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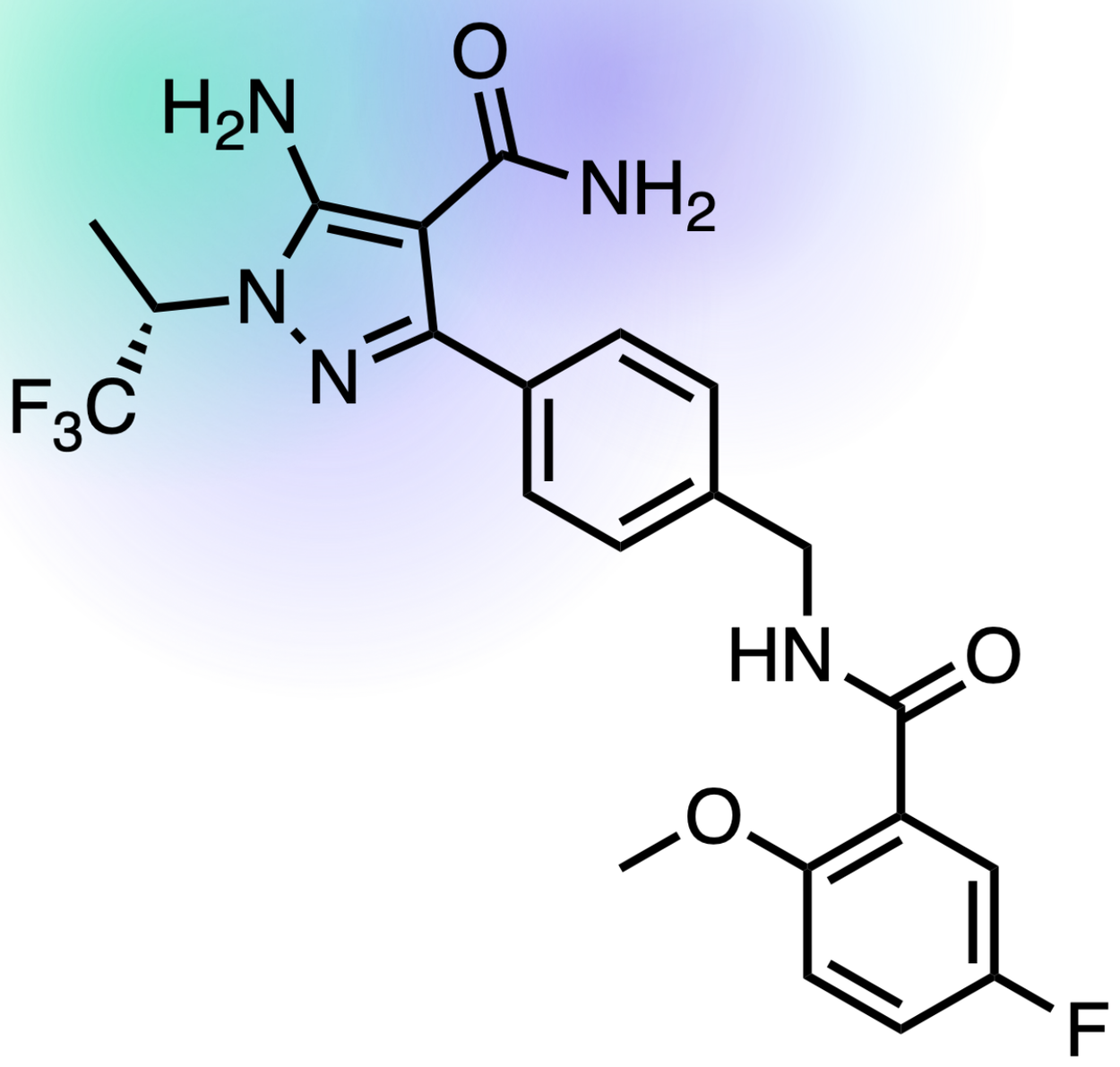
Small Molecules of the Month

