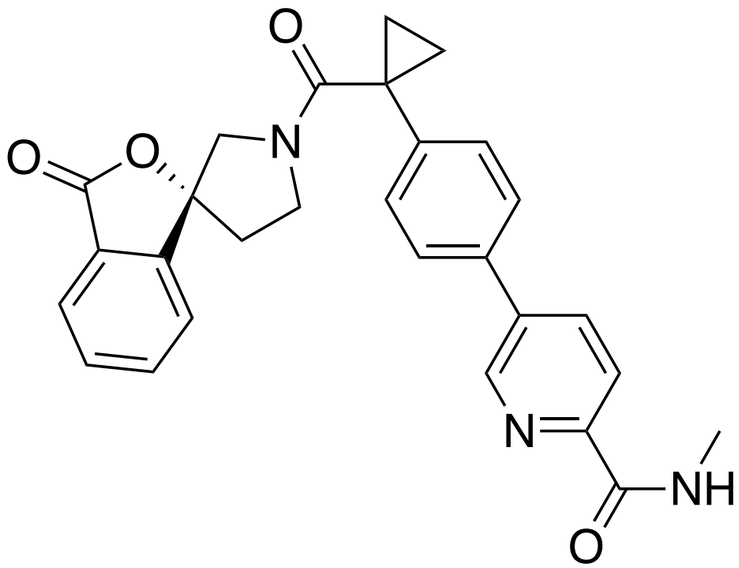
Preclinical Pharmacology. Following administration of the RXFP3-selective agonist R3/I5, the effect of RLX-33 on feeding behaviors was evaluated by analyzing food intake across a 4-hour session following drug administration (10 mg/kg) in male Wistar rats. Treatment was found to significantly affect food intake (P = 0.0001) while a significant main effect on feeding time was also seen (P = 0.0005). When studying drugs that affect eating behaviors it is important to look at the amount of food and time eating because the drug can affect how much food is eaten and the time spent eating. Here, animals ate less in the first 2 hrs than those without the drug in the same time period. Two-way ANOVA revealed a significant main effect on treatment (P <0.0001) and significant interaction (P = 0.04). No significant main effects of treatment on total water consumption were observed.

Clinical Development. Preclinical.

Patent. Not found.

INCB13739

11β-HSD1



oral tissue-targeting 11β-HSD1 inhibitor

Ph. II in T2DM/obesity completed (QD)

scaffold-hopping from cortisone and prior 11β-HSD1 inh.

Bioorg. Med. Chem. Lett.

Incyte Research Institute, Wilmington, DE

paper DOI: https://doi.org/10.1016/j.bmcl.2022.128782

Context. INCB13739 (Incyte) is an oral 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) antagonist. Inhibition of the tissue-specific generation of cortisol using agents that antagonize 11β-HSD1, the key metabolic enzyme involved in the conversion of inactive glucocorticoids to active ones, has been considered a desirable therapeutic strategy for type 2 diabetes and metabolic syndrome. Indeed, at least 25 compounds from natural or synthetic sources have been evaluated in preclinical and some Ph. I-II studies over the last decade, although none have progressed to Ph. III studies. Early clinical data reported by Incyte scientists have demonstrated the successful reduction of HbA1C and fasting plasma glucose levels by INCB13739, while the compound was also found to improve hyperlipidemia and hypertriglyceridemia; however, the molecule appears to have been long discontinued and no information is currently available on the continued development of the compound.

Editor's Comments: The molecule represents an interesting human proof-of-concept for adipose-tissue targeting based on high drug lipophilicity, and the recent article shares a wealth of interesting clinical information. The 11β-HSD1 target is no longer as hotly pursued today but there is still limited clinical activity in this space, with the most recently completed trial in 2021 (AZ). One agent is being explored in Alzheimer's Disease (Xamamem, Actinogen).

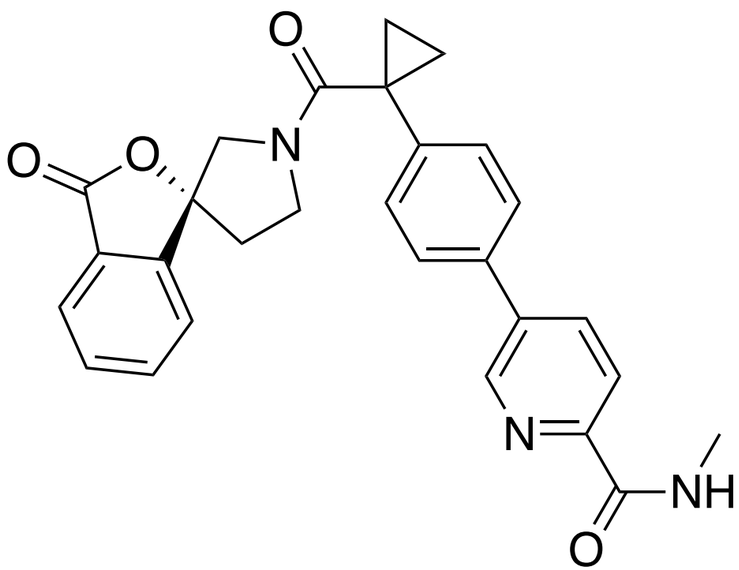
Target. 11β-hydroxysteroid dehydrogenase (11β-HSD) exists in two isoforms, with both acting to regulate the levels of cortisol in key metabolic tissues. One of the isoforms, 11β-HSD1, catalyzes the conversion of inert cortisone to the active cortisol, and is expressed predominantly in liver, adipose tissue, muscle, pancreas, brain, bone, and ocular tissues. Based on the well-established role of glucocorticoids, such as cortisol in the suppression of insulin action, leading to hypoglycemia, 11β-HSD1 is regarded as a desirable target for type 2 diabetes therapeutics. The role of 11β-HSD1 in glucocorticoid metabolism has also led to the implication of the protein in metabolic syndrome. Indeed, 11β-HSD1 knockout mice have been associated with improved glucose tolerance and lipid/lipoprotein profile, increased insulin sensitivity, and resistance to stress/diet-induced hyperglycemia.

