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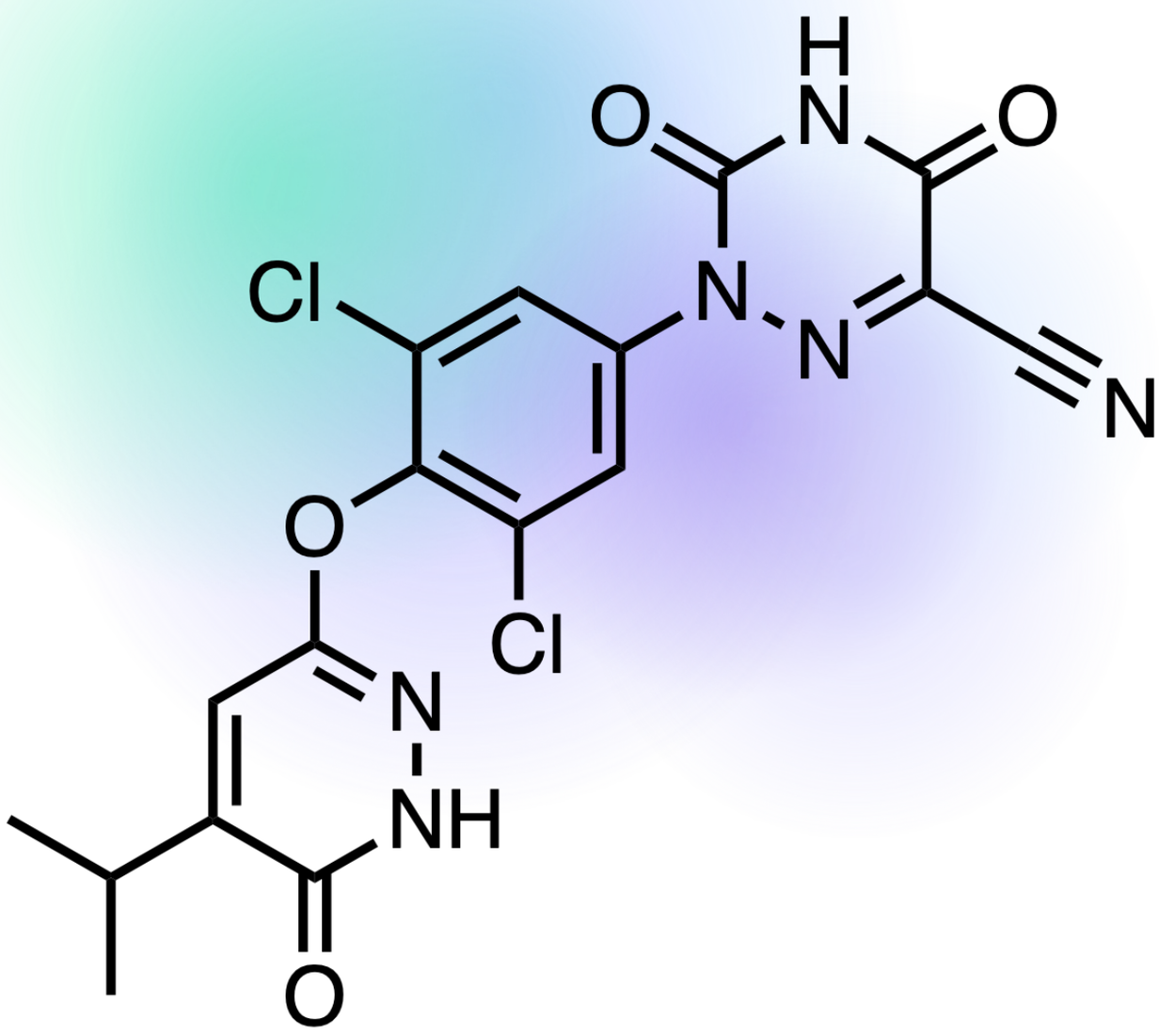
# Small Molecules of the Month

## December 2022

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# resmetirom

## THR-β

once daily, oral THR β-selective agonist
Ph. III for NASH
opt. of triiodothyronine
*Company Announcement,* December 19, 2022
MADRIGAL PHARMACEUTICALS, PA
paper DOI: https://doi.org/10.1021/jm4019299

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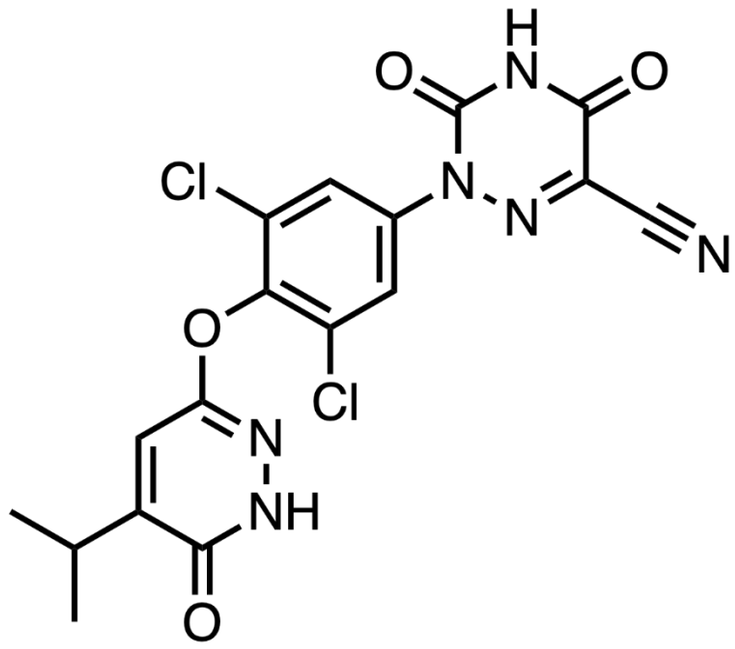
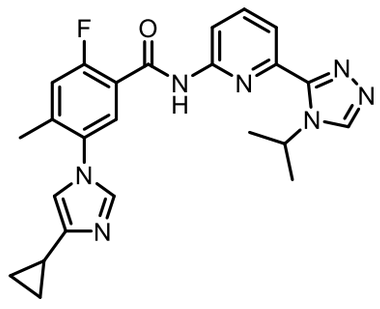
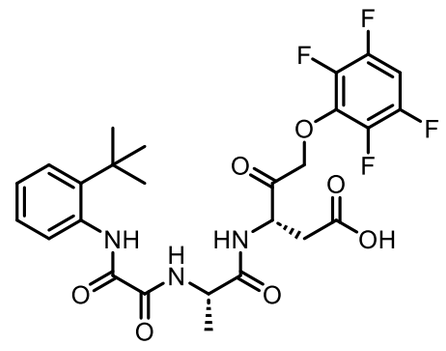
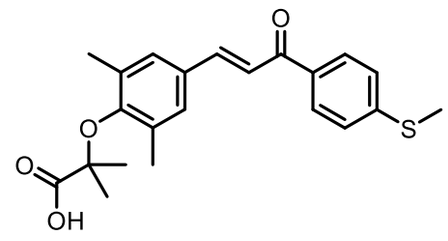




**selonsertib**
ASK1 inhibitor



**emricasan**
pan-caspase inhibitor

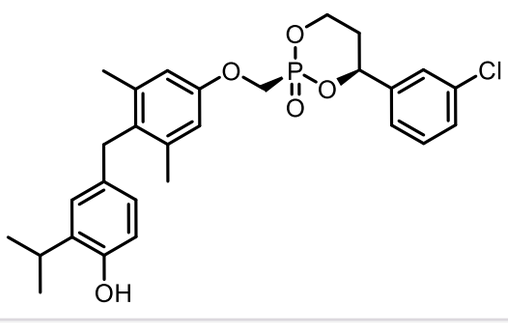


**elafibranor**
dual PPARα/δ agonist

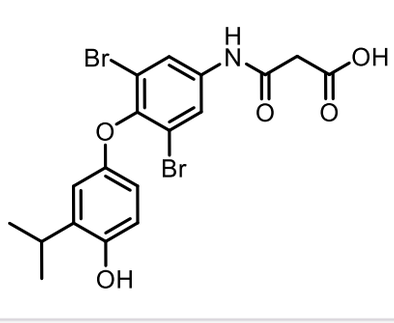
**A first-in-class drug for NASH?** Resmetirom, an oral, liver-targeting, once-daily THR-β-selective agonist, recently made headlines for meeting both primary surrogate endpoints in an early Ph. III readout for NASH, demonstrating NASH resolution and no worsening of fibrosis by biopsy after 52 weeks in 26% of patients at 80 mg QD (n=316) vs. 10% for placebo (n=318), and demonstrating improvement in fibrosis with no worsening of NASH (24% at 80 mg vs. 14% for placebo). These unexpectedly positive results skyrocketed the valuation of Madrigal (MDGL) to over $5B. Resmetirom could become the first drug to achieve accelerated approval for NASH, with an NDA to be filed in '23.

**Why is this a big deal?** Non-alcoholic steatohepatitis (NASH) impacts an estimated 3-5% of the global population, is one of the most common drivers of liver transplants and has no FDA-approved treatments. It has been challenging to develop drugs for NASH because demonstrating improvement would require showing improved liver outcomes, prevention of cirrhosis, and improved survival. All of these milestones require a drug to be safe and effective for potentially decades, as disease progression to cirrhosis can manifest upwards of 10 years. Furthermore, most NASH patients are undiagnosed, and sequential liver biopsies are required for assessment of the condition, complicating trial enrollment and retention. The high safety bar required for success is exemplified by Intercept's FXR agonist, obeticholic acid, which was approved in a rare disease but rejected for NASH despite meeting a fibrosis endpoint due to safety issues, including pruritus and liver injury. A drug like resmetirom that demonstrates efficacy on surrogate biomarkers, such as improvements in liver fibrosis and safety when taken chronically, has a better chance of demonstrating benefit over the long periods of time required for full FDA approval and commercial success in NASH. The sub-100 mg dose of resmetirom is also associated with a lower risk of drug-induced liver injury, an important factor for any chronic drug.

**What else has been tried before?** Numerous molecules, pathways, and targets have been explored for NASH, with limited success despite attempts to demonstrate efficacy with various surrogate endpoints, such as improvements in fibrosis or resolution of NASH without fibrosis. Recent high-profile clinical programs were terminated due to low efficacy, including Gilead's ASK1 inhibitor, selonsertib, Conatus' pan-caspase inhibitor, emricasan, and Genfit's dual PPARα/δ agonist, elafibranor, among others, leading to skepticism about resmetirom's success. Intercept recently resubmitted its application for previously rejected FXR agonist obeticholic acid, which faces an uphill battle with the FDA due to aforementioned safety issues combined with lack of NASH resolution.



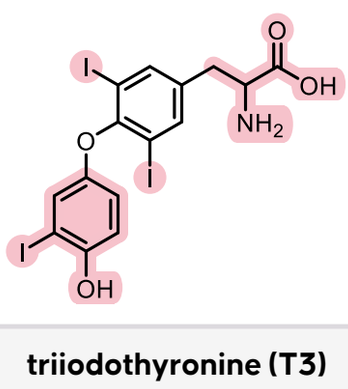
**VK2809 (MB07811)**



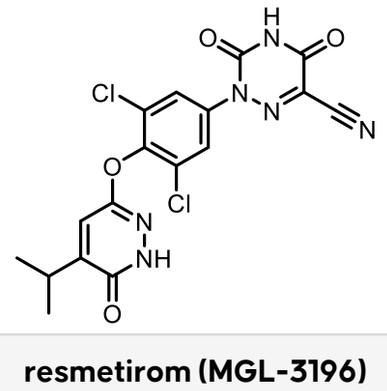
**eprotirome**

**How does resmetirom work?** Madrigal's resmetirom is an agonist of thyroid hormone receptor β (THR-β), which like many other NASH targets (e.g., FXR, PPAR, ACC, SCD1, DGAT2, FGF) influences liver fatty acid metabolism. As a selective THR-β agonist, resmetirom mimics the roles thyroid hormones play in regulating liver lipid metabolism, reducing liver fat and ultimately progression to liver fibrosis. Due to its 28-fold functional selectivity over THR-α, resmetirom avoids the adverse effects of thyroid hormone in the heart and bone, as verified by THR-α-mediated gene expression in the heart in rodents. The liver-targeting property of resmetirom (8:1 liver to plasma ratio in mice) reduces systemic exposure, contributing to safety by avoiding the pituitary gland, where THR-β plays a central role in the thyroid system. Resmetirom is the most advanced THR-β agonist in clinical trials for NASH, with Viking Therapeutics' THR-β agonist VK2809 (a liver-targeting prodrug, also derived from thyroid hormone, formerly known as MB07811) following closely in Ph. IIb trials (NCT04173065). Earlier THR-β agonist eprotirome lowered LDL cholesterol by 15-41% in clinical trials, but development was halted due to cartilage damage observed in canines.

**How was resmetirom discovered?** Resmetirom was identified ("compound 53") by Roche scientists (Nutley) and patented in 2007. Like most THR modulators, the starting point was the thyroid hormone, triiodothyronine (T3). The Roche campaign employed models based on the cocrystal structure of T3 to THR-β (PDB: 3GWS). A functional FRET-based coactivator assay was used to assess selectivity since binding assays do not provide information on relative functional agonism. Roche licensed the candidate to VIA Pharmaceuticals in 2008, along with a DGAT1 inhibitor, for VIA's metabolic disease platform. The program was then acquired by Madrigal in 2011 through the buyout of VIA.