January 2023



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pirtobrutinib

BTK

oral, reversible BTK inhibitor
approved for R/R MCL, in Ph. II/III for CLL/SLL, NHL
activity against C^{481} BTK mutants
FDA approval, January 27, 2023
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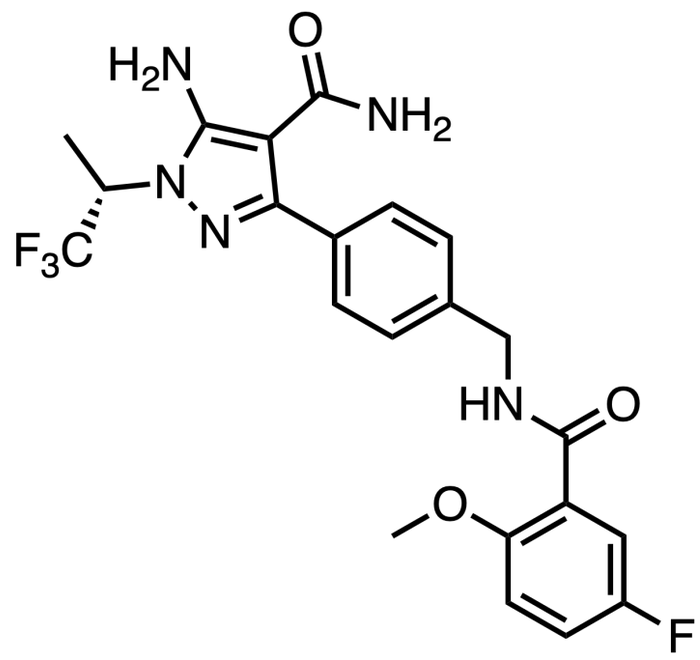

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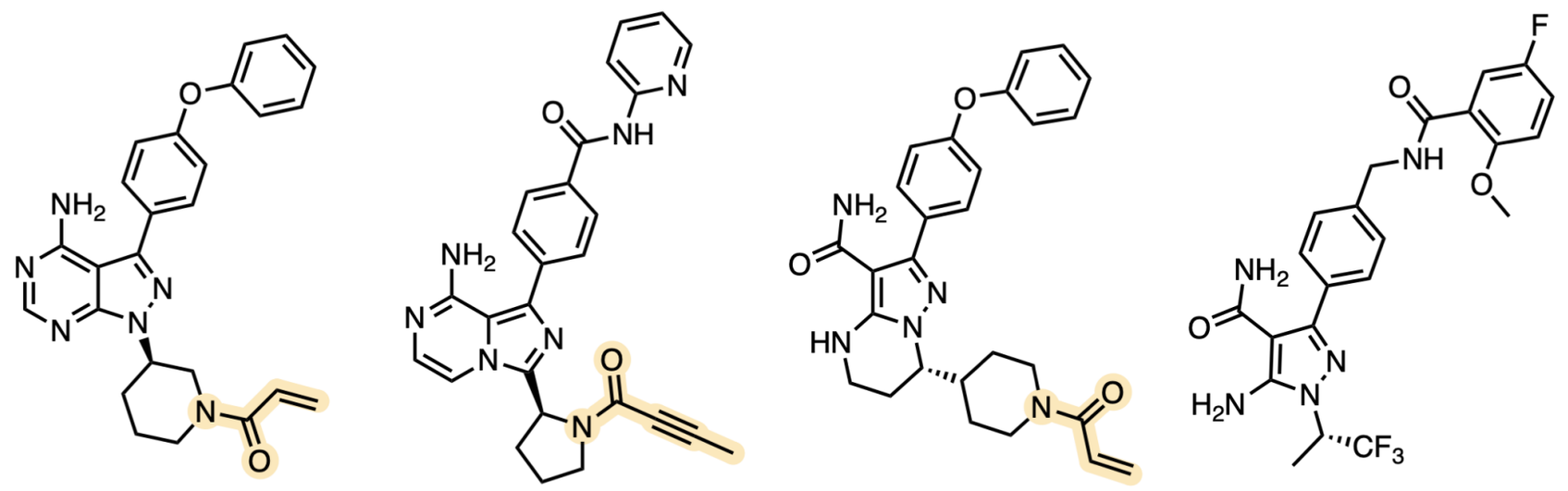
The first approved non-covalent BTK inhibitor. As the nexus of the B cell receptor (BCR) signaling pathway, BTK has been a hotly pursued target in B-cell-mediated cancers and immunological diseases, as evidenced by the many BTK inhibitors we've highlighted in just the past few years. Recently, pirtobrutinib (Jaypirca, LOXO-305) received accelerated FDA approval based on its promising efficacy (50% ORR, 13% CR, n=120) in mantle cell lymphoma (MCL) patients who had previously received another BTKi treatment. The non-covalent mechanism enables several new advantages including greater selectivity and thus its "remarkable" tolerability, as well as resistance mitigation, due to previously approved BTK inhibitors requiring the Cys^{C481} mutation for covalent binding. Even further, this BTKi prolongs BTK target coverage irrespective of BTK resynthesis rates. All of these properties render this molecule unexpectedly valuable, as evidenced by its acquisition history. Pirtobrutinib was one of three development-stage assets acquired by Lilly from Loxo in a hefty $8B transaction in 2019. While Loxo had acquired the BTKi just two years earlier from struggling Redx Pharma in 2017 for only $40M.