Mechanism of Action. In the presence of NADPH, 11β-HSD reductively converts the 11-ketone moiety of cortisone to a 11β-OH group, generating cortisol. Typically, 11β-HSD1 inhibitors partially inhibit this process, reducing cortisol levels. It is thought that these inhibitors selectively lower cortisol levels in tissues without affecting normal plasma levels during the stress response. Due to their lipophilicity, molecules in this series had high fat-to-brain ratios (280x for INCB13739), which help prevent undesired hypothalamic-pituitary-adrenal axis activation.

Hit-Finding Strategy. An interesting three-point pharmacophore was developed without a crystal structure, based on 3D modeling of 11β-HSD1 and cortisone. A scaffold-hopping approach utilizing this model and the structurally distinct 11β-HSD1 inhibitors carbenoxolone, BVT-2733, and MK544 led to the starting point.

INCB13739

11β-HSD1



oral tissue-targeting 11β-HSD1 inhibitor

Ph. II in T2DM/obesity completed (QD)

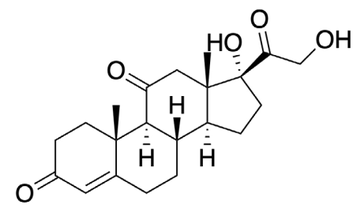
scaffold-hopping from cortisone and prior 11β-HSD1 inh.

Bioorg. Med. Chem. Lett.

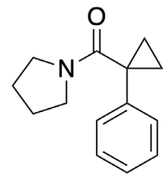
Incyte Research Institute, Wilmington, DE

paper DOI: https://doi.org/10.1016/j.bmcl.2022.128782

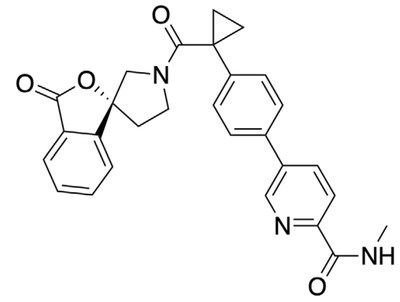
Lead Optimization. The authors employed an interesting pharmacophore reconstruction approach from a small amide-containing starting point (compound 1, 9.4 μM), reincorporating key elements of cortisone into the lead series. The central carbonyl group of cortisone is mimicked by an amide and a cyclopropyl group was inserted to mimic the methyl group of cortisone. Appending lipophilic aryl groups in the form of the isobenzofuranone and formation of a biaryl compound increased potency. hERG, CYP induction, and PXR induction liabilities were addressed by introducing polar motifs including a pyridine nitrogen and amide group resulting in the selection of clinical candidate INCB13739 (3.2 nM).



cortisone



compound 1



INCB13739

Preclinical Pharmacology. In PK studies done in rats and cynomolgus monkeys, INCB13739 demonstrated desirable oral bioavailability (F% = 51 in rats, 43 in monkeys). In rats, the molecule was highly distributed in the adipose tissue (fat to plasma ratio: 2.8:1) and poorly distributed to the brain (brain to plasma ratio: 0.01:1) 1 h after treatment. The molecule was also well-tolerated in both species.

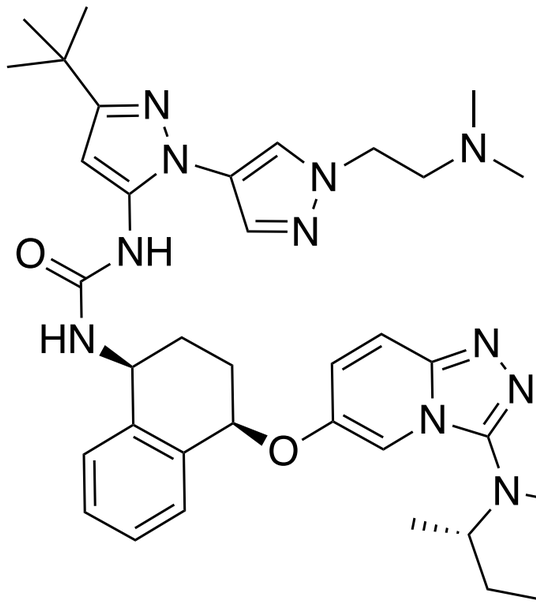
Clinical Development. INCB13739 was studied in three trials. A Ph. 1 study NCT00398619 was completed in 2007, and two Ph. 2 studies NCT00478322 and NCT00698230 completed in 2008 and 2009. In the most recent study, 302 patients with type 2 diabetes, whose metformin monotherapy was failing, were randomized in a double-blind placebo-controlled paralleled trial in once-daily INCB13739 at 5, 15, 50, 100, 200 mg or placebo arms administered orally concomitant with a mean dose 1500 mg metformin. The primary endpoint was the change in A1C. The 200 mg treatment arm achieved significant reductions in hemoglobin A1C (-0.6%), fasting plasma glucose (-24 mg/dL), in HOMA-IR (-24%) compared with placebo. Total cholesterol, LDL cholesterol, and triglycerides were all significantly decreased in hyperlipidemic patients. Positive effects were observed mainly in obese subjects, highlighting the probable importance of 11β-HSD1 activity in adipose tissue to cardiometabolic sequelae of obesity.