**triiodothyronine (T3)**
THR-β $EC_{50}$ = 15 nM
THR-α $EC_{50}$ = 10 nM



**resmetirom (MGL-3196)**
THR-β $EC_{50}$ = 210 nM
THR-α $EC_{50}$ = 3.7 μM

**What's next?** The positive clinical data for resmetirom may be an inflection point in NASH drug discovery, with surrogate endpoints achieved with a drug that appears to be well-tolerated. It seems to be only a matter of time before an accelerated approval is issued in the space. This suggests that clean molecules targeting related mechanisms, such as ACC inhibition, DGAT2, inhibition, or FXR agonism, may similarly be capable of resolving NASH and fibrosis. The next big question will be whether molecules that achieve accelerated approval on surrogate endpoints ultimately lead to improved clinical outcomes with respect to liver cirrhosis, survival, and long-term quality of life. Given the time it takes to gather these data, this will be an interesting and competitive area to watch for many years.

**Relevant patents.** Hoffmann-La Roche Ltd. currently holds two patents for thyroid hormone analogs (WO2014043706A1, 2014 and WO2007009913A1, 2007 with Madrigal Pharmaceuticals, Inc.).

December 2022

# RMC-5552

## mTORC1

selective, bi-steric mTORC1 inhibitor
Ph. I/Ib for R/R solid tumors
opt. of rapalog+linker+mTOR inh
*J. Med. Chem.,* December 19, 2022
REVOLUTION MEDICINES, REDWOOD CITY, CA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01658

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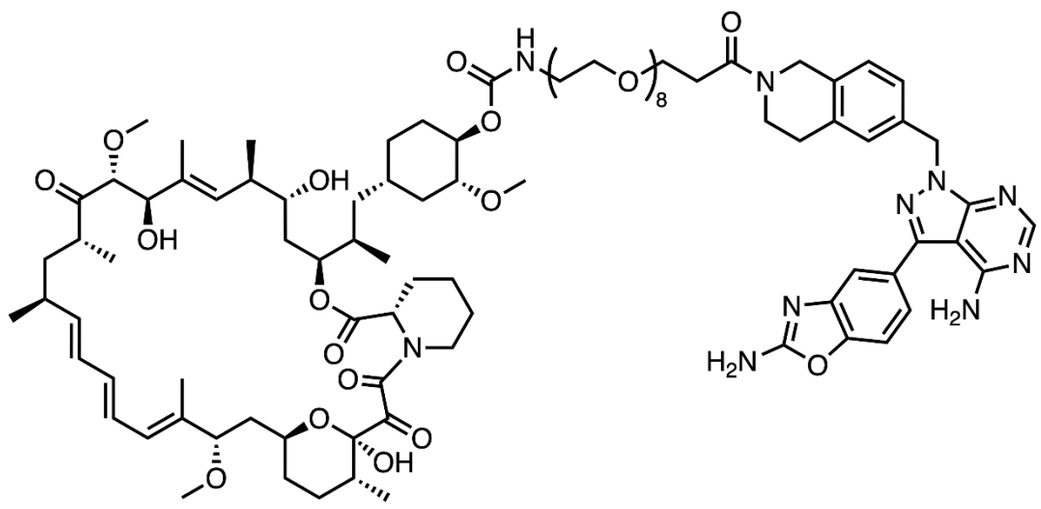
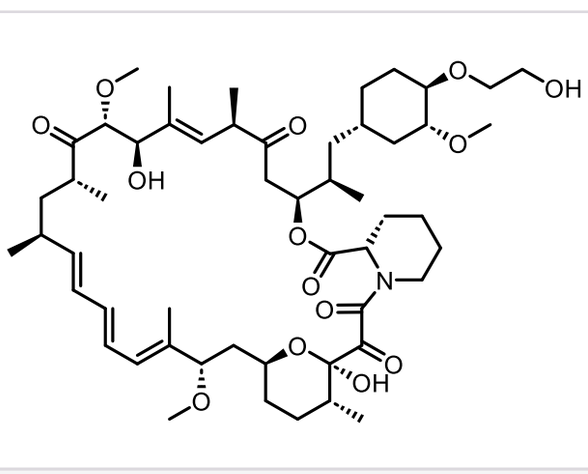


**What is it?** RMC-5552 is a potential first-in-class, bi-steric, mTORC1-selective inhibitor in Ph. I/IIb (NCT04774952, NCT05557292) as an IV agent to treat tumors with hyperactive mTORC1 signaling. This previously highlighted molecule is a bi-steric inhibitor containing an allosteric C32-hydroxy rapamycin derivative linked to an orthosteric, second-generation mTOR inhibitor, sapanisertib (MLN0128, INK128). Inspired by the first reported bi-steric mTOR inhibitor RapaLink-1 from the Shokat lab, RMC-5552 was optimized to have 40-fold selectivity for mTORC1 over mTORC2 to address adverse effects commonly associated with pan-mTOR inhibition.

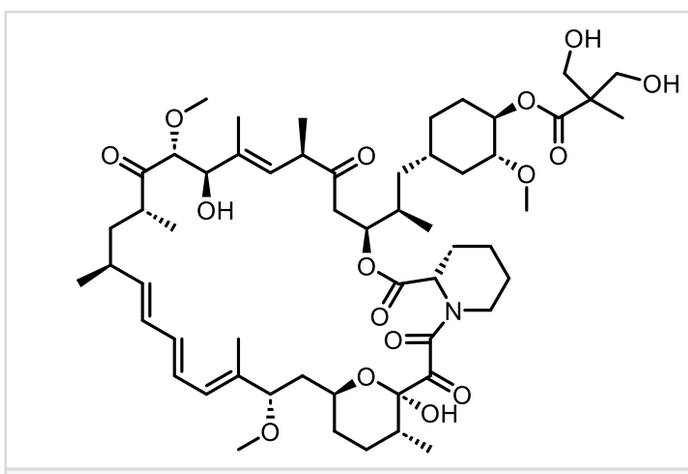
**Why do we care?** The phosphatidylinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway is one of the most pursued targets in cancer therapy, but many candidates have been withdrawn due to toxicity issues and resistance mechanisms. Strategies to inhibit mTOR allosterically (rapamycin analogs) or orthosterically (ATP-competitive kinase inhibitors) have resulted in marginal clinical activity and dose-limiting toxicities. All three approved mTOR therapies are monosteric rapamycin analogs (rapamycin, everolimus, and temsirolimus). RMC-5552 is interesting both as a scientific proof-of-concept for a novel modality (bi-steric inhibitor of both allosteric and orthosteric sites simultaneously) and as a potential new therapy both alone and in combination with targeting two sites, bi-steric inhibitors may be able to overcome traditional mTOR resistance mutations.

A cryo-EM structure of mTORC1-RMC-5552-FKB12 was solved with 2.9 Å resolution (**PDB:** 8ERA). The rapalog portion of RMC-5552 recruits FKB12 to an allosteric binding site on FRB, which is blocked in the complex of mTORC2 by Rictor-Sin1. Within the ATP-binding site of mTOR, the aminopyrazolo[3,4-*d*]-pyrimidine orthosteric warhead of RMC-5552 engages with the mTOR hinge region like a classical ATP-competitive kinase inhibitor.

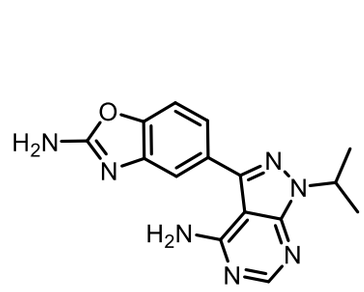
**How was it discovered?** RapaLink-1 was the first example of a bi-steric inhibitor but demonstrated only 4-fold selectivity for mTORC1. Optimization focused on mTORC1-selectivity enhancement. Different orthosteric inhibitors with a C40-ether triazole linker were initially evaluated, with the most potent and selective being "rigid MLN," a tetrahydroisoquinoline analog of MLN0128 (sapanisertib). Bi-steric molecule "compound 21," for example, had picomolar activity with 20-fold selectivity for mTORC1. The triazole was replaced with a carbamate linker to avoid safety hazards of scaling the azide precursor. Additional efforts involved mitigating ring opening



**everolimus (Afinitor)**



**temsirolimus (Torisel)**

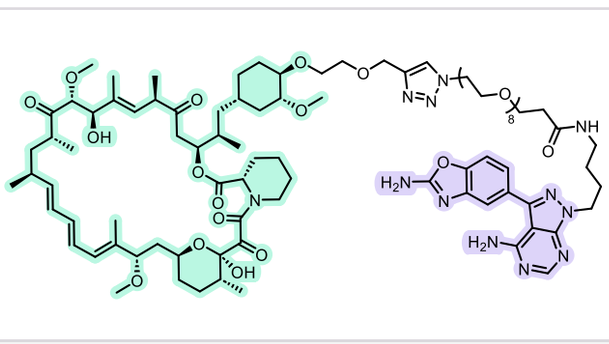


**sapanisertib (MLN0128, INK128)**

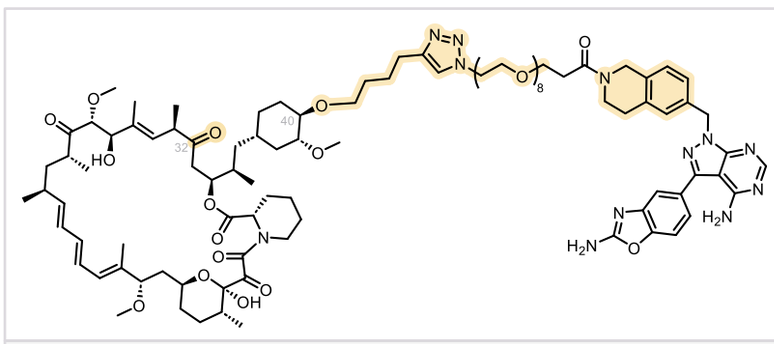
other drugs to overcome resistance. Since our last update, RMC-5552 has entered the clinic (NCT04774952, NCT05557292) with promising preliminary data showing a 71% disease control rate in several tumor types with hyperactive mTORC1 signaling when given at 6 or 8 mg doses IV weekly.

**How does it work?** mTOR plays a critical role in regulating metabolism, growth and proliferation of both normal and cancer cells. mTOR combines with other proteins to form one of two complexes, mTORC1 and mTORC2. Activation of mTORC1 leads to 4EBP1 phosphorylation and release of eIF4E, a primary driver of growth and proliferation, whereas mTORC2 regulates glucose metabolism, cell growth and survival. The general design of a bi-steric mTOR inhibitor contains an allosteric rapamycin or rapalog linked to an orthosteric mTOR inhibitor. By to seco-analogs in vivo by replacing the C32 ketone with an alcohol, which serendipitously enhanced mTORC1 selectivity to 40-fold for RMC-5552 ("compound 38"). In the structure of RMC-5552 below/above, the green portion refers to the allosteric binding portion and purple is the orthosteric ligand.

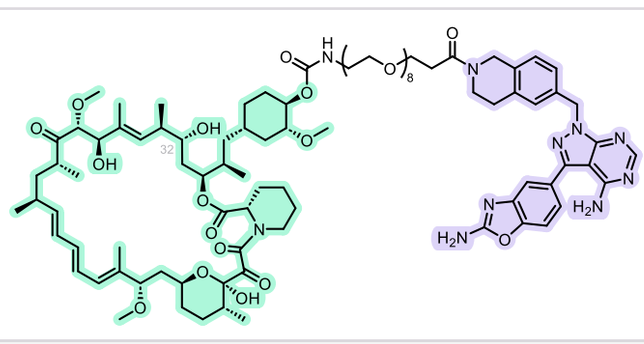
**Patents.** Revolution Medicines, Inc. currently holds two patents for rapamycin analog mTOR inhibitors (WO2018204416A1, 2018 and WO2019212990A1, 2019).



**RapaLink-1**
mTORC1 p4EBP1 $IC_{50}$ = 1.7 nM
mTORC2 pAKT $IC_{50}$ = 6.7 nM
mTORC1/2 selectivity = 4X



**compound 21**
mTORC1 p4EBP1 $IC_{50}$ = 0.44 nM
mTORC2 pAKT $IC_{50}$ = 8.8 nM
mTORC1/2 selectivity = 20X



**RMC-5552 (compound 38)**
mTORC1 p4EBP1 $IC_{50}$ = 0.48 nM
mTORC2 pAKT $IC_{50}$ = 19 nM
mTORC1/2 selectivity = 40X

December 2022

# inavolisib

## mutant PI3Kα

isoform-selective mutant PI3Kα degrader
oral <9 mg QD, Ph. III in HR+/HER2- BC
from cellular characterization of PI3Ki and opt.
*J. Med. Chem.,* December 1, 2022
GENENTECH, SO. SF, CA
paper DOI: https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01422

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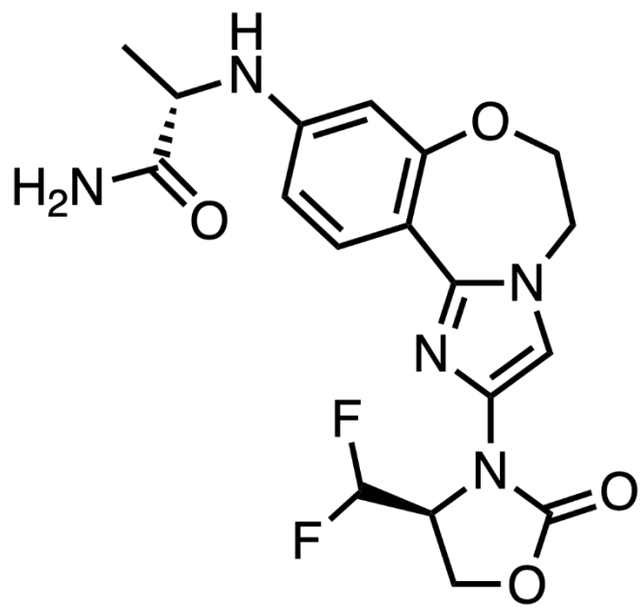
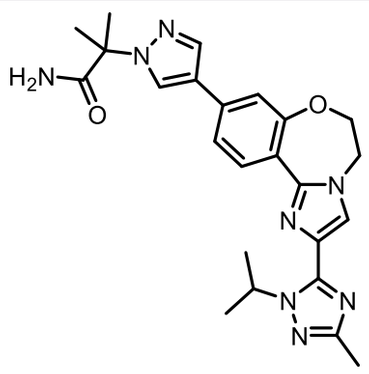


**What is it?** Inavolisib is a PI3Kα isoform-selective kinase inhibitor and monovalent degrader of the mutant p110α catalytic subunit of mutant PI3Kα. The molecule selectively depletes mutant p110α in cancer cells with active receptor tyrosine kinase (RTK) signaling and is in several ongoing or planned Ph. III trials for breast cancer.