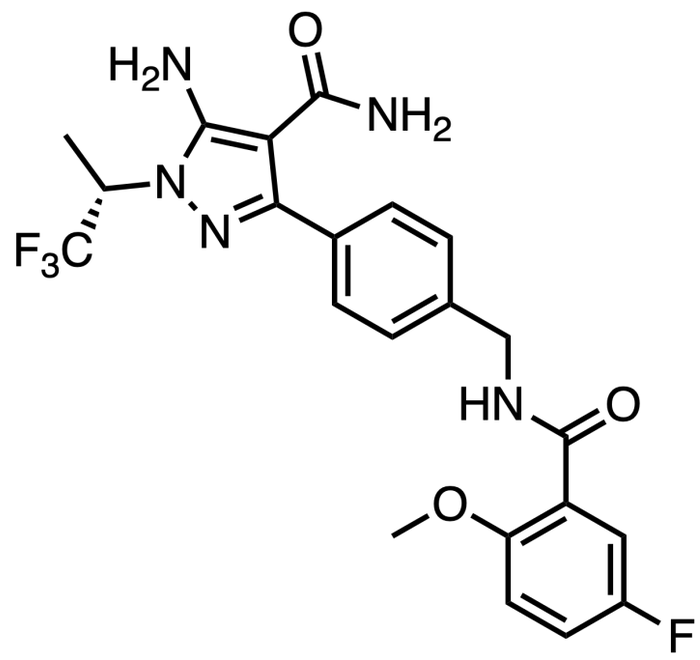
BTK inhibitors	ibrutinib	acalabrutinib	zanubrutinib	pirtobrutinib
dose (mg)	560 mg QD	100 mg BID	160 BID/320 QD	200 mg QD
WT BTK IC_{50}	1.5 nM	5.1 nM	0.5 nM	<5 nM
Mut. BTK IC_{50} (C481S)	100 nM	n.d.	n.d.	≤10 nM
Mut. BTK IC_{50} (C481T)	inactive	n.d.	n.d.	≤10 nM
ITK / TEC IC_{50}	11 nM / 78 nM	>1,000 nM / 93 nM	30 nM / 1.9 nM	>5,000 nM / 1,234 nM
PPB (Hu) f_u	2.7%	2.5%	6%	4%
human CL	2000 L/h	159 L/h	182 L/h	2.0 L/h
human $T_{1/2}$	4-6 h	1.4 h	2-4 h	19 h
human *F*(%)	3.4%	25%	15%	86%
human C_{max} (ng/mL)	156 ng/mL	563 ng/mL	314 ng/mL	6,460 ng/mL
grade III+ diahrr. (all)	5% (51%)	3.2% (31%)	0.8% (23%)	0% (19%)
grade III+ rash (all)	3% (25%)	0.8% (18%)	0% (36%)	0% (14%)
grade III+ AFib (all)	3.7%	1.1% (4.1%)	0.6% (2%)	1.0% (2.5%)

Comparison of key properties of FDA-approved BTK inhibitors including first-generation covalent inhibitor ibrutinib, second-generation covalent inhibitors acalabrutinib and zanubrutinib, and reversible, non-covalent inhibitor pirtobrutinib (LOXO-305). Since each molecule is approved based on different populations/settings, efficacy is discussed in the text. Sources: FDA drug labels, selectivity data, and mutant activity.

January 2023

pirtobrutinib

BTK



oral, reversible BTK inhibitor
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A potentially better tolerability profile for patients. The first approved BTK inhibitor, ibrutinib (Imbruvica), has been remarkably successful both clinically and commercially, accruing a mind-boggling >$230 billion in sales over the first 6 years since its 2013 launch. Despite this success, a metastudy of MCL patients undergoing long-term treatment with ibrutinib (median duration on treatment = 7.8 months with follow-up at median = 16.1 months) showed ibrutinib had an 84% overall discontinuation rate, with primary reasons for discontinuation being treatment intolerance due to off-target toxicities (22% in 3L) and disease progression. Drug tolerability is especially important given the indefinite nature of BTKi treatment for cancer - long-term follow-up showed 42% of ibrutinib patients remained on treatment after 8 years.