Human PK/PD. In healthy volunteers, a half-life of 11 h was observed. A single dose of 50 mg to obese insulin-resistant patients indicated >90% target inhibition at least 24 h post-dose, based on an ex-vivo whole tissue assay. Interestingly, responses were greater in patients with greater body mass, supporting the thesis that increased adipose tissue activity was key. Normal cortisol levels were maintained.

Patent. US7304081B2/WO2005110992A1 assigned to Incyte describes 11-β hydroxyl steroid dehydrogenase type 1 antagonists.

Compound 4e

p38α/β MAPK



inhaled p38α/β MAPK inhibitor

effective in the rat LPS-induced lung inflamm. model

SBDD and focus on slow dissociation kinetics

J. Med. Chem.

Chiesi Farmaceutici, Parma, IT

paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00115

Context. "compound 4e" (Chiesi Farmaceutici) is an inhaled p38α/β mitogen-activated protein kinase (MAPK) inhibitor being developed for lung inflammatory diseases. As a regulator of key proinflammatory mediators, p38 MAPK has been extensively explored as a therapeutic target for inflammatory diseases, including those of the lung. However, despite the description of the first p38 MAPK inhibitor nearly 3 decades ago, no agent has been approved targeting the protein, likely attributable to selectivity and/or tolerability issues. Regardless, the pursuit of these inhibitors has not waned and more than 20 candidates are currently in various stages of clinical development, the most advanced of which is Fulcrum Therapeutics' losmapimod which, although failed in chronic obstructive pulmonary disease (COPD) trials, is seeing some success in the muscular dystrophy space. To mitigate systemic exposure which has been a major challenge encountered with these p38 MAPK inhibitors, "compound 4e" is among a small subset of agents being developed as an inhaled product. The compound has demonstrated promising efficacy in early preclinical experiments and it would be interesting to see how it will perform in more complex disease models of inflammatory lung diseases.

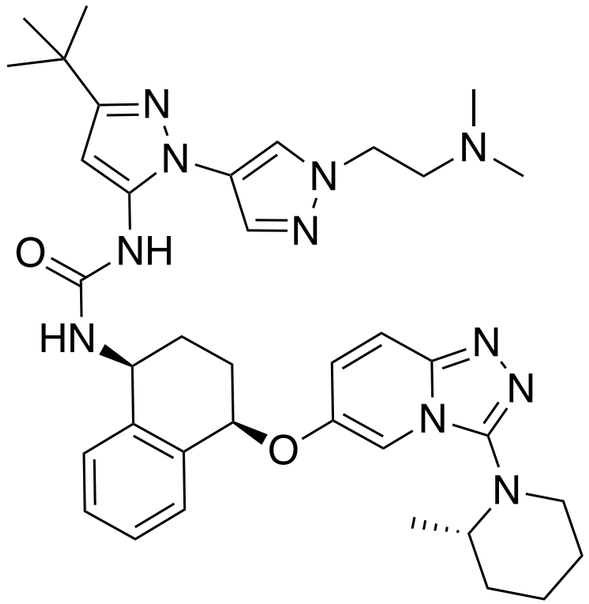
Target. The four human isoforms of p38 MAPKs (p38α, p38β, p38γ, and p38δ) differ in their expression patterns, regulation of kinase activation, and downstream substrate specificities. The p38α isoform is the best characterized and is among the isoforms and is expressed in most cell types. Based on sequence similarity, p38α and p38β are classified under one subgroup, while p38γ and p38δ make up the other subgroup. The role of p38α/β in the pathogenesis and progression of several diseases has been widely researched. In inflammatory lung diseases, such as COPD, the involvement of p38 in the upregulation of pro-inflammatory cytokines has been validated in both animal models and human studies.

Mechanism of Action. Inhibitors of p38α/β MAPK are hypothesized to mediate the exit of targeted cells from the cell cycle, preclude their differentiation and stop the cells from responding to external stimuli. Chiesi Farmaceutici scientists prioritized the development of agents that dissociated from the enzyme in a slow manner to ensure the prolonged duration of action in the lungs.

Hit-Finding Strategy. High-throughput screening identified a pyrazole urea-based inhibitor of p38 MAP kinase. Following a rational drug design approach, a novel tetrahydronaphthalene series was identified, characterized by nanomolar inhibition of p38α with selectivity over p38γ and p38δ isoforms.

Compound 4e

p38α/β MAPK



inhaled p38α/β MAPK inhibitor

effective in the rat LPS-induced lung inflamm. model

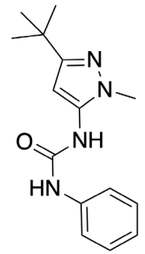
SBDD and focus on slow dissociation kinetics

J. Med. Chem.

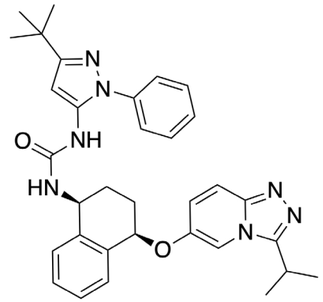
Chiesi Farmaceutici, Parma, IT

paper DOI: https://doi.org/10.1021/acs.jmedchem.2c00115

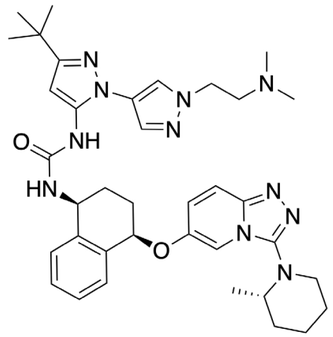
Lead Optimization. Compounds were designed to bind to the enzyme with a DFG-out binding mode and optimized to achieve a limited free fraction in plasma by targeting high hepatic turnover along side high plasma protein binding (PPB). Optimization of 1c was conducted focusing on SAR where improvements in potency against p38α and ligand-enzyme dissociation kinetics were observed, leading to several compounds showing pronounced anti-inflammatory effects in vitro (inhibition of TNFα release). The exchange of tolyl group for pyrazolyl-ethanamine group and isopropyl group for methylpiperidine group improved solubility, as well as PK/PD properties.