**Why do we care?** PI3K is an extensively studied oncology target, and over 2 million cancer patients are diagnosed annually with mutations in PIK3CA, the gene encoding p110α. Despite the unmet medical need, it has been challenging to identify drugs for mutant PI3Kα with significant benefit due to the toxicities associated with wild-type PI3K-family inhibition and a negative feedback loop triggered by PI3K inhibition that activates RTK signaling, counteracting drug activity. By being highly selective for PI3Kα (>300-fold over δ) and selectively degrading mutant p110α by a mechanism that depends on RTK activation, inovalisib achieves a greater preclinical therapeutic window than previous PI3K inhibitors, including the recently approved PI3Kα inhibitor alpelisib. Given the importance of combination therapy in breast cancer, a greater therapeutic window in the clinic could allow for better outcomes due to the inherently toxic nature of most other combination agents like CDK4/6 inhibitors. The novel molecule is also an important demonstration of monovalent targeted protein degradation, an emerging drug modality of significant interest.

**How does it work?** Mutant p110α is more vulnerable than its WT counterpart degradation due to its shorter half-life, ubiquitination in the membrane, and proteasome-mediated turnover. RTK activity further promotes p110α degradation by recruiting it to the membrane. It has been suggested that inavolisib may enhance membrane binding through inducing conformational changes in p110α, leading to an acceleration in membrane-based ubiquitination and proteasome-mediated degradation. The combination of intrinsic isoform selectivity for PI3Kα with the functional selectivity imparted by selective degradation of mutant protein helps inavolisib achieve a greater therapeutic window against WT PI3K inhibition.

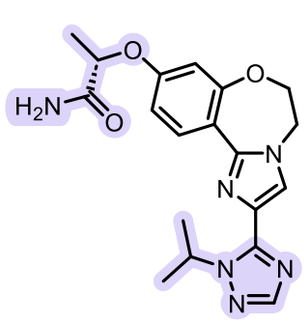
**How was it discovered?** While profiling PI3K inhibitors, Genentech scientists discovered that a prior clinical candidate, taselisib, was a much stronger antiproliferative agent in PIK3CA-mutant cancer cell lines than other PI3K inhibitors, which was not explained by isoform selectivity or general cellular potency. Inavolisib emerged from a campaign to discover a next-generation inhibitor to retain the mutant-selective degradation profile and favorable PK profile of taselisib, while improving PI3K isoform selectivity to avoid toxicities such as GI toxicities attributable to PI3Kδ inhibition.



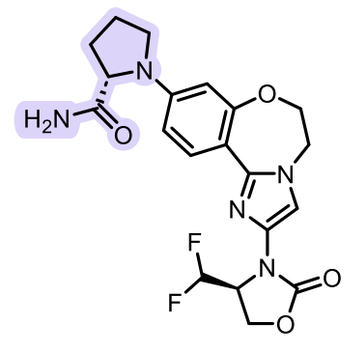
taselisib

**Key steps in lead optimization.** While previously reported "compound 1" (GDC-0326), a related analog of taselisib, exhibited 20-fold selectivity for PI3Kα over PI3Kδ, selectivity for mutant p110α was not observed. Replacement of oxygen-linked carboxamide with pyrrolidine-carboxamide linker improved bioactivity ($K_i$ = 0.026 nM) and exhibited degradation activity in a HCC1954 (breast cancer) cell line. To reduce TPSA for improved permeability and oral bioavailability, the triazole was replaced with a library of nonaromatic heterocycles equipped with a carbonyl to engage in a water-mediated hydrogen bond with Tyr836 and Asp 810 within the back pocket of the active site.

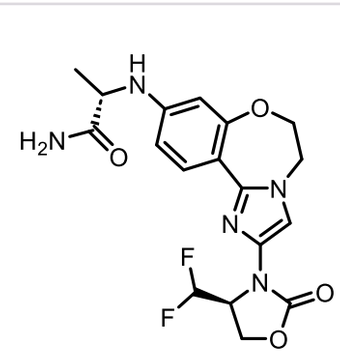
Installation of a 4-substituted-oxazolidinone in "compound 22" led to promising metabolic stability (HHep $CL_{hep}$ = 0.8 mL/min/kg) and in vitro permeability (MDCK $P_{app}$ = 4.3 x $10^{-6}$ cm/s) without compromising isoform selectivity. In addition, selective degradation of mutant p110α was observed for "compound 22" via western blot measuring phosphorylation reduction of



**compound 1 (GDC-0326)**
PI3Kα $K_i$ = 0.35 nM
PI3Kδ/PI3Kα activity = 22x
mutant/WT PI3Kα selectivity = 1.4x



**compound 22**
PI3Kα $K_i$ = 0.053 nM
PI3Kδ/PI3Kα activity = 34x
HHep $CL_{hep}$ = 0.8 mL/min/kg
F = 35%



**compound 32 (GDC-0077, inavolisib)**
PI3Kα $K_i$ = 0.034 nM
PI3Kδ/PI3Kα activity = 361x
HHep $CL_{hep}$ = 1.4 mL/min/kg
F = 58%

PRAS40 (pPRAS40) in mutant (PIK3CA-H1047R) vs WT (HDQ-P1). Finally, modification of the pyrrolidine-carboxamide to an alanine resulted in a compound with enhanced isoform selectivity (>300-fold) to yield "compound 32" (GDC-0077, inavolisib) with no detected WT protein degradation. The increased isoform selectivity is hypothesized to be due to improved binding energy from a non-canonical hydrogen bond between a fluorine and Ser774 within the P-loop of PI3Kα, which is absent in the equivalent Ser754 of PI3Kδ due to a 2.5 Å shift away from the active site (**PDB:** 8EXV). Within the oxazolidinone, the oxygen forms a hydrogen bond with water through the catalytic lysine (Lys802).

**Clinical application in mutant specific solid tumors.** Inavolisib currently has several ongoing Ph. I-II studies for various solid tumors, including HER+ and PIK3CA mutant breast cancers (NCT05306041, n=170), ER+/HER2- breast cancer with progression after CDK4/6i treatment (NCT04802759, n=510), and advanced or metastatic solid tumors with KRAS G12C mutation (NCT04449874, n=498). Two Ph. III trials for HR+/HER2-, PIK3CA-mutated locally advanced or metastatic breast cancer are currently in the works, the first assessing the combination of inavolisib plus a chemotherapy agent (palbociclib) and the ER antagonist fulvestrant after progression with adjuvant endocrine therapy (NCT04191499, n=400). The next Ph. III trial to initiate will assess the combination of inavolisib (9 mg PO QD) plus fulvestrant after progression with a CDK4/6i, with progression free survival as the primary outcome measure (NCT05646862, n=400).

**Patents.** Genentech holds patents for the synthesis of benzoxazepin oxazolidinone compounds (WO2022251567A1, 2022) and the treatment of breast cancer with combinations containing gdc-9545 and gdc-0077 (WO2022177844A1). Genentech and Hoffman-La Roche hold a patent for the treatment of HER2+ cancer with combination therapies (WO2022125483A1, 2022).

December 2022

# BI 685509

## sGC

oral sGC activator
Ph. II for CKD & DKD
discovery not disclosed
*J. Pharmacol. Exp. Ther.,* December 6, 2022
BOEHRINGER INGELHEIM PHARMACEUTICALS, CT
paper DOI: https://doi.org/10.1124/jpet.122.001423

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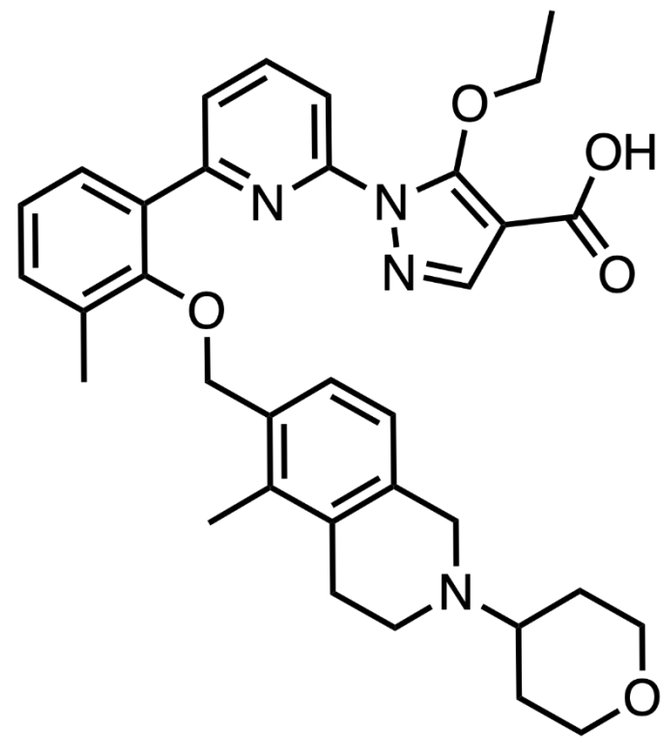
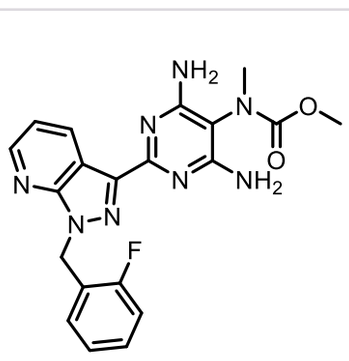
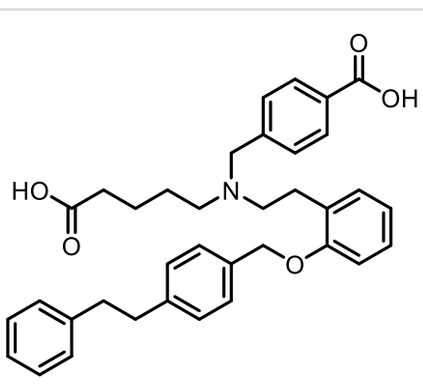


**A newly disclosed sGC activator in Ph. II for CKD/DKD.** This month, researchers at BI reported promising preclinical results for a newly disclosed sGC activator, BI-685509, which is in several Ph. II trials including for treatment of chronic kidney disease (CKD) and diabetic kidney disease (DKD) (NCT04736628, NCT04750577). In rodents, the molecule demonstrates efficacy both in combination with a standard therapy for CKD/DKD, enalapril, and anti-fibrotic activity as a single agent.

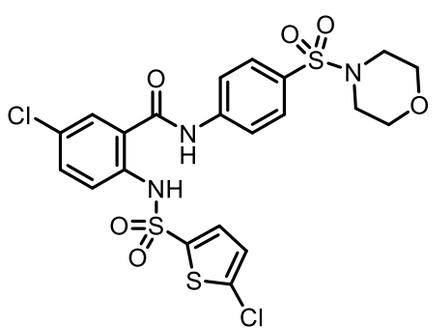
**Why is it important?** Nitric oxide (NO) is a key signaling molecule for cardiovascular functions, acting through a key mediator, soluble guanylate cyclase (sGC), in the NO-sGC-cGMP pathway. This pathway has been modulated by drugs for almost 150 years, from nitric oxide itself, to PDE5 inhibitors like sildenafil (Viagra) which degrade cGMP downstream of sGC. Small molecules that activate sGC in the presence of NO are called sGC stimulators, and have been approved for pulmonary hypertension (riociguat, 2013) and recently to treat heart failure (vericiguat, 2021). In contrast, small molecules that activate sGC independent of NO are called sGC activators, bind in the absence of heme to stabilize the sGC enzyme in an active state, have distinct pharmacology, and have not yet been approved in any indication, despite starting points being identified as early as 1997. First-generation sGC activators cinaciguat (BAY 58-2667) and ataciguat (HMR1766) entered Ph. II trials for treatment of cardiovascular conditions and neuropathic pain, but the trials were discontinued due to negative hypotensive events and other limitations. With its distinct chemical structure, BI- 685509 represents another opportunity to find clinical benefit in the field and evaluate whether previously observed adverse effects can be avoided or managed in different settings.



**riociguat (BAY 63-2521)**
sGC stimulator



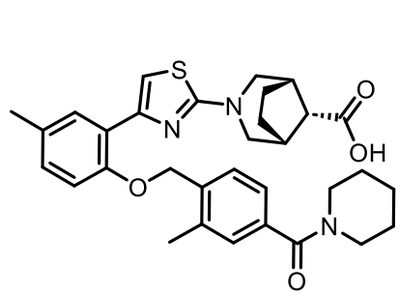
**cinaciguat (BAY 58-2667)**
sGC activator



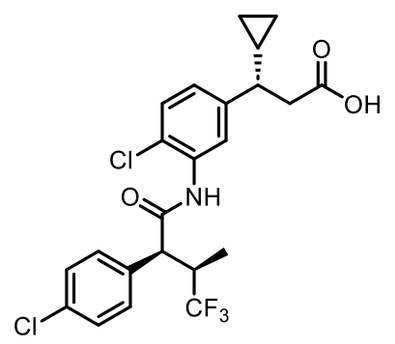
**ataciguat (HMR1766)**
sGC activator

**What other sGC activators are out there?** BI previously disclosed sGC activator BI-703704 in 2015, but there have been no subsequent studies or trials reported. Another sGC activator and a Molecule of the Month in April of 2021, Bayer's runcaciguat (BAY 1101042), is also in Ph. II trials for CKD and diabetic retinopathy (NCT04507061, NCT04722991). While the BI-685509 discovery story has yet to be disclosed, it is noteworthy that all disclosed "second-generation" sGC activators are monocarboxylic acids, suggestive of a related binding mode.