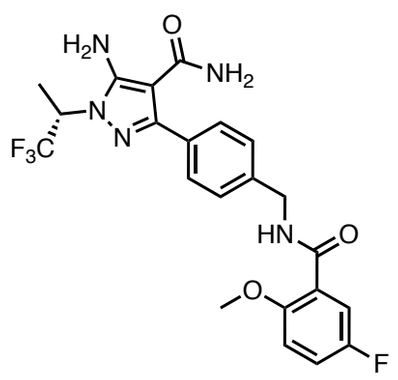
The more selective second-generation covalent inhibitors are better tolerated, and in recent head-to-head trials in CLL, discontinuation rates due to treatment-emergent adverse events were lower for zanubrutinib (7.8%) and acalabrutinib (14.7%) than ibrutinib (13-21.3%). For MCL, medical record data shows that at least 25% of patients discontinue covalent BTK inhibitor treatment due to toxicity. In contrast, only 1% of MCL patients discontinued treatment due to treatment-related AEs in Ph. I/II studies with pirtobrutinib (n=618, median follow up = 8 months). Furthermore, in a study of patients who were previously intolerant to a covalent BTK inhibitor, most patients on pirtobrutinib did not experience a recurrence of the high-grade AEs that led to their discontinuation on previous BTK inhibitors.

A reversible MOA for better kinase selectivity and patient tolerability. The adverse events leading to BTK inhibitor discontinuation in MCL and CLL have been attributed to the inhibition of off-target kinases possessing a BTKi-binding cysteine residue. For example, ibrutinib binds to 19 other kinases, with off-target engagement with ERBB2/HER2 and ERBB4/HER4 implicated in ibrutinib's side effect of atrial fibrillation (4% grade ≥3, vs. 1% for pirtobrutinib and 0.5% for placebo). Although second-generation covalent BTK inhibitors, like acalabrutinib (Calquence) and zanubrutinib (Brukinsa), have demonstrated increased selectivity and reduced adverse effects relative to ibrutinib, acalabrutinib still suffers from significant cardiac risks and zanubrutinib from decreased neutrophil counts and infections, which may be linked to undesired kinase activity. Pirtobrutinib is a highly selective kinase inhibitor with 300-fold selectivity over 98% kinases tested, exemplified by its significantly greater selectivity over ITK and TEC (Table 1). The actual selectivity difference is likely greater in vivo than detectable in vitro, given the time-dependent nature of covalent inhibition and the prolonged exposure that patients have to BTK inhibitors when in treatment over years.

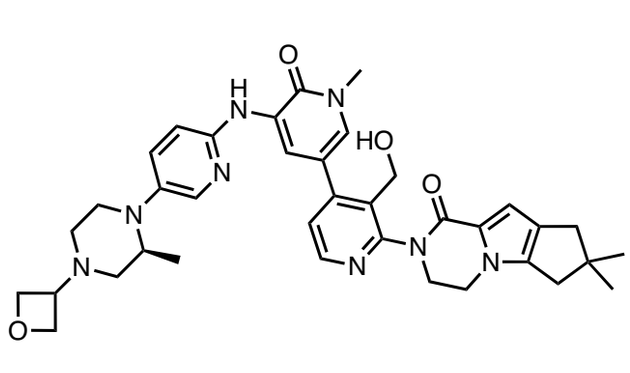
A BTKi with significantly improved pharmacokinetics. The improved tolerability of pirtobrutinib may be attributable to its high bioavailability and long half-life, enabling sustained target coverage (IC_{90} coverage over 24 h) with a "flatter" PK profile (i.e., lower peak-to-trough ratio). Pirtobrutinib is significantly more bioavailable (absolute *F*(%) = 86) compared to ibrutinib (absolute *F*(%) = 3) or acalabrutinib (absolute *F*(%) = 25). The half-lives of ibrutinib, acalabrutinib, and zanubrutinib are in the range of 1 to 6 h, whereas pirtobrutinib has a half-life of 19 h. In principle, the prolonged trough coverage of BTK by a reversible inhibitor could also contribute to efficacy by preventing resistance due to increased rates of BTK synthesis, which can be as high as 31% per day in some.