Pyrazole urea



Compound 1a



Compound 4e

Binding Mode. X-ray cocrystal structure of 4e analogs compounds, the trans 1S,4S enantiomer compound 1b (PDB:6QDZ), and cis-1S,4R enantiomer compound 1c (**PDB:6QE1**), revealed some interesting features as DFG-out binding mode and similar regions occupation by both molecules despite the different ring stereochemistry.

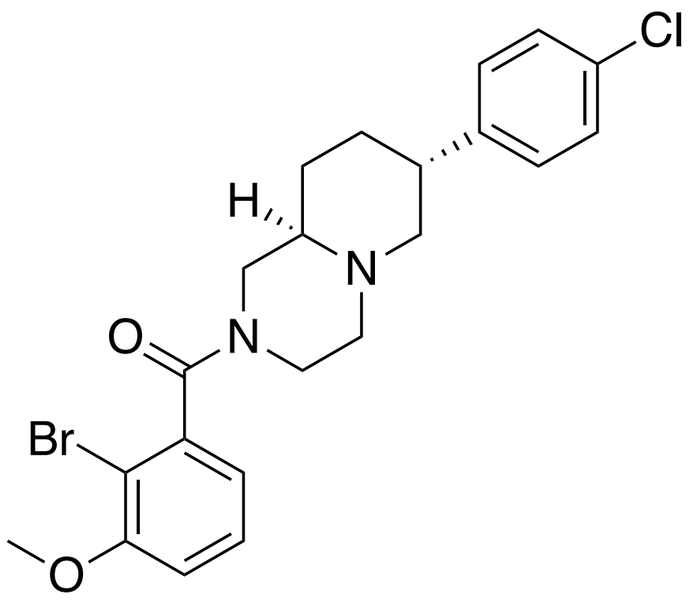
Preclinical Pharmacology. A rat lipopolysaccharide-induced inflammatory model was used for in vivo safety and efficacy studies. The animals were dosed with compound 4e at 0.03, 0.1, and 0.3 mg/kg, given by intratracheal instillation, followed by a 3 μg/mL lipopolysaccharide challenge after 1 h. The 0.1 mg/kg dose mediated the effective reduction in neutrophil levels in challenged animals.

Clinical Development. Preclinical.

Patent. Chiesi Farmaceutici SpA patents to p38 /β MAPK inhibitor series: US8907094B2/WO2013083604A1.

MAGLi 432

MAGL



CNS penetrant, intraperitoneal, reversible MAGL inh. in vivo target engagement in brain but lack of efficacy from opt. of a screening hit

bioRxiv

Roche, Basel, CH

paper DOI: https://doi.org/10.1101/2022.05.04.490688

Context. MAGLi 432 (Roche) is a novel, reversible monoacylglycerol lipase (MAGL) inhibitor. There has been increasing interest in the role of inflammation and blood-brain barrier dysfunction in neurology. The appeal of MAGL as an anti-neuroinflammatory therapeutic target comes from its hallmark role as the enzyme that links the endocannabinoid and eicosanoid systems together by modulating the degradation of the most abundant endocannabinoid in the brain, arachidonoylglycerol (2-AG), to arachidonic acid which is a precursor of prostaglandins and other pro-inflammatory molecules. Unsurprisingly, inhibitors targeting MAGL are being evaluated as nociceptive, anxiolytic, anti-inflammatory, and even anti-cancer agents. At least 20 MAGL inhibitors have been reported, the most advanced of which is Lundbeck's Lu AG06466 (ABX-1431 prior to being acquired) which, despite disappointing in a Ph. II study in patients with Tourette syndrome, is being evaluated in other studies. Unlike most MAGL inhibitors reported which are irreversible agents, MAGLi 432 binds reversibly to the enzyme. Roche scientists believe this will reduce cross-tolerance with CB1 cannabinoid receptor agonists, and make it more selective overall against serine hydrolases (supported by ABPP). In a Ph. I study, FAAH serine hydrolase inhibitor BIA 10-2474 led to fatal neurotoxicity in a patient, as an example of potential off-target concern. However, preclinical data revealed the inability of MAGLi 432 to exert its expected anti-inflammatory effects in the animal model used.

Target. In the latest article, the Roche authors show that MAGL is expressed in the microvasculature (predominantly pericytes) for the first time (previous studies primarily focused on neurons and glial cells). MAGL catalyzes the hydrolysis of 2-AG, the most common endocannabinoid in the brain. 2-AG acts as a full agonist for the widely-expressed and well-characterized CB1 and CB2 cannabinoid receptors, and functions broadly to regulate synaptic function throughout the nervous system. Hydrolysis of 2-AG by MAGL results in the generation of a large pool of glycerol and arachidonic acid in the brain which can be converted to prostaglandins by cyclooxygenases. MAGL knockout mice or inhibition of MAGL action by pharmacological agents have been associated with accumulation of 2-AG and subsequent reduction in arachidonic acid levels. In studies, 2-AG has been found to have neuroprotective effects through reduction of blood-brain barrier permeability, while increased levels of the endocannabinoid have been associated with beneficial effects in brain injury through the reduction of arachidonic acid and associated prostaglandins and leukotrienes.