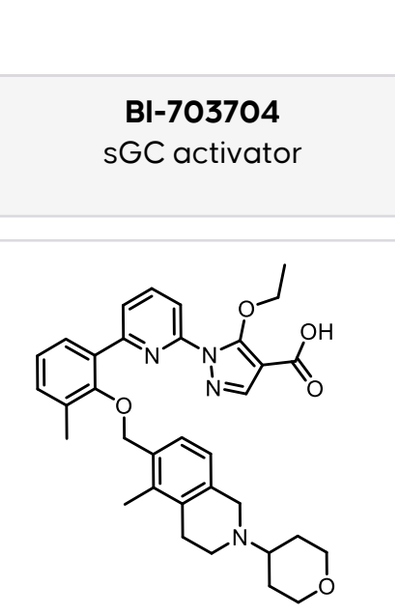
The overall structures of the Bayer and BI compounds are quite different however, suggesting different starting points or discovery strategies. GSK previously reported a tool compound (GSK2181236A) with a similar core structure in 2012.



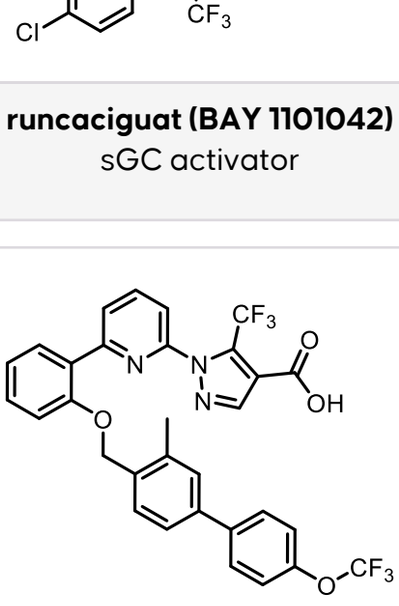
**BI-703704**
sGC activator



**runcaciguat (BAY 1101042)**
sGC activator



**BI-685509**
sGC activator



**GSK2181236A**
sGC activator

**Why do sGC activators differentiate from sGC stimulators?** Reduced cGMP levels have been implicated in the pathophysiology of various diseases involving oxidative stress, including diabetes, cardiovascular and kidney disease. Thus, restoring cGMP levels by enhancing sGC activity is proposed to be a therapeutic solution for these indications. sGC is a heterodimeric protein with both α- and β-subunits and an NO-binding heme unit. When NO binds to the heme, a conformational change of this oxidized state activates the catalytic site, converting GTP to cGMP, which acts as a secondary messenger and key modulator of several downstream targets. Stimulators and activators both increase sGC enzymatic activity, but in different ways: stimulators enhance activity of the oxidized heme ($Fe^{3+}$) form of sGC activity in the presence of NO, and activators enhance activity of the reduced form ($Fe^{2+}$) of sGC, independently of NO. Though sGC stimulators have demonstrated greater clinical successes to-date, they are inactive in states of oxidative stress. Under these conditions, the sGC heme can be lost, rendering the target unresponsive to both endogenous and exogenous NO or sGC stimulation due to the requirement of NO binding to the heme for this MOA.

**What's next for sGC activators?** With the recent approval of a second sGC stimulator, increasing sGC activity is now well-validated. The question now is, in what settings will sGC activators provide greater benefit than sGC stimulators? Biological studies enabled by the now available high-quality molecules will point to different indications, and empirical clinical data in the different settings will be closely watched.

**Patents.** Boehringer Ingelheim currently holds two patents for alkoxy pyrazole soluble guanylate cyclase activators (WO2014039434A1, 2014 and WO2020011804A1, 2020)

December 2022

# DN-1289

## DLK/LZK

selective, CNS-penetrant dual DLK/LZK inhibitor
neuroprotective effects for in vivo + in
vitro models for ALS
LBDD/SBDD + opt. from known molecule
*J. Med. Chem.,* December 5, 2022
DENALI THERAPEUTICS, SO. SF, CA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01056

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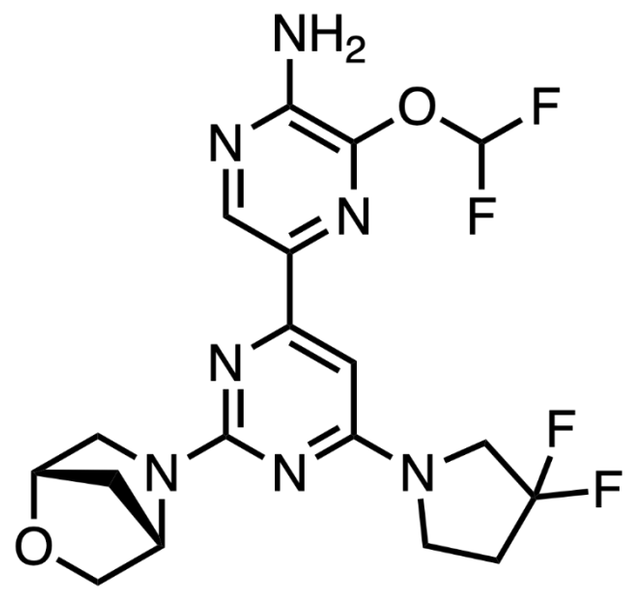
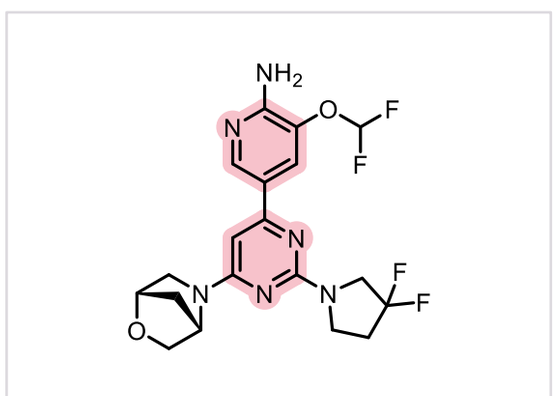


**What is it?** DN-1289 is a CNS-penetrant, ATP-competitive DLK/LZK-selective dual kinase inhibitor intended to treat amyotrophic lateral sclerosis (ALS). The molecule is structurally closely related to Genentech's recent Ph. I candidate, GDC-0134.

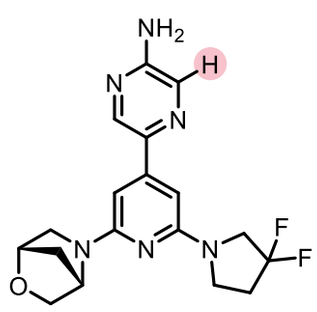
**Why do we care?** Dual leucine zipper kinase (DLK) and leucine zipper-bearing kinase (LZK) are involved in both neurodegeneration and axon growth, and DLK in particular is a target of interest for neurodegenerative diseases including AD, Parkinson's, and ALS. DLK regulates neuronal apoptosis through JNK kinase activation, and loss of DLK signaling has been shown to be neuroprotective in at least 12 different preclinical models. The leading DLK inhibitor in clinical development, Genentech's GDC-0134, recently completed Ph. I trials (NCT03807739, NCT03237741, NCT02655614), with dosing up to 1200 mg daily.

**A target marred by ocular toxicity.** Unfortunately, development of DLK/LZK inhibitor GDC-0134 was discontinued due to an unacceptable safety profile for ALS. In particular, despite unremarkable findings in the 28-day MAD portion of the study, a single 69-year-old patient developed irreversible bilateral blindness from optic ischemic neuropathy at 600 mg BID, the highest dose level, and no patients continued the study at the lower doses. The findings were believed to be drug-related, and ocular exams found a potential association between GDC-0134 and retinal or optic nerve toxicity. While Genentech had observed retinal abnormalities in non-human primates (NHPs), they were distinct from those observed in humans. The publication of this molecule by Denali and associated preclinical data show Denali has also had a keen interest in DLK as a target but may have also paused development based on Genentech's findings. However, Denali has been successful in advancing a molecule (DNL201) in the past where previous safety data posed a major barrier, so this will be an area to continue to watch.

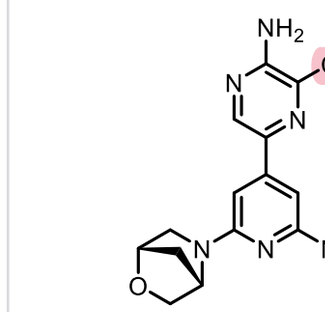


**GDC-0134**

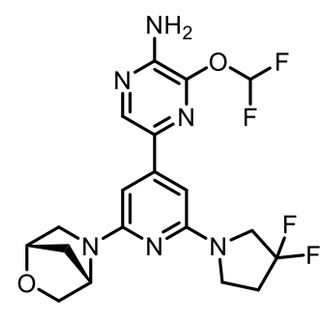
**How was DN-1289 discovered?** Beginning with GDC-0134, Denali scientists replaced the electron-rich pyridine with a pyrazine and changed the connectivity of the bottom pyrimidine. The difluoromethoxy group on the pyrazine had a remarkable influence on potency (>10x potency improvement relative to $-CF_3$, >50x relative to -H) (PDB of analog: 8DEG). Difluoromethoxy groups are often proposed but due to the challenge of their synthesis, this is a nice example of this group being required.



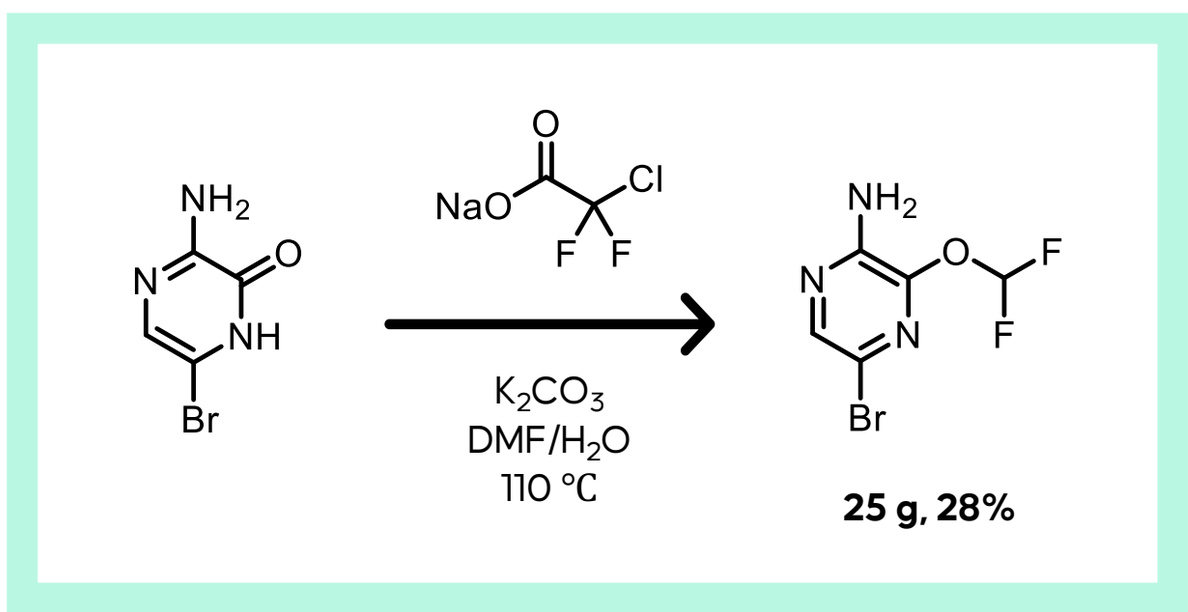
**compound 5**
DLK $IC_{50}$ = 201 nM
LZK $IC_{50}$ = 247 nM



**compound 6**
DLK $IC_{50}$ = 53 nM
LZK $IC_{50}$ = 149 nM



**compound 7**
DLK $IC_{50}$ = 4 nM
LZK $IC_{50}$ = 7 nM



December 2022

# paltusotine

## SST2

oral, non-peptide SST2 agonist
Ph. III for acromegaly + Ph. II for NETs
SAR on in-house library hit
*ACS Med. Chem. Lett.,* December 10, 2022
CRINETICS PHARMACEUTICALS, CA
paper DOI: https://doi.org/10.1021/acsmedchemlett.2c00431