Built-in resistance mitigation: mutant Cys^{C481} no longer required for binding for this BTKi. One of the clearest advantages of a reversible inhibitor in this setting is avoiding resistance due to loss of the cysteine (C481) required for covalent inhibitor binding, which is a major clinical resistance mechanism in CLL and other cancers. In one study, 80% of relapsing CLL patients were found to have the C481S mutation (n = 37 of 46). While this was one of the main rationales for pursuing a reversible BTK inhibitor in oncology, C481 mutations are less common in MCL (17%), suggesting additional properties such as greater target coverage or tolerability may contribute to the clinical response rate observed in MCL (50% ORR, 13% CR). Pirtobrutinib inhibits C481 mutants (i.e., C481S, C481T, C481R) with nanomolar potency (IC_{50} ≤10 nM) whereas ibrutinib has significantly weaker activity on C481S mutant BTK (IC_{50} = 100 nM) and essentially no activity on C481T and C481R mutants. Clinically, the overall response rate in CLL patients treated with pirtobrutinib with prior covalent BTK inhibitor resistance was 67% (n = 53 of 79), and on BTK C481-mutant cancers was 71% (n = 17 of 24). Although the molecule is not immune to resistance mechanisms, it meets an urgent need for new treatment options against R/R MCL and CLL.

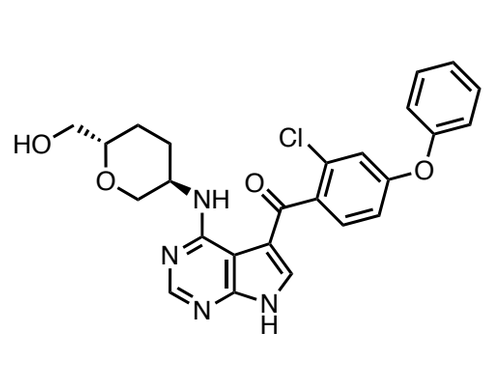
Reversible Inhibitors in Clinical Development:



pirtobrutinib
(LOXO-305)
200 mg QD
Ph. II NHL
Ph. III MCL and CLL/SLL
FDA accelerated approval for MCL



fenebrutinib
(GDC-0853)
50/150 mg QD or 200 mg BID
Ph. I CLL and DLBCL
Ph. II CSU, SLE, RA
Ph. III RMS



nemtabrutinib
(ARQ 531, MK-1026)
65 mg QD
Ph. II CLL/SLL, RS, MZL, MCL, RL, and WM

January 2023

pirtobrutinib

BTK

oral, reversible BTK inhibitor
approved for R/R MCL, in Ph. II/III for CLL/SLL, NHL
activity against C^{481} BTK mutants
FDA approval, January 27, 2023
REDX, ALDERLY PARK, UK (LOXO/LILLY)
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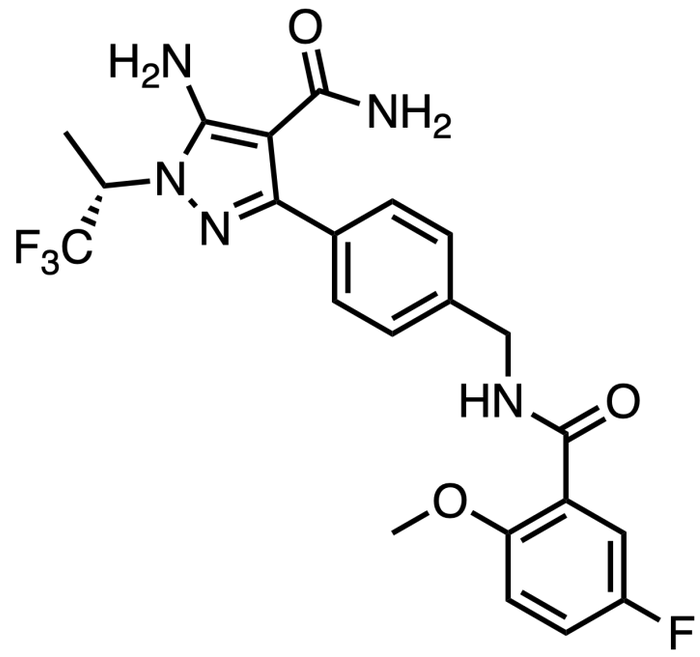
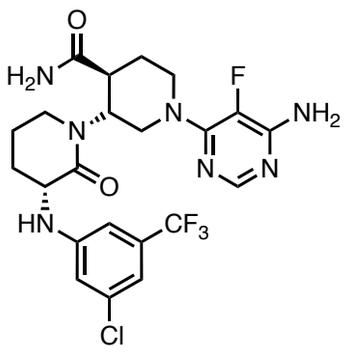
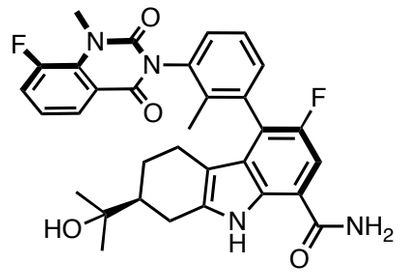
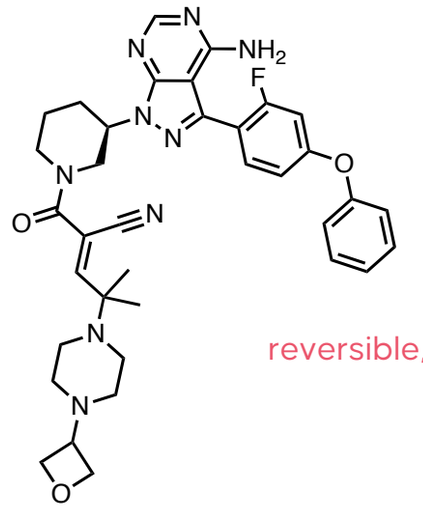