Mechanism of Action. In the brain, inhibition of MAGL activity results in impaired synthesis of arachidonic acid which further precludes the generation of pro-inflammatory prostaglandins and thromboxane through the action of the cyclooxygenase enzymes. MAGL inhibitors either bind irreversibly through covalent bond formation with the catalytic Ser122 residue of the enzyme or are reversible inhibitors. In an in vivo study, chronic treatment with an irreversible inhibitor resulted in a loss of its activity and cross-tolerance to CB1 agonists in mice.

Hit-Finding Strategy. The bicyclopiperazine derivative MAGLi 43 was optimized from a screening hit, but little more was disclosed.

Binding Mode. Human MAGL complexed with MAGLi 432 crystal structure confirms the non-covalent binding mode (PDB:7ZPG). A water molecule positioned near the oxyanion hole mediates the interaction between the Ser122 residues and MAGLi 432 amide carbonyl group.

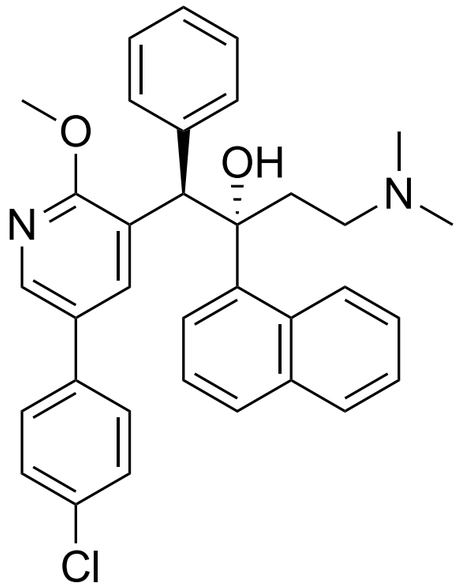
Preclinical Pharmacology. Target engagement in the brain was evaluated with MAGLi 432 at 1 mg/kg (IP) in CD-1 mice. Brain tissue analysis confirmed the inhibition of MAGL activity; mice that received MAGLi 432 were found to accumulate about 10-fold more 2-AG than vehicle controls by LC-MS, while arachidonic acid levels were significantly reduced in LPS-treated groups regardless of MAGLi 432 treatment. The anti-inflammatory activity of the compound by preventing leakage through the blood-brain barrier was investigated using tracing of a FITC dextran tracer injected after the 3-day treatment period. Tissue analysis showed vascular leakage of the tracer in both groups of LPS-treated animals regardless of MAGLi 432 treatment. Additional experiments analyzing brain lysates showed that the levels of the pro-inflammatory cytokines IL-1β and IL-6 induced by LPS treatment were not reduced by treatment with MAGLi 432 treatment.

Clinical Development. Preclinical.

Patent. Roche has submitted the following patent to regulatory offices WO2019134985A1/US20210024546A1

Sudapyridine (WX-081)

mycobacterial ATP-Synthase



oral ATP-synthase inhibitor

Ph. I candidate for pulmonary tuberculosis

toxicol. risk mitigation from prev. disclosed inhibitor

Bioorg. Med. Chem. Lett.

WuXi AppTec, Shanghai, CN

paper DOI: https://doi.org/10.1016/j.bmcl.2022.128824

Context. Sudapyridine (WuXi AppTec) is an oral mycobacterial ATP synthase inhibitor being developed for *tuberculosis* (TB). Despite the significant success achieved with the discovery of antibiotic drugs in the last several decades, drug resistance, particularly related to agents targeting organisms such as *M. tuberculosis*, remains a predominant challenge. To make matters worse, potent agents such as Janssen's first-in-class diarylquinoline, bedaquiline, which was approved in 2012 and sanctioned by the WHO in 2018 for multidrug resistance TB, or the TB Alliance's pretomanid which is the latest approved TB antibiotic, are associated with serious adverse events such as unexplained mortality and QT prolongation. These challenges highlight the need for continuous development of improved antibacterials targeting *M. tuberculosis*. Developed from bedaquiline as the starting point, sudapyridine is off to a promising start. The low cytotoxicity, better lung exposure, and lower QT prolongation effects of the drug reported preclinically will position it well in the TB therapy space if the properties translate clinically. A Ph. II trial evaluating sudapyridine is currently underway, and it may not be long before we get answers to these questions.

Editor Comments: This case study is an interesting example of addressing clinical QTc risk while still having an in vitro hERG signal with metabolites (see Preclinical Pharmacology). A Rabbit fibers assay and dog ECG studies were used to de-risk the molecule. The lower risk may be due to differences in calcium or other channel activity.

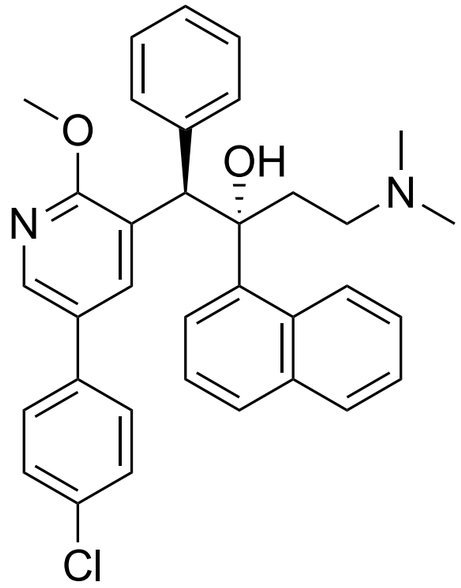
Target. Bacterial ATP synthase mediates ATP generation through the utilization of the free energy of an electrochemical gradient of H^+/Na^+ ions. The transmembrane protein comprises a soluble catalytic F_1 region and a membrane-embedded proton translocating F_0 region. The *M. tuberculosis* ATP synthase has been well-validated as an effective antibiotic target, with the diarylquinoline class of agents being well-researched inhibitors. Recent structural data on the *M. tuberculosis* ATP synthase bound to bedaquiline are also shedding more light on other regions of the enzyme that can be targeted.