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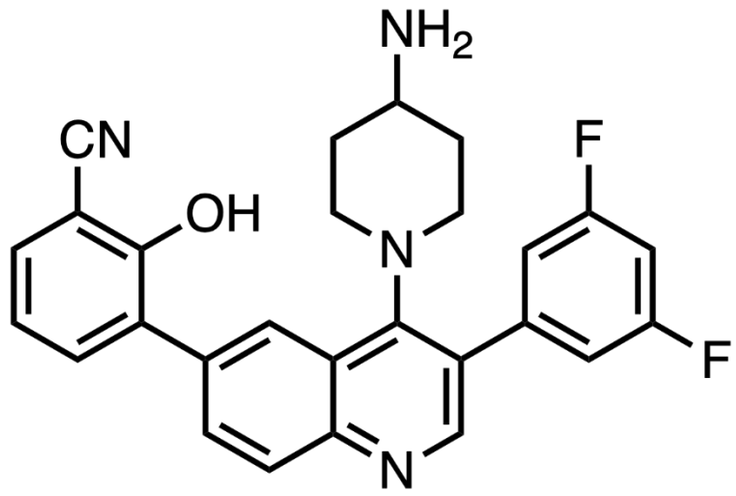
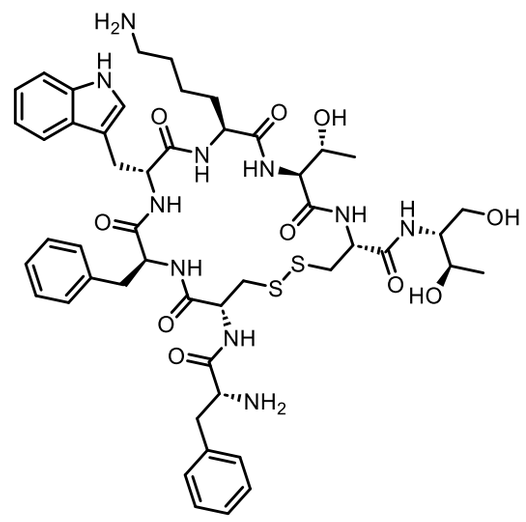
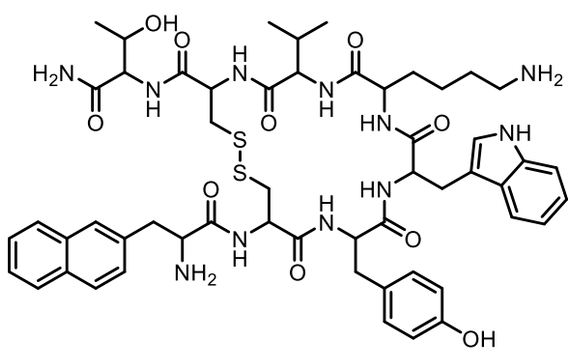
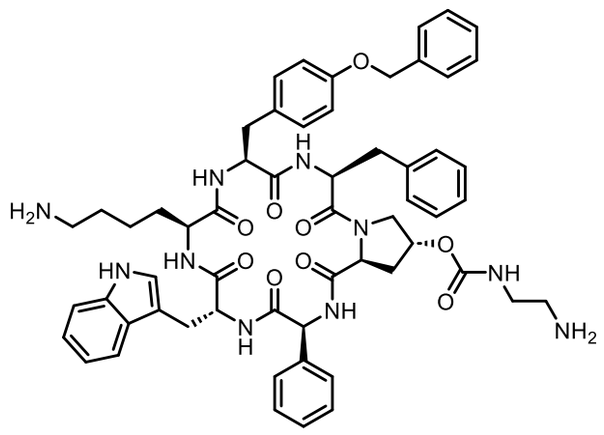




**octreotide**



**lanreotide**



**pasireotide**

**Toward an oral, once-daily non-peptide STT2 agonist.** Paltusotine (CRN00808), a novel oral somatostatin receptor 2 (SST2) agonist and Crinetics' lead investigational drug (WO2022251212A2), is intended to treat acromegaly and neuroendocrine tumors (NETs) in carcinoid syndrome. Both diseases have been treated with surgery and injectable somatostatin analogues for disease control, such as octreotide (Sandostatin), lanreotide (Somatuline), targeting somatostatin receptor subtype 2 (SST2) and pasireotide (Signifor), targeting SST5. Although some of these peptide injectables are long-acting (monthly), delivered IM or SC, the burden of injectables detrimentally affects patient quality of life and treatment adherence. An oral option would be preferable for patients as evidenced by the fact that in Ph. II studies, paltusotine administered as a once-daily oral medication was preferred by 89% of patients (n = 32) over injections. Interestingly for chemists, this oral molecule is likely zwitterionic with an acidic cyanophenol and basic amine.

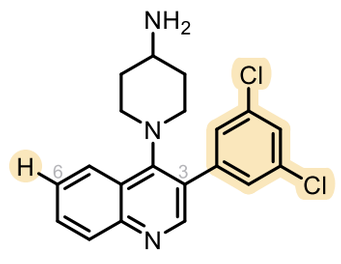
**Controlling overexpression of growth hormone in rare diseases by targeting STT2.** Normally within the pituitary gland, SST2 modulates antisecretory effects by inhibiting GH secretion, decreasing IGF-1 production in the liver. Acromegaly, which affects 2.8–13.7 per 100,000 people per year, is caused by a pituitary adenoma, which results in prolonged overexpression of GH and high secretion of IGF-1. NETs, which affect 35 per 100,000 people per year, arise from heterogeneous neoplasms leading to malignancies throughout the body, where STT2 receptors are highly expressed in the tumors. SST2 agonism inhibits the secretion of growth hormone, which drives both diseases. In healthy adults, paltusotine significantly reduces stimulated GH secretion at doses between 2.5 and 20 mg. In a completed Ph. II trial (NCT03789656) for acromegaly, patients were switched from injectable SRLs to paltusotine for 13-weeks and given once-daily doses of 10 mg up to 40 mg/day. At the end of the study, 87% of patients (n = 20) maintained IGF-1 levels lower than baseline or within a 20% window. Paltusotine has advanced to two Ph. III trials for acromegaly, one measuring safety and efficacy in patients after previous treatment with SRLs (NCT04837040) and the other in patients that were previously untreated (NCT05192382).

**How was it discovered?** "Compound 8" (hSST2 $EC_{50}$ = 140 nM) was discovered as a hit from a small, in-house library of diverse scaffolds using an in vitro human SST2 cAMP functional assay. The lead optimization campaign focused on obtaining an orally available candidate with desirable drug-like properties while preserving the 4-(4-amino-piperidinyl)-quinoline core. After exchanging the 3,5-chlorines for fluorines on the 6-phenyl to optimize drug-likeness, initial SAR studies at the 6-aryl position of "compound 8" revealed that installation of a phenyl ring enhanced potency 50-fold ($EC_{50}$ from 1600 nM to 9.2 nM). Additional substitutions at the 3-aryl position demonstrated the need for a lipophilic moiety exchange to an isosteric 2-methoxypyridine reduced potency 200-fold ($EC_{50}$ = 50 nM), leaving the 3,5-disubstituted fluorophenyl as the optimal ligand. Further efforts to functionalize the 6-phenyl ring with a 3-cyano-2-hydroxy group resulted in "compound 22" (paltusotine) with subnanomolar potency ($EC_{50}$ = 0.25 nM) and >4000 selectivity over other somatostatin receptors. Paltusotine exhibited metabolic stability (LM (human) $t_{1/2}$ = 66 min), low hERG inhibition ($IC_{50}$ values > 10 µM), and minimal CYP liabilities ($pIC_{50}$ = 5.1 and 5.3 µM for CYP2C9 and CYP2C19, respectively, no inhibition detected for CYP2D6 and CYP3A4 up to 10 µM).

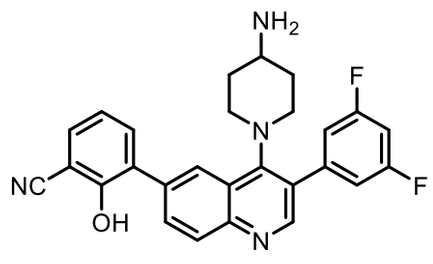
**Oral availability in dogs and humans despite poor rat bioavailability.** Interestingly, rats exhibited poor bioavailability (F = 8.8%) with a short half-life ($t_{1/2}$ = 2.8 h) compared to studies in beagles (F = 48%, $t_{1/2}$ = 7.5 h). Efflux of paltusotine as a P-gp substrate in rats is thought to contribute to its poor oral bioavailability. Using daily dosing at 20 mg, the half-life in humans was 50 h compared to 30 h for a single 20 mg dose, both supporting an oral once-daily dosing strategy. Paltusotine exhibited high absolute oral bioavailability (F = 70%) in humans using an oral 20 mg dose in five healthy males in a Ph. I study. This is a nice example of a drug being successful in man despite P-gp efflux and low rat bioavailability.

**What's next?** An improved oral formulation (i.e., spray dried dispersion tablet) of the drug will be used in Ph. III studies to enable higher doses up to 80 mg and reduces the fasting requirement to 1h. An ongoing, Ph. II open label extension study for acromegaly (NCT04261712) has shown that paltusotine is well tolerated and was able to reduce and normalize IGF-1 concentrations for up to 103 weeks. Paltusotine is also being explored for efficacy against NETs in a Ph. II trial using 40 and 80 mg doses of drug (NCT05361668).

**What else is out there?** Pasireotide exhibits greater efficacy than octreotide but suffers from a safety profile related to SST5 and its effect on glucose metabolism (i.e., hyperglycemia), and so is not prescribed first-line. Octreotide, which has been used for the treatment of acromegaly for over 30 years, was approved in 2020 as a twice-daily oral formulation leading to Chiasma's acquisition by Amryt Pharma in 2021. Apart from paltusotine, which is currently in Ph. II/III trials, there are only a few SRLs under investigation, including somatropim (also referred to as DG3173, completed Ph. II, SC), ONO-5788 (in Ph. I, oral) and ONO-ST-468 (preclinical, oral).



**compound 8**
hSST2 $EC_{50}$ = 140 nM
hSST4 $EC_{50}$ = >10,000 nM



**paltusotine (compound 22)**
hSST2 $EC_{50}$ = 0.25 nM
hSST4 $EC_{50}$ = ~1,000 nM

December 2022

# SPR720

## GyrB

oral GyrB/ParE inhibitor
Ph. II for MAC pulmonary disease
HTS using a GyrB ATPase assay
*bioRxiv,* December 9, 2022
SPERO THERAPEUTICS, CAMBRIDGE, MA
paper DOI: https://doi.org/10.1101/2022.12.08.519697

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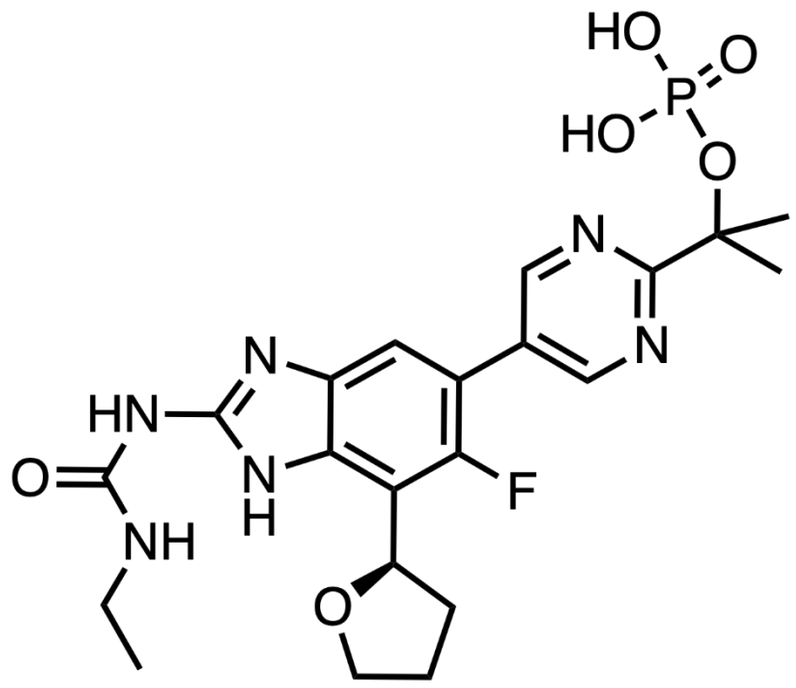


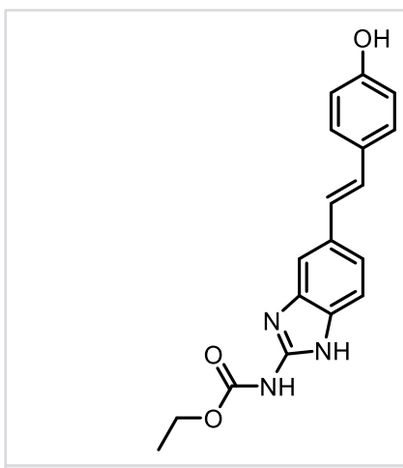
**What is it?** SPR720 (pVXc-486) is an oral prodrug of a dual inhibitor of the bacterial DNA gyrase and topoisomerase IV ATPase catalytic domains, GyrB and ParE. The molecule was in-licensed by Spero Therapeutics, a 2014 "Fierce 15" startup, from Vertex (WO2014014845A1, US20140031318), and is now Spero's lead development program after Spero was issued a CRL for tebipenem and out licensed tebipenem to GSK. Last year, the FDA lifted its clinical hold on SPR720, which was originally placed due to mortality findings in non-human primates (NHPs), despite demonstration of safety in a Ph. I trial (NCT03796910, 500-1,500 mg QD). The molecule now continues its Ph. II study in nontuberculous mycobacterial pulmonary disease (NTM-PD) due to *Mycobacterium avium* complex (MAC) (NCT05496374).

**Why do we care?** If successful, SPR720 would become the first oral antibiotic approved for NTM-PD. NTM-PD is a rare but increasingly prevalent condition with no approved oral antibiotic options that currently requires 12-24 months of therapy with combinations of primarily unapproved antibiotics and is frequently complicated by tolerability and/or toxicity issues. Non-tuberculous mycobacteria can cause respiratory failure in patients with compromised immune systems or lung disease. Preclinically, the molecule has shown activity in clinical isolates, activity against a virulent multidrug-resistant *M. abscessus* strain, and combination activity with clarithromycin (23S ribosome inhibitor) and ethambutol (cell wall synthesis inhibitor). It has also been shown to penetrate human monocytes, which is important for targeting intracellular bacteria, and not always trivial to achieve given the polar nature of bacteria-penetrating compounds. NTM-PD treatment often includes rifamycins, RNA polymerase inhibitors which have serious side effects and cause drug-drug interactions due to potent CYP450 and transporter induction.

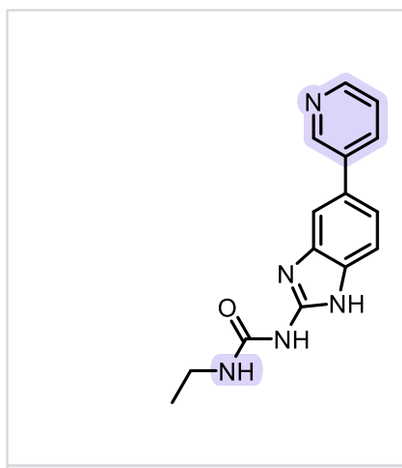
If SPR720 continues to demonstrate efficacy with limited side effects, it has the opportunity to substitute for rifamycins as a new standard of care and provide a new option for treating drug-resistance bacterial infections in other indications.