vecabrutinib
(SNS-062)
25 mg escalated to 500 mg
Ph. II B cell lymphoma
(discontinued)



BMS-986142
200 or 300 mg QD
Ph. II RA and SS



reversible, covalent

rilzabrutinib
(PRN1008)
400 mg BID
Ph. II wAIHA, IgG4-RD, asthma, CSU, and atopic dermatitis,
Ph. II/III ITP
Ph. III pemphigus
(terminated)

A reversible inhibitor with a surprisingly simple structure. From a chemistry perspective, pirtobrutinib has a remarkably simple structure compared to other reversible BTK inhibitors like fenebrutinib and BMS-986142 that had entered development. The prevailing idea was that noncovalent inhibitors needed to be larger to simultaneously access the H3 selectivity pocket and hinge region of the active site in BTK for selectivity. Pirtobrutinib much more compact in size by many measures (MW = 479 vs. fenebrutinib's 665, ring count = 3 vs. 8 for fenebrutinib) but has a similar topological polar surface area (TPSA = 125 Å) to fenebrutinib (119 Å), though no data have been reported to date on whether pirtobrutinib is brain-penetrant like other BTK inhibitors. Given its small size, effective target coverage with a reversible mechanism, and good tolerability profile, pirtobrutinib may be a harbinger for future BTK drugs with better tolerability in non-oncology indications.

From discounted $40M pipeline candidate to $8 billion dollar acquisition. Pirtoburtinib's (formerly RXC005) originator was Redx Pharma (WO2017103611A1, 2017) who sold the program to Loxo Oncology in July of 2017 for $40M to reduce debt. Loxo's pipeline development of pirtoburtinib (WO2022056100A1, 2021; WO2021113497A1, 2020; WO2020028258A1; 2019) and other clinical stage candidates were then sold to Eli Lilly for $235 per share or $8B in Q1 of 2019.

What's next? The drug has advanced to Ph. III for MCL, is under investigation in Ph. II trials for NHL, and has been applied in several Ph. III expansion studies for CLL/SLL, for which it seems likely to gain additional approvals based on efficacy and safety data so far.

Another reversible, non-covalent BTKi in Ph. II for R/R hematologic malignancies is nemtabrutinib (ARQ 531, MK-1026), which was acquired by Merck via a $2.7B purchase of ArQule in 2019, highlighting the perceived value of reversible BTK inhibitors. The success of this reversible inhibitor in oncology suggests that applications of reversible BTK inhibitors in non-oncology indications may not be far off. BTK inhibitors continue to be explored in immunological and neuroimmunological conditions including multiple sclerosis, in which the bar for safety is higher than for oncology. For example, fenebrutinib a potentially brain-penetrant BTK inhibitor ($k_{p,uu,CSF}$ = 0.15, $C_{max,\ CSF}$ = 12.9 ng/mL, 10 mg/kg PO in NHPs) was originally explored in oncology and is now in Ph. III for the treatment of MS (NCT04586023). While Sanofi's rilzabrutinib (PRN1008), a reversible covalent inhibitor and a Molecule of the Month from March 2022, recently did not reach its primary or secondary endpoints in a Ph. III trial for lack of efficacy in pemphigus (NCT03762265), however, it is still under investigation for several autoimmune diseases in Ph. II and Ph. III.