Mechanism of Action. Bedaquiline, the precursor of sudapyridine, inhibits ATP generation by blocking the rotary movement of the membrane-embedded F_0 region which halts the activity of the catalytic F_1 region. This has been supported by structural data showing that the drug induces conformational changes in the F_0 region, leading to futile proton cycling and effectively crippling the protein's energy generation ability.

Hit-Finding Strategy. Bedaquiline, previously known as TMC207, R207910, or compound J, belongs to the diarylquinoline drug class, which was first discovered by Andries et *al.* in 2005 (Johnson & Johnson, Belgium) in a high-throughput phenotypic screening for compounds active against the saprophytic mycobacteria.

Sudapyridine (WX-081)

mycobacterial ATP-Synthase



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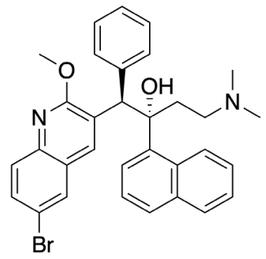
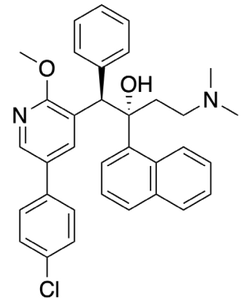
Lead Optimization. Based on bedaquiline, modifications on this scaffold were employed to discover a novel diarylpyridine series, which led to the identification of sudapyridine.



Bedaquiline



Sudapyridine

Preclinical Pharmacology. Bedaquiline has clinical QTc interval prolongation due to a major metabolite with an extremely long half-life in humans. WX-081 produces a similar major metabolite that also inhibits hERG (WX-081-M3). While both of these metabolites inhibit hERG, WX-081-M3 appears to be less active on the Cav1.2 channel, and is less potent in the Rabbit Purkinje fibers assay. In 14-day GLP dog ECG studies, there were no ECG changes with WX-081, but QTc prolongation was observed in bedaquiline dogs.

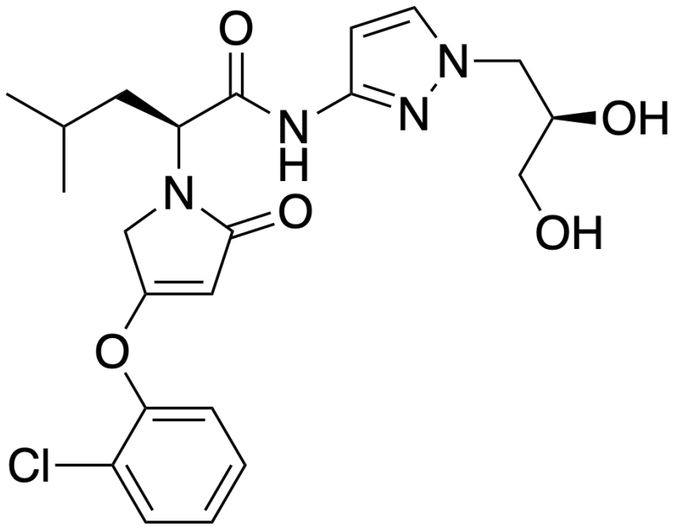
Both acute and chronic tuberculosis infection mouse models were used for efficacy studies. Mice were treated with sudapyridine (5, 10, and 20 mg/kg), bedaquiline (5, 10, and 20 mg/kg), and rifampin (15 mg/kg) QD during a 5-day period per week for 4 weeks. In the acute infection model, lung bacterial load as measured by colony-forming units (CFUs) was significantly reduced with both sudapyridine and bedaquiline in a dose-dependent manner, with complete pathogen clearance achieved with the 20 mg/kg dose. In the chronic infection model, both drugs again were associated with a reduction in bacterial load compared to the control animal, although the infection was more difficult to treat in this model.

Clinical Development. Sudapyridine is currently being evaluated in a Ph. II trial (NCT04608955), which will consist of several experimental arms to evaluate the early bactericidal activity, safety, and tolerability of sudapyridine in drug-naive and drug-resistant tuberculosis, as well as efficacy in participants with drug-resistant tuberculosis. The treatment arm will receive a dose of 150 mg or 300 mg or 450 mg PO QD sudapyridine or standard care for 2 weeks. A subset of patients with drug-resistant tuberculosis will receive either sudapyridine 400 mg PO QD or bedaquiline 400 mg PO QD. In the second stage, the efficacy will be investigated in participants with drug-resistant tuberculosis. The patients will receive sudapyridine 150 mg PO QD and a multi-drug background treatment or bedaquiline 200 mg PO QD and multi-drug background treatment for 6 weeks.

Patent. The drug patent is held by CISEN PHARMACEUTICAL and Shanghai Jia Tan Pharmatech Co Ltd (originator) US9878982B2/WO2016008381A1.

Dorzagliatin

Glucokinase



oral allosteric glucokinase activator

Ph. III candidate in T2DM

HbA1c reduction vs placebo

Nat. Med.