**How does it work?** DNA gyrase and topoisomerase IV (topoIV) are well-established antibiotic targets that are essential for bacterial replication but have no human homologs. SPR719 works via a novel mechanism by targeting the ATP-binding sites of the ATPase domains of gyrase and topoisomerase IV (GyrB and ParE, respectively). In contrast, fluoroquinolone drugs inhibit the catalytic subunits of gyrase and/or topoIV. It is statistically much less likely for resistance to develop to a dual inhibitor of two independently essential targets (~1 in $10^{14}$ bacteria). Given the distinct MoA of SPR719 from existing drug classes and its dual inhibition property, combinations involving SPR719 may have durable activity and counteract resistance in this setting.

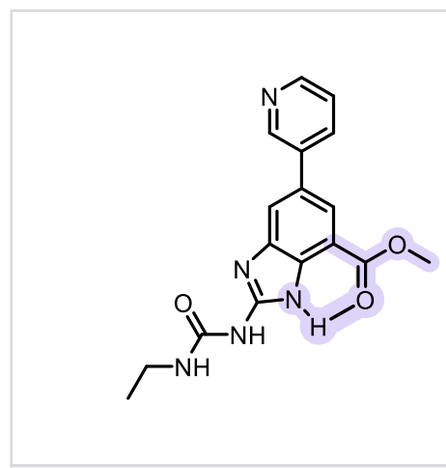
**How was it discovered?** The benzimidazole urea starting point was discovered by Vertex (2008) via a 30k compound HTS in high-throughput ATPase assay targeting GyrB. Crystal structures of the structurally unrelated natural product novobiocin bound to *E. coli* GyrB (PDE: 1EI1) were used to guide optimization. Modeling suggested that changing the carbamate to a urea would change a repulsive interaction with an aspartate into an attractive one. Modeling also identified an opportunity to form a hydrogen bond with an arginine by introducing a hydrogen-bond acceptor into the styrene group. The combination of these changes resulted in a whopping 250-fold potency increase ("compound 3"), highlighting how a few atoms can make all the difference.



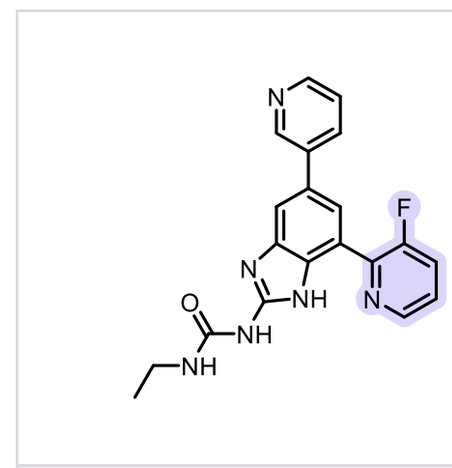
**compound 1**
*E. coli* GyrB $IC_{50}$ = 20 µM
*E. coli* topoIV $IC_{50}$ = >60 µM
serum-free *S. aureus* MIC = >16 µg/mL



**compound 2**
*E. coli* gyrase $IC_{50}$ = 81 nM
*E. coli* topoIV $IC_{50}$ = 2300 nM
serum-free *S. aureus* MIC = 16 µg/mL



**compound 10**
*E. coli* gyrase $IC_{50}$ = <4 nM
*E. coli* topoIV $IC_{50}$ = 35 nM
serum-free *S. aureus* MIC = 0.063 µg/mL



**compound 15**
*E. coli* gyrase $IC_{50}$ = <4 nM
*E. coli* topoIV $IC_{50}$ = 23 nM
serum-free *S. aureus* MIC = 0.031 µg/mL
50% HS *S. aureus* MIC = 0.5 µg/mL
*poor ADME properties*

December 2022

# SPR720

## GyrB

oral GyrB/ParE inhibitor
Ph. II for MAC pulmonary disease
HTS using a GyrB ATPase assay
*bioRxiv,* December 9, 2022
SPERO THERAPEUTICS, CAMBRIDGE, MA
paper DOI: https://doi.org/10.1101/2022.12.08.519697

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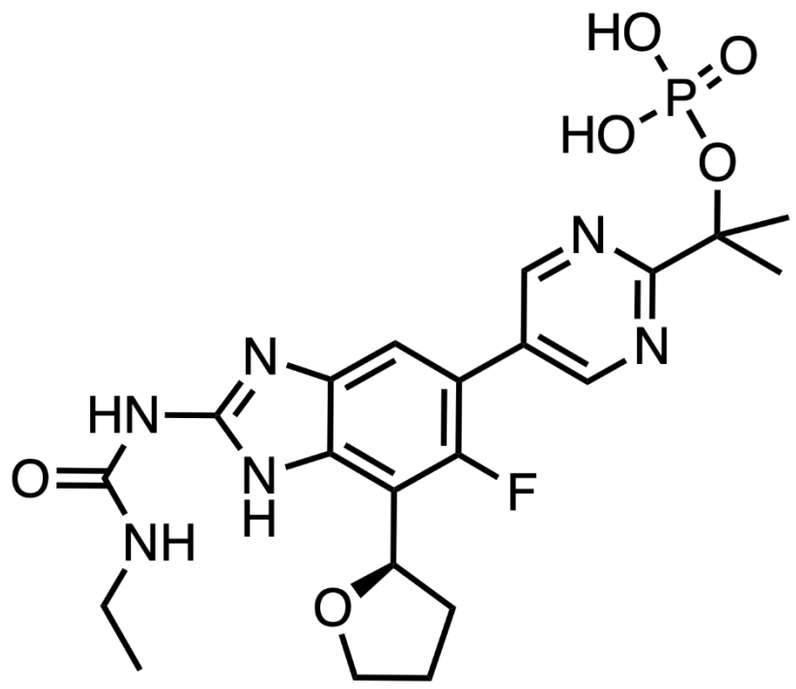
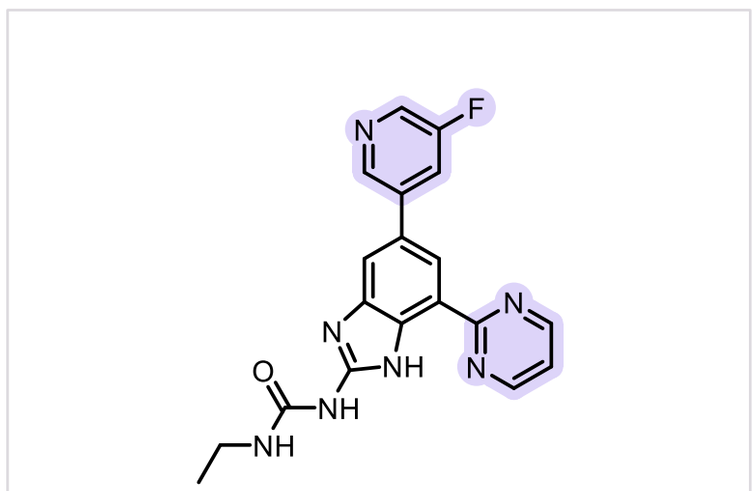



The urea binds to an aspartate in a bidentate manner, and the pyridine hydrogen bonds to an arginine (related analog PDB: 4P8O). Filling space with an ester ("compound 10") led to a surprisingly large increase in potency against both gyrase and topoisomerase. An intramolecular hydrogen bond with the imidazole enforcing a planar orientation was found to be important, so hydrogen-bonding isosteres were evaluated, with 2-pyridyl being optimal. Finally, fluorination of the pyridine ("compound 15") was found to reduce serum-shift in potency, leading to Vertex's first-generation in-vivo tool compound.

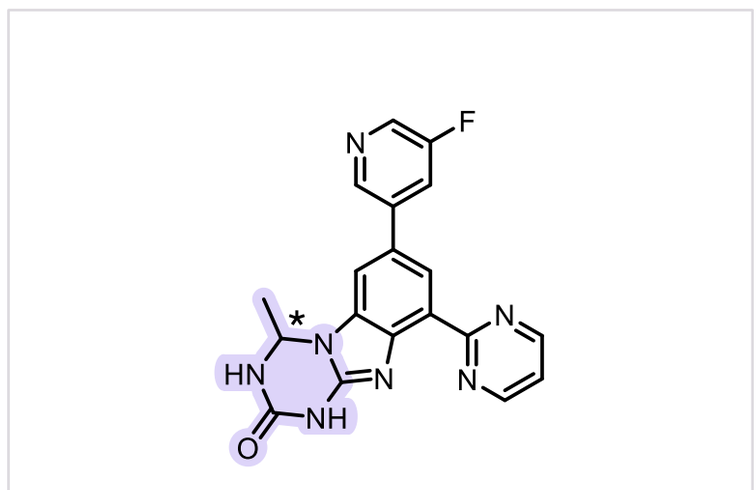
**Addressing a surprising reactive metabolite from the urea with a "metabolic shift" approach.** "Compound 15" was potent against bacterial strains in rodent models, but was rapidly metabolized, had poor physicochemical properties, and potently inhibited CYP3A4. By moving the fluorine atom to a neighboring ring and changing one of the pyridines to the privileged pyrimidine heterocycle, the resulting "compound 3" had low nanomolar activity against GyrB and ParE ($IC_{50}$ = 5 nM, <6nM) and significantly improved ADME, but was found to covalently modify liver proteins both in vivo and in vitro, a safety liability.

It turned out the *N*-alkylurea was responsible for labeling due to oxidation to a reactive imine intermediate, which is stabilized through cyclization onto the benzimidazole. Unfortunately, despite numerous attempts, any modification of the urea abolished activity. A "metabolic shift" strategy was employed instead, introducing metabolically labile groups elsewhere in the molecule to change the route of metabolism away from the urea. The tetrahydrofuran group served this purpose, maintaining potency but shifting metabolism to this moiety instead. Unfortunately, the compound had poor solubility, and to address this, a team at Vertex created a water soluble prodrug by changing the tertiary alcohol to a phosphate ester, resulting in SPR720.

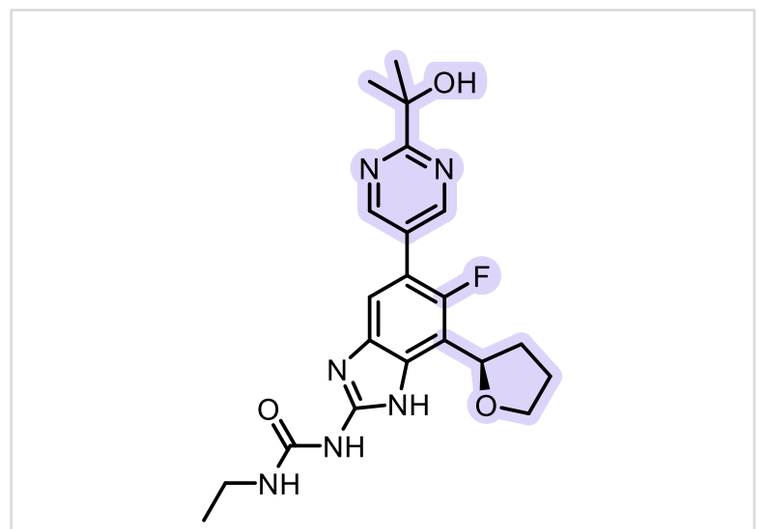



**covalent liver protein labeling**

**compound 3**
GyrB $IC_{50}$ = 5 nM
ParE $IC_{50}$ = <6 nM




**reactive intermediate**




**lack of liver labeling**

**compound 34 (SPR719, VXc-486)**
GyrB $IC_{50}$ = 6 nM
ParE $IC_{50}$ = <12 nM

December 2022

# emraclidine

## M4

M4 positive allosteric modulator
Ph. II for schizophrenia
discovery not disclosed
*Lancet,* December 17, 2022
CEREVEL THERAPEUTICS, CAMBRIDGE, MA
paper DOI: https://doi.org/10.1016/S0140-6736(22)01990-0

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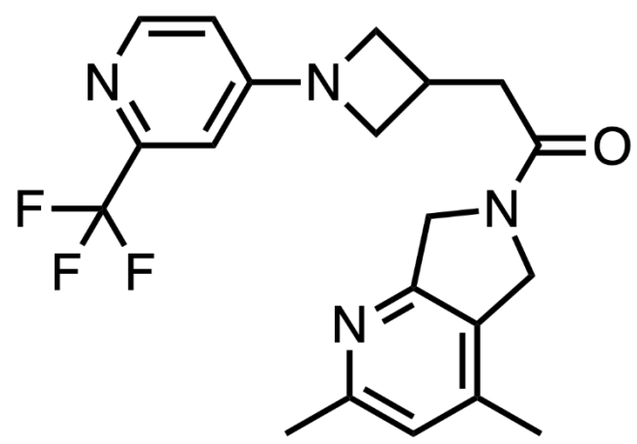


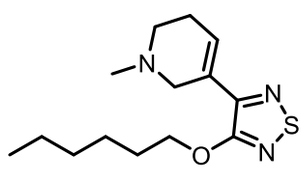
**What is it?** Emraclidine (CVL-231) is a selective positive allosteric modulator (PAM) of the cholinergic M4 muscarinic receptor (GCPR) and is the only selective M4 PAM in clinical development. The molecule was highlighted by Cerevel at JPM '23 as it has shown promising early results in schizophrenia with several Ph. II trials enrolling (NCT05443724, NCT05227703, NCT05227690), and is starting elderly healthy volunteer studies to support development in Alzheimer's Disease psychosis (ADP) at 2-30 mg QD. The molecule demonstrates high brain penetration and target engagement based on PET studies in rhesus macaques using an M4 PAM radioligand ([11C]MK-6884).