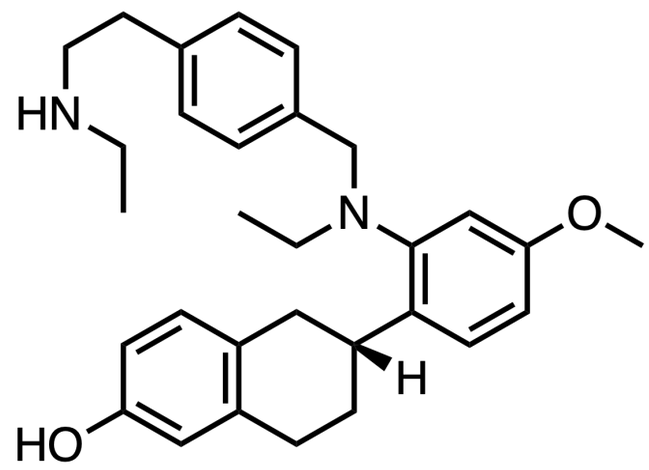
Lessons learned. Pirtobrutinib is an excellent case study highlighting how reversible inhibitors can offer several properties that differentiate them from covalent inhibitors in oncology, including different resistance profiles, potential for greater selectivity and tolerability, and the potential for more durable target coverage in the face of target resynthesis.

January 2023

elacestrant

estrogen receptor (ER)

oral, non-steroidal SERD/SERM
approved for ER+, HER2-, mESR1 adv./metastatic BC
degrades ER alpha in dose-dependent manner
FDA approval, January 27, 2023
EISAI, TOKYO, JP (STEMLINE/MENARINI)
recent approval: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-elacestrant-er-positive-her2-negative-esr1-mutated-advanced-or-metastatic-breast-cancer



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What is it? Elacestrant (Orserdu) is an oral, selective estrogen receptor modulator and degrader (SERM/SERD) that was recently approved for ER-positive, HER2-negative, estrogen receptor α (ESR1)-mutated advanced or metastatic breast cancer (345 mg PO QD) based on progression-free survival (PFS) data from EMERALD (NCT03778931). As the first novel endocrine therapy approved in over 20 years and the first orally approved SERD, the drug marks an inflection point in the exciting and highly competitive pursuit of oral SERDs and a milestone for breast cancer treatment in general. The drug's activity against difficult-to-treat ER-mutant tumors, thanks to a novel binding mode, allows it to improve survival among patients with ESR1-mutations detected in the ligand-binding domain via a circulating tumor DNA (ctDNA) companion diagnostic. The use of a blood-based cancer diagnostic for the drug is also notable, as a significant improvement over invasive biopsy-based diagnoses.

Why do we care? Endocrine therapy, which targets the cancer-driving estrogen receptor in ER+ cancers, has been a key component of ER+ breast cancer treatment for over 40 years. The oral SERM tamoxifen, for example, which binds to ERα to antagonize ERα-related gene transcription, was approved in 1977. Tamoxifen, however, induces stabilization of the ER protein, which can lead to agonist signaling and the development of secondary cancer. Selective estrogen receptor degraders (SERD) such as fulvestrant (2002) not only antagonize ER function but also induce degradation via the proteosome, more effectively inhibiting ER. However, fulvestrant's poor PK requires it to be administered in a long-acting intramuscular depot formulation, limiting its exposure for maximal target engagement and efficacy.

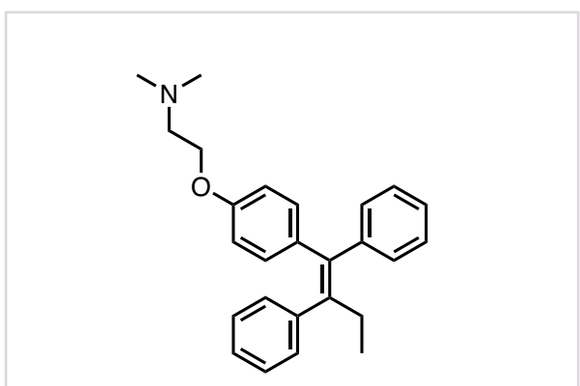
Next-generation oral SERDs could provide greater efficacy, quality of life, address resistance, and enable all-oral combinations with now standard-of-care CDK4/6 inhibitors. Commercially, these properties have resulted in oral SERDs being characterized as a "multi-billion dollar opportunity." Oral SERDs naturally turned into an intensively competitive field within the industry, with molecules from AstraZeneca, Lilly, Sanofi, Genentech, Arvinas, and many others late in development. Given the competition, elacestrant has become a "dark horse" with its first positive clinical data and approval, given its development by less well-known biotech, Radius Health and Italian commercial partner, Menarini. The fact that the molecule is active in ER mutants (present in up to 40% of ER+, HER2- advanced or metastatic BC), is generally well-tolerated, is and active after progression on CDK4/6 therapy bodes well for further development of SERDs in earlier breast cancer settings with larger patient populations.

Where did it come from? While the discovery of elacestrant hasn't been disclosed, its patent was filed by Eisai in 2005 (US7612114B2) and licensed to Radius Health in 2006. Interestingly, the molecule was originally intended to relieve hot flashes and prevent osteoporotic bone loss in postmenopausal women (NCT02653417).