Hua Medicine, Shanghai, CN

paper DOI: https://doi.org/10.1038/s41591-022-01802-6

Context. Dorzagliatin (Hua Medicine) is an oral dual-acting full glucokinase (GK) activator being developed for type 2 diabetes. More than 50 years since the first report of the role of GK in glucose metabolism, no agents targeting this enzyme have been approved, although not for lack of trying. We recently covered a partial GK activator, BMS-820132 (BMS), with more context. Roche's piragliatin was the first GK activator to be studied in patients with type 2 diabetes, but the compound, like many to succeed it, disappointed in the clinic due to increased risk of hypoglycemia and gradual loss of efficacy. Alternative strategies such as the development of hepatoselective molecules and partial activation of the enzyme have been tried and some of these agents are still in early clinical development. As a hepatic and pancreatic dual-acting full GK activator, dorzagliatin seems to be able to exert its therapeutic effect while allowing GK to maintain its key ability as a glucose sensor, which could be the factor previous failed molecules may have missed. The potential first-in-class molecule is currently being evaluated in a Ph. III study and is the most clinically advanced GK activator.

Reviewer or Editor Commentary. The molecule was nominated by reviewer Romyr Dominique. Reviewer Ron Li says, "I noticed that the drug was studied in a population of drug naive type 2 diabetes patients. This makes the study less interesting clinically, since we already have several drugs that work well as first line therapy for lowering HbA1c. A more helpful study would be to look at effectiveness among patients who have not responded to one or more oral anti-hyperglyemic drugs (to show that it would be a good alternative to insulin). In general, there is a need for oral therapies for patients with type 2 diabetes who have persistently high HbA1c despite lifestyle modification and being on a first and/or second line oral agent. At this point, there is usually a conversation about starting insulin, so an effective alternative would be desirable. This study just shows dorzagliatin decreasing HbA1c in a drug naive patient population, which would also likely respond well to any of the available oral antihyperglycemic agents." However, there is a Ph. III head to head study planned with metformin and insulin (see Clinical Development).

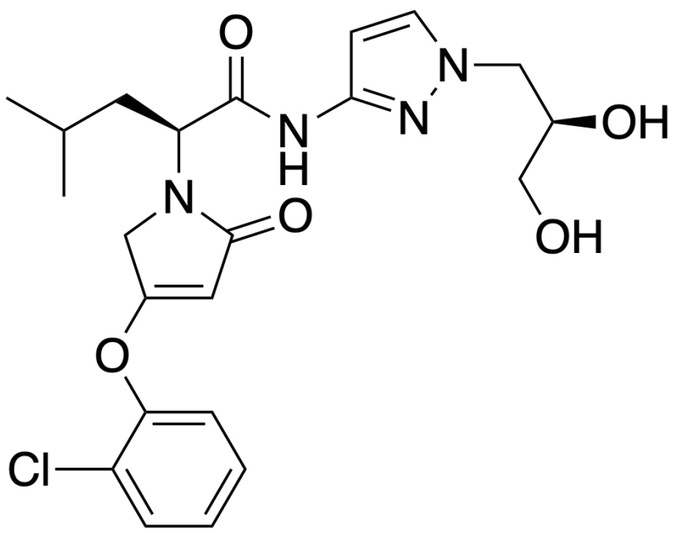
Target. Expressed predominantly in hepatocytes and pancreatic β-cells, glucokinase (or hexokinase IV) catalyzes the phosphorylation of glucose to glucose-6 phosphate. At physiological glucose levels, GK is not saturated which allows the enzyme to "sense" glucose levels. GK was validated thanks to human genetics prior to its human clinical trials; loss-of-function mutations have been associated with a decrease in enzyme activity and subsequent increase in blood glucose, while gain-of-function mutations have been linked with hyperglycemia.

Mechanism of Action. GKs activators have been classified as full activators, partial activators, liver-selective, or pancreas and liver dual-acting agents. Dorzagliatin is a dual-acting full GK activator that acts on pancreatic and hepatic GK in a glucose-dependent manner. Preclinical experiments showed that the molecule's antidiabetic effect is exerted through the mitigation of insulin resistance and an increase in insulin sensitivity. Hua Medicine contends that the drug's properties allow it to increase GK enzymatic efficiency at effective concentrations while maintaining the enzyme's key role as a glucose sensor where needed.

Hit-Finding Strategy. The Roche starting point was an HTS of a library of 120,000 structurally diverse synthetic compounds screened for the ability to increase GK enzymatic activity. Compounds that increased the enzymatic activity of GK were selected, and chemical optimization of one of these initial molecules led to the synthesis of RO-28-0450 as a lead GK activator (GKA).

Dorzagliatin

Glucokinase



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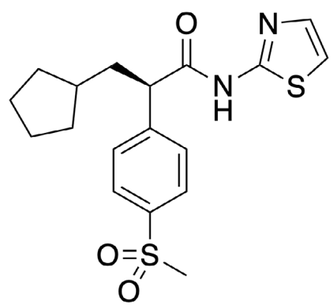
Nat. Med.

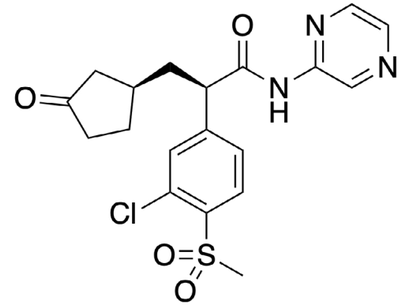
Hua Medicine, Shanghai, CN

paper DOI: https://doi.org/10.1038/s41591-022-01802-6

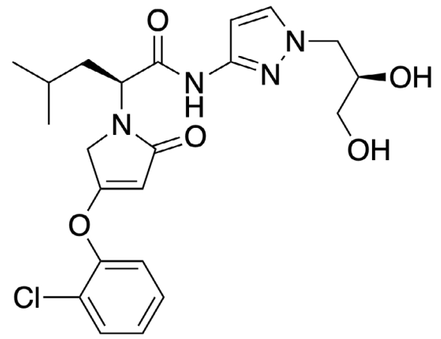
Lead Optimization. The R- and S- RO-28-0450 enantiomers were tested. Activation of GK was exquisitely sensitive to the chirality of the molecule: The R-enantiomer, RO-28-1675, was a potent GKA, whereas the S-enantiomer, RO-28-1674, was inactive. Little more has been disclosed about the discovery of HMS5552 (Dorzagliatin), a fourth-generation GKA.