**Why do we care?** Schizophrenia affects an estimated 24 million people worldwide. For decades, treatments focused on modulating D2 dopamine receptors which are associated with psychosis symptoms, and current antipsychotics are all thought to antagonize various dopamine receptors. These dopamine-modulating antipsychotics, however, come with a plethora of side effects including weight gain and cardiovascular issues in the long-term. Outcomes with antipsychotics are varied, as some patients will experience prolonged remission and may reach a possibility of non-treatment, while the majority of patients will remain at risk of relapse (~80%) with some experiencing treatment resistance in extreme cases. If a drug with a novel MoA like emraclidine is able to demonstrate durable antipsychotic efficacy with fewer side effects, it could see significant adoption in schizophrenia as well as other neurological diseases where psychosis manifests, including Alzheimer's and Parkinson's. So far, results from the Ph. Ib (NCT04136873) trial showed emraclidine treatment improved symptoms as measured by PANSS and CGI-S scores at 30 mg PO QD and 20 mg PO BID, with similar levels of adverse events across treatment and placebo groups (5-30 mg PO QD and 5-20 mg PO BID).

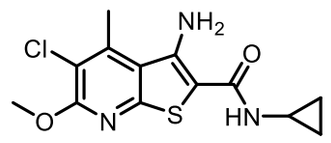
**How does it work?** As a positive allosteric modulator of M4, emraclidine activates M4 in the presence of acetylcholine, indirectly lowering dopamine output without direct D2/D3 receptor inhibition that is suspected to cause unwanted motor side effects of existing treatments. It could therefore help address psychosis, agitation, and other neurological issues in schizophrenia and which manifest in diseases like Alzheimer's or Parkinson's. The therapeutic hypothesis originates from clinical findings in the 90's with xanomeline, a full M1/M4 muscarinic agonist, which serendipitously showed dose-dependent improvements in psychosis, cognition, agitation, and aggression in schizophrenia and Alzheimer's. However, its development was halted due to severe GI side effects attributable to M2 and M3 receptor activation, leading to a >50% discontinuation rate. M1 activation has also been linked to undesired cardiovascular and GI issues. M4 knockout experiments have supported the notion that a more selective M4 activator would be effective without the same degree of GI effects.

**Why is this different?** Emraclidine is >390x selective for M4 over M1/2/3/5 in vitro. In contrast to full agonists, as a positive allosteric modulator, its activity is dependent on acetylcholine levels and may not lead to excessive stimulation of M4-expressing neurons, receptor desensitization, and loss of efficacy. Emraclidine is also claimed to have a low potential for drug-drug interactions which is important in schizophrenia where numerous drugs are used in combination.

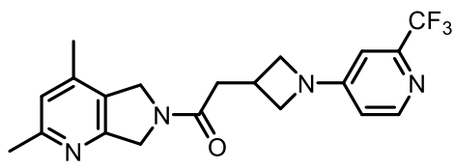
**How was it discovered?** The molecule is one of several Pfizer neurology assets that were spun-off via licenses to Cerevel (WO2018002760A1, compound 11, 2017; US20210309659A1, 2021). The molecule is structurally related to Lilly's original M4 PAM tool molecule first reported in 2008 from screening.



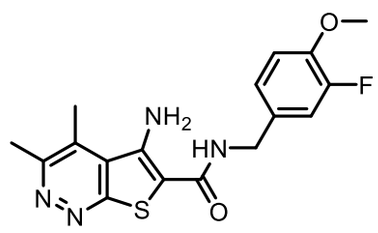
**xanomeline**
M1/M4 receptor agonist



**LY2033298**



**emraclidine**



**VU0467485/AZ13713945**

**What else is out there?** Xanomeline continues to be developed now by Karuna Therapeutics (KRTX) in a combination treatment (KarXT) with trospium, a non-brain-penetrant, orthosteric muscarinic antagonist, to mitigate the activation of muscarinic receptors outside of the brain. In 2019, Karuna disclosed positive Ph. II data in schizophrenia, an important proof-of-concept. As of the date of publication, Karuna had a nearly $7B public valuation in a depressed biotech market, suggesting a favorable view of the likelihood and consequence for success in this area. Other mAChR agonists in development include Neurocrine's HTL0016878, a selective M4 receptor agonist in Ph. I (NCT04935320) and Maplight's dual M1/M4 receptor-preferring agonist ML-007 in Ph. I. Preclinical molecules include orthosteric M4 agonists from Sosei Heptares and Sumitomo Dainippon and various M4 PAMs such as from Addex Therapeutics and Vanderbilt University.

**What's next?** A main concern for an M4 modulator would be on-target cholinergic side effects, and in its Ph. I study, transient treatment-related increases in heart rate and blood pressure were observed following single doses of emraclidine (>10 mg), which may be due to on-target activity in either the CNS or PNS. In Dec. 2017, Pfizer had already completed a Ph. I SAD trial (C2561001) on the safety and tolerability of emraclidine at doses between 0.3-30 mg, showing side effects were mild to moderate. Dose-related, asymptomatic, and transient effects on blood pressure and heart rate were detected, especially at the highest 30 mg dose. A single moderately severe hypotension event occurred at 30 mg but resolved within 2 h without intervention. In a 13-week canine study, the heart rate increases were found to mostly resolve over longer dosing periods.

Given the reversible, monitorable, and quickly resolved nature of the effects, Cerevel believes they can be addressed through dose titration. To better understand human PK/PD and optimize the dose, Cerevel is measuring target occupancy in the brain in a healthy population (NCT04787302, n=15). To assess safety/tolerability in anticipation of Alzheimer's disease trials, an elderly healthy volunteer study is also being conducted (NCT05644977, n=50).

December 2022

# compound 25

## LRRK2

selective, CNS-penetrant LRRK2 kinase inhibitor
preclinical, low projected human dose
opt. of MLi-2 series, from HTS
*J. Med. Chem.,* December 7, 2022
MERCK, BOSTON, MA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01605

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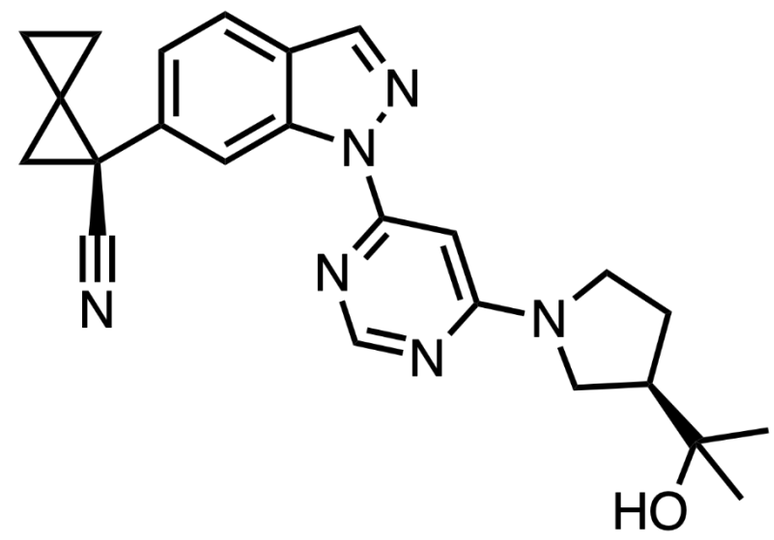
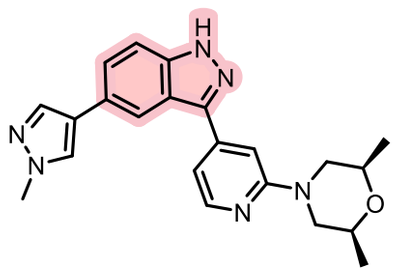


**What is it?** "Compound 25" is a selective, CNS-penetrant, ATP-competitive LRRK2 inhibitor that entered preclinical candidate enabling studies but was discontinued for undisclosed reasons. The molecule possesses two interesting chemical features: a "bow-tie" spirocyclopropyl group that made a significant impact on potency and overall properties, and a non-classical C-H hinge binder. The molecule was nominated by reviewer Dennis Koester, who says:

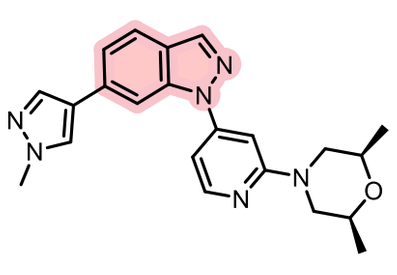
"Although they terminated the compound for undisclosed but scientific reasons, I think it is a wonderful drug discovery story. Of note for a CNS target, they only looked at BCRP late in the program and discovered the compound to be a BCRP substrate and inhibitor leading to a low $Kp_{u,u}$ in NHPs compared to rats. The compound was not an MDR1 substrate. I found the entire article super interesting."

**Why do we care?** LRRK2 is a well-established target for Parkinson's, for which several molecules have been highlighted, including Merck's MLi-2, the progenitor of this compound. Preclinical safety studies with Merck and Genentech's compounds led to concerns about the target, but Denali has re-opened the field with progress on a clinical candidate. This molecule is interesting both as a potential indicator that Merck continued research on LRRK2 after its NHP findings with MLi-2, and more fundamentally as a case study for lead optimization.

**How was it discovered?** The molecule comes from optimization of indazole MLi-2, which in turn originated from a high throughput screening hit from the Merck collection. MLi-2 had poor cross-species PK resulting in a high projected human dose (EDE = 3.9 g BID), and its chemical series carried safety risks including for drug-induced liver injury due to bioactivation and genotoxicity. Optimization focused on PK improvements with a focus on CNS-penetration (e.g., lower HBA/HBD counts, molecular efficiency) and lower human dose using early dose estimates based on unbound cell $IC_{50}$ and allometric scaling from rat PK data. Interestingly, the pyrazole hinge binder was replaced with an initially less potent reverse indazole nonclassical C-H hinge binder to eliminate a hydrogen bond acceptor. Fortunately, the drop in potency was compensated for later with increases in potency from other regions such as the spirocyclopropylnitrile.

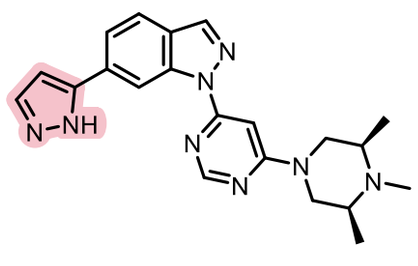


**compound 7**
LRRK2 $IC_{50}$ = 0.3 nM
cell $IC_{50}$ = 4 nM

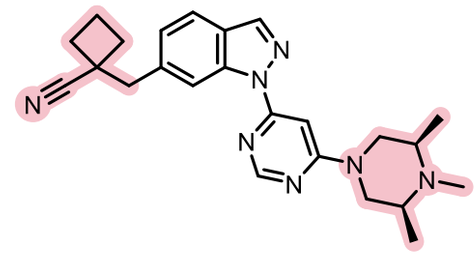


**compound 10**
LRRK2 $IC_{50}$ = 100 nM
cell $IC_{50}$ = 120 nM

**What does the spirocyclopropyl nitrile do, and how is it made?** Initial leads used a hydrogen-bonding pyrazole group to compensate for the loss of potency seen with the non-classical C-H hinge binder. The pyrazole brought along unfavorable properties including a hydrogen bond acceptor and poor solubility, so alternative motifs were screened through nickel-catalyzed $sp^3$-$sp^2$ cross-coupling chemistry or metallophotoredox cross-coupling, leading to the identification of modestly potent nitrile replacements.

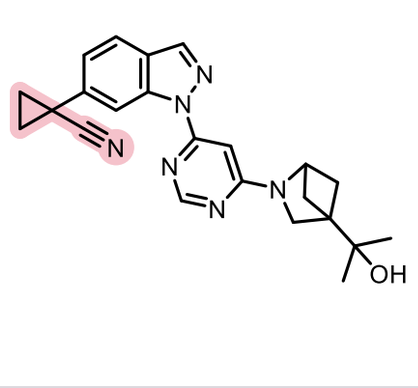


**compound 12**
LRRK2 $IC_{50}$ = 21 nM

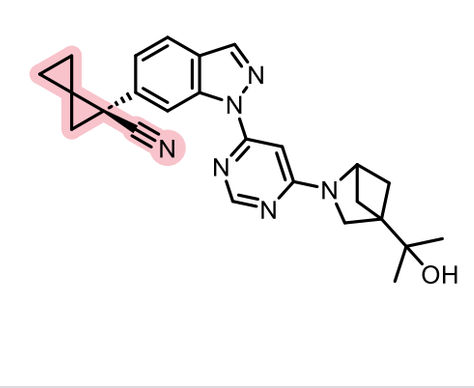


**compound 15**
LRRK2 $IC_{50}$ = 275 nM

The spirocyclopropyl group ("compound 24") led to a 20x improvement in biochemical potency and 75x improvement in cellular potency relative to unsubstituted "compound 20", due to conformational locking of the nitrile in a bioactive conformation co-planar to the indazole. The nitrile displaces a key water molecule while engaging in a Lys-based water network, and the spirocyclopropane group occupies a small lipophilic pocket ("compound 25," PDB: 8E81). The spirocyclopropyl group also reduced clearance by acting as "stable lipophilicity," lowering unbound clearance while increasing unbound volume, enhancing mean residence time (MRT).