RO-28-0450



Piragliatin



Dorzagliatin

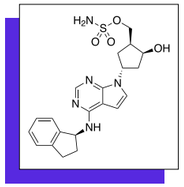
Preclinical Pharmacology. In vivo experiments were done in diabetic rats divided into four groups: control, diabetic, low-dose (10 mg/kg) HMS5552-treated diabetic, and high-dose (30 mg/kg) HMS5552-treated diabetic. The treatment was administered daily intragastrically for one month. The molecule exerted significant glucose-lowering effects in both treated groups within 2 hr of administration. The molecule was also associated with a significant decrease in the levels of fasting insulin, triglycerides, and total cholesterol. Tissue analysis showed that treatment with dorzagliatin was associated with a significant increase in both the number of GK-immunopositive cells in the liver and insulin-immunopositive cells in pancreatic β-cells.

Clinical Development. Dorzagliatin completed several Ph. I and II clinical trials and two Ph. III trials DAWN and SEED (NCT03141073 and NCT03173391). The results from the SEED trial (NCT03173391) were recently disclosed. In this study, subjects with type 2 diabetes were randomly assigned to receive dorzagliatin 75 mg PO BID or a placebo. The primary efficacy endpoint was reached after 24 weeks with a statistically significant reduction in HbA1c levels over placebo. Dorzagliatin was well-tolerated during this period. A Ph. III study (NCT05098470) is currently recruiting aiming to study the effect of dorzagliatin, metformin, and insulin to determine if they improve nighttime blood sugar levels.

Patent. Hua Medicine holds the rights of dorzagliatin through the granted patents US11266630B2/WO2018108128A1

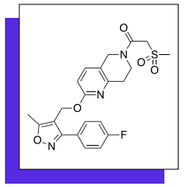
Small Molecules of the Month

May 2022
drughunter.com



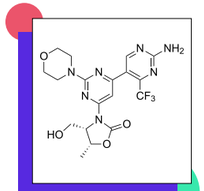
Pevonedistat | NEDD8-activating enzyme (NAE)

intravenous NEDD8-activating enzyme inhibitor
Ph. III candidate for oncology
from HTS and optimization
Drug Metab. Dispos.
Takeda, Lexington, MA



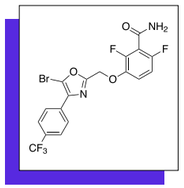
Compound 20 | α5-GABA$_A$

oral α5-GABAA receptor NAM
oral efficacy in CIAS rodent models
scaffold hopping from literature starting point
J. Med. Chem.
Gedeon Richter, Budapest, HU



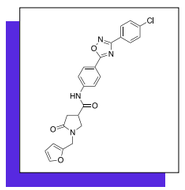
NVP-CLR457 | pan-class IA PI3K

oral pan-class IA PI3K inhibitor
Ph. I in advanced solid tumors
opt. from previous clinical candidate
J. Med. Chem.
Novartis (NIBR), Basel, CH



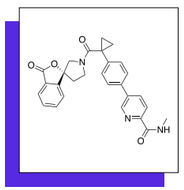
TXA6101 | FtsZ

gram-negative FtsZ inhibitor
in vitro efficacy against E. coli
literature starting point and opt
J. Antibiot.
TAXIS Pharmaceuticals, Monmouth Junction, NJ



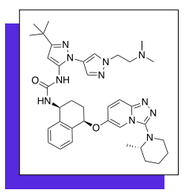
RLX-33 | RXFP3

intraperitoneal Relaxin-3/RXFP3 antagonist
in vivo efficacy in induced food intake rats models
from 19000 compound HTS and opt.
J. Med. Chem.
Research Triangle Institute, Durham, NC



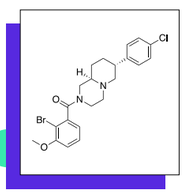
INCB13739 | 11β-HSD1

oral tissue-targeting 11β-HSD1 inhibitor
Ph. II in T2DM/obesity completed (QD)
scaffold-hopping from cortisone
and prior 11β-HSD1 inh.
Bioorg. Med. Chem. Lett.
Incyte Research Institute, Wilmington, DE



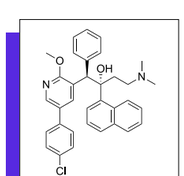
Compound 4e | p38α/β MAPK

inhaled p38α/β MAPK inhibitor
effective in the rat LPS-induced lung inflamm. model
SBDD and focus on slow dissociation kinetics
J. Med. Chem.
Chiesi Farmaceutici, Parma, IT



MAGLi 432 | MAGL

CNS penetrant, intraperitoneal, reversible MAGL inh.
in vivo target engagement in brain but lack of efficacy
from opt. of a screening hit
bioRxiv
Roche, Basel, CH



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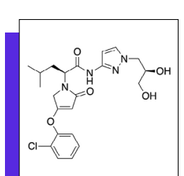

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Nat. Med.
Hua Medicine, Shanghai, CN

drug hunter

drug hunter

discover together

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info@drughunter.com