**compound 20**
LRRK2 $IC_{50}$, cell $IC_{50}$: 14 nM, 30 nM
MRT(rat) = 0.5 h
EDE BID = >10,000 mg



**compound 24**
LRRK2 $IC_{50}$, cell $IC_{50}$: 07 nM, 0.4 nM
MRT (rat) = 3 h
EDE BID = 8 mg

December 2022

# compound 25

## LRRK2

selective, CNS-penetrant LRRK2 kinase inhibitor
preclinical, low projected human dose
opt. of MLi-2 series, from HTS
*J. Med. Chem.,* December 7, 2022
MERCK, BOSTON, MA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01605

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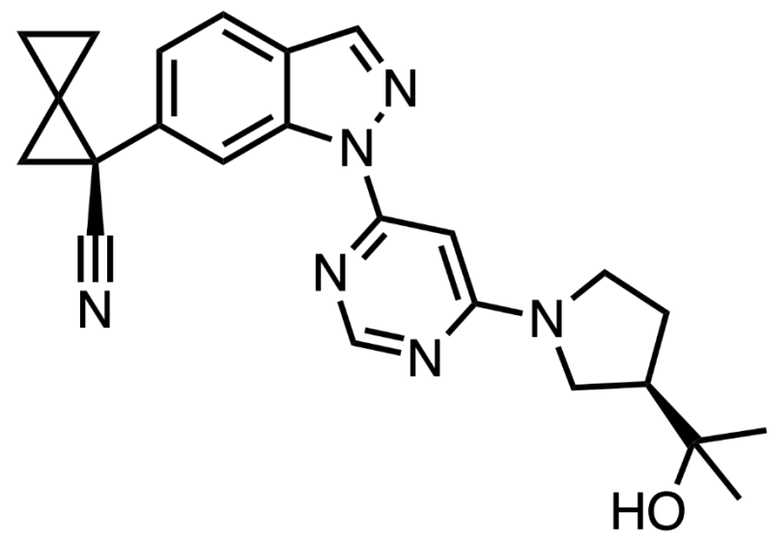
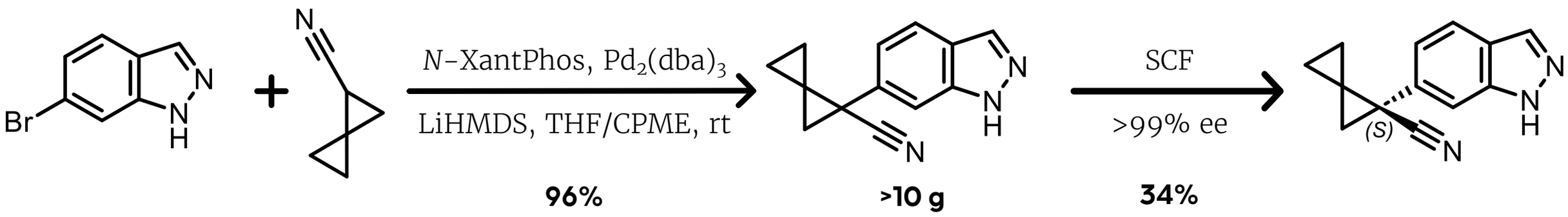
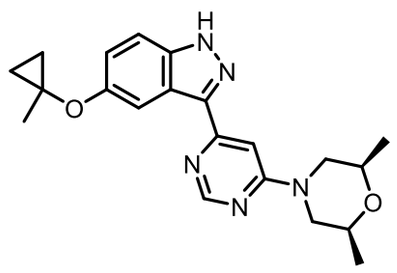




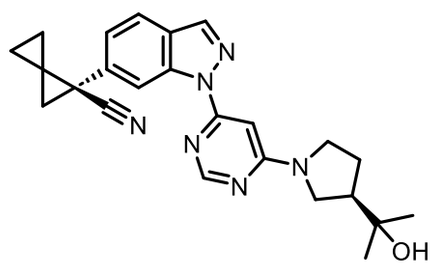

The spirocyclopropylnitrile is accessed through Pd-catalyzed coupling of the anion with *N*-XantPhos as a ligand, and enantioenriched via chiral supercritical fluid (SFC) chromatography.

**Preclinical characterization.** Merck states that with its picomolar potency in human PBMCs, "compound 25" is one of the most potent LRRK2 inhibitors they have evaluated. It is >100-fold selective against 267 other measured kinases and >1000-fold selective against all measured off-targets (ion channels and CYP enzymes) and was tolerated with no major findings in a 7-day rat DLT study up to 100 mg/kg QD. The compound dose-dependently lowers LRRK2 pS935 in rat brain striatum ($EC_{50}$ = 0.18 nM), which correlates with ex vivo PBMC activity, suggesting PBMCs may be acceptable surrogate biomarkers for CNS target engagement clinically.

The rat $Kp_{uu,brain}$ was moderate (0.43), and while the molecule was not found to be a rat P-gp substrate, it was found to be a mouse BCRP substrate and potent human BCRP inhibitor (BCRP $IC_{50}$ = 120 nM). Since non-human primates (NHPs) have increased BCRP expression relative to rats and humans, brain penetration in NHPs was lower ($Kp_{uu,brain}$ = 0.1).



**MLi-2**
LRRK2 $IC_{50}$, cell $IC_{50}$: 0.5 nM, 1.2 nM
Rat, NHP $Kp_{uu,brain}$: 0.5, 0.4
Rat PK MRT = 2 h
Dog PK MRT = 7 h
Rhesus PK MRT = 1.5 h
EDE BID = 3900 mg



**compound 25**
LRRK2 $IC_{50}$, cell $IC_{50}$: 0.9 nM, 0.3 nM
Rat, NHP $Kp_{uu,brain}$: 0.4, 0.1
Rat PK MRT = 2.5 h
Dog PK MRT = 3 h
Rhesus PK MRT = 3 h
EDE BID = 15 mg

December 2022

# compound 49

## CD73

orally bioavailable CD73 inhibitor
single agent, dose-dependent antitumor efficacy in mice
opt. of known CD73 inhibitor
*J. Med. Chem.,* December 18, 2022
CALITHERA BIOSCIENCES, CA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01287

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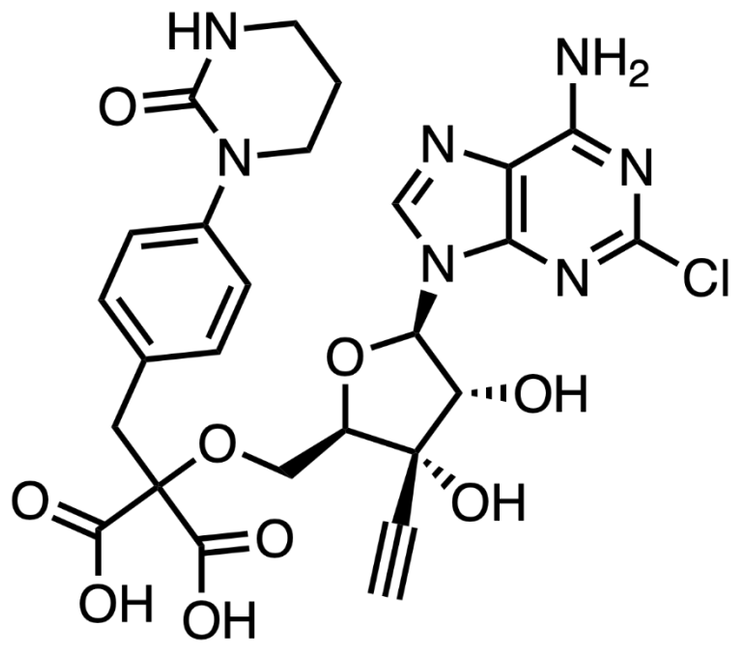


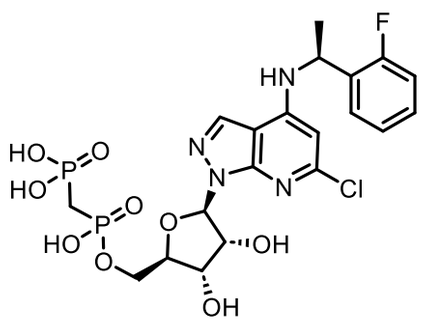
**What is it?** Compound 49 is an orally available CD73 inhibitor with dose-dependent PK/PD and an immune-mediated antitumor mechanism of action. The molecule is highly potent in human plasma (0.38 nM), and despite having a high free fraction (42% unbound) and possessing several highly polar or charged groups, rings, hydrogen bond acceptors and donors, is orally available with a long half-life in rodents. Given the recently approved liquidation of Calithera, development will need to continue by a different company.

**Why do we care?** Calithera is a Bay Area company that focused on programs for immuno-oncology and targeted oncology therapy, including an arginase program (partnered with Incyte) and an oral CD-73 inhibitor program (CB-703, partnered with Antengene). While the structure of CB-703 is undisclosed, Calithera Biosciences holds a patent for ectonucleotidase inhibitors targeting CD73 WO2018049145A1 (2018), so the molecule is likely to be related to compound 49. The adenosine signaling pathway mediated by CD39 and CD73 ectonucleotidases has been well-studied and actively pursued from a number of angles for potential applications in cancer immunotherapy. This molecule is particularly interesting given its oral route of administration (in contrast to first-to-clinic nucleoside AB680), which is very surprising given the presence of two acids, its overall size, and the presence of 16 oxygen or nitrogen atoms including at least five hydrogen bond acceptors and 13 hydrogen bond acceptors.

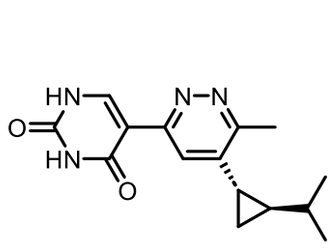
**What else is out there?** The first-to-clinic small molecule CD73 inhibitor is Arcus Biosciences' AB680, an IV phosphonate-based analogue that has completed Ph. I-II evaluation for pancreatic (NCT04104672), colorectal (NCT04660812), and prostate cancers (NCT04381832). Lilly had an oral, non-competitive CD73 inhibitor (LY-3475070) of a different chemotype in development in collaboration with Merck, but this program appears to have been discontinued (NCT04148937). Some examples of other preclinical molecules in the field include competitive nucleoside inhibitors and malonate analogues from Vitae Pharmaceuticals, mono-phosphonate OP-5244 from ORIC Pharmaceuticals, and a benzotriazole analogue from Arcus Biosciences.

**How was it discovered?** The only available X-ray structure with hCD73 was with well-known inhibitor AMPCP (PDB: 4H2I) at the start of the program. Boehringer Ingelheim had patented malonic acid inhibitors (e.g., "compound 2", US10654884B2). Calithera had first attempted to optimize these compounds, but while potency was improved, ADME struggled. Introduction of an ethynyl group surprisingly led to a 59-145x improvement in potency for "compound 20". This potency boost was attributed in part to an unusual intramolecular C-H-$\pi$ interaction between the alkyne and adenine ring, stabilizing a bioactive conformation.

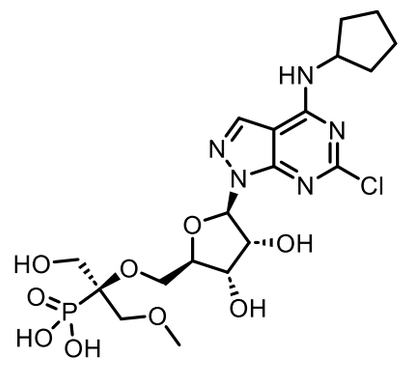
**Oral efficacy despite low bioavailability.** Addition of a cyclic urea to the benzyl group led to the more potent "compound 49", which showed a remarkably low shift between plasma potency (0.38 nM) and enzyme activity ($IC_{50}$ = 0.17 nM) thanks to its polarity and low free fraction. The polar molecule also exhibited limited in vitro metabolism. The very high aqueous solubility (3.9-151 mg/mL) across pH, long half-life, and high plasma potency more than compensated for the low percent bioavailability (F = 2.8% at 50 mpk), enabling 24 h oral coverage of CD73 (IC90 = 3 nM). While it is more typical to see programs focus on achieving high F% with more lipophilic molecules, this provides a nice example of how low F% can be compensated for with other properties inherent to polar molecules.



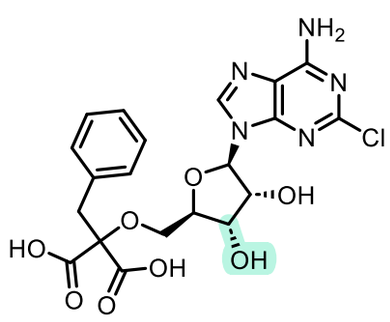
**AB680**
Arcus Biosciences
IV



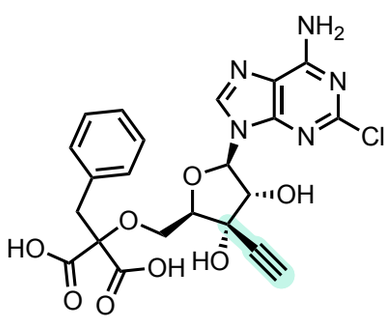
**LY-3475070**
Elly Lily and Company
oral



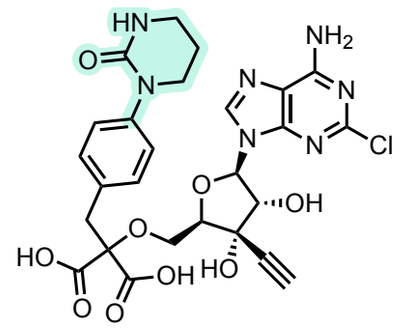
**OP-5244**
ORIC Pharmaceuticals
oral



**compound 2**
Human CD73 $IC_{50}$ = 100 +/- 39 nM
Human cell surface $IC_{50}$ = 434 nM
cLogP = -0.37



**compound 20**
Human CD73 $IC_{50}$ = 1.7 +/- 22 nM
Human cell surface $IC_{50}$ = 2.8 +/- 0.41 nM
Human plasma CD73 $IC_{50}$ = 5.6 nM
cLogP = 0.74



**compound 49**
Human CD73 $IC_{50}$ = 0.17 +/- 0.02 nM
Human plasma CD73 $IC_{50}$ = 0.38 +/- 0.11 nM
Human cell surface $IC_{50}$ = 0.21 +/- 0.12 nM
cLogP = 0.67

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