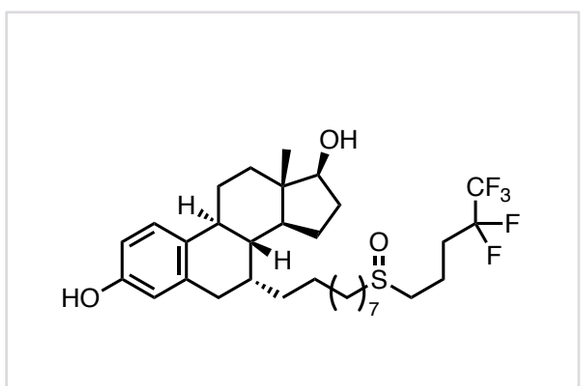
Tissue selectivity. In contrast to prior-generation SERMs like tamoxifen and raloxifene, elacestrant does not exhibit agonist activity in the uterus, and instead antagonizes the uterus-stimulating properties of E2 estrogen.

Human PK and CNS penetration. Elacestrant is more bioavailable than fulvestrant but still only has a human bioavailability of 10%. This is made up for with a long half-life ($T_{1/2}$ = 30-50 h). In contrast to older SERMs and SERDs which are known to be ineffective at crossing the BBB, preclinical studies with elacestrant suggested that it may be brain penetrant based on efficacy in intracranially implanted tumors. However, in humans, PET imaging suggests elacestrant does not significantly engage ER in the brain and has low CSF concentrations (below limit of quantitation in CSF at 200 mg/d). The lack of human brain penetration may be a reason the molecule was repurposed from treating hot flashes (which would have required BBB penetration) to oncology. This is likely an area that future SERDs will have an opportunity to differentiate with.

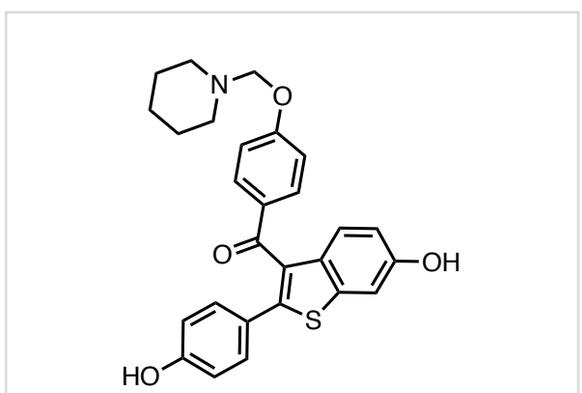
What's next for oral SERDs? While elacestrant's approval is an important milestone, there continue to be opportunities for other SERDs to demonstrate best-in-class properties. Elacestrant appears to still have ER agonist activity in bone, suggesting that degraders with more consistent full antagonism across tissues could be more effective or safer. The molecule is currently primarily approved based on activity in ESR1-mutant tumors, which limits its patient population to late-stage patients. A molecule that demonstrates efficacy in earlier settings, with more complete target degradation relative to fulvestrant could have a bigger impact in the adjuvant setting, for example. The molecule also has a relatively high dose - lower dose molecules may be safer, which would be important for chronic/indefinite treatment of breast cancer patients in earlier settings who can survive for a long time. To treat brain metastases, a SERD that is clearly brain-penetrant would also likely contribute to overall survival. In summary, this first milestone is exciting, but there are many more opportunities for the field ahead.



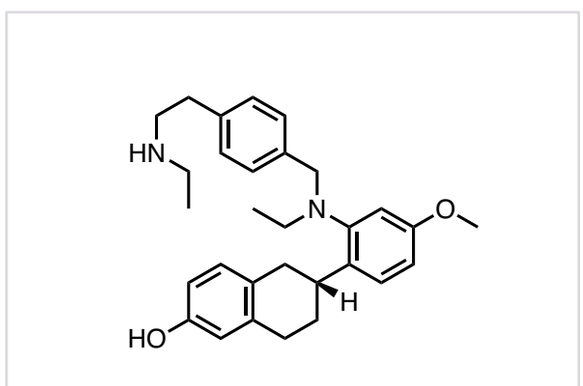
tamoxifen
oral SERM



fulvestrant
injected SERD



raloxifene
oral SERM



elacestrant
oral, non-steroidal SERD/SERM

January 2023

MK-8189

PDE10A

oral PDE10A inhibitor
Ph. IIb for schizophrenia
from fragment screen + SBDD
J. Med. Chem., January 10, 2023
MERCK, WEST POINT, PA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01521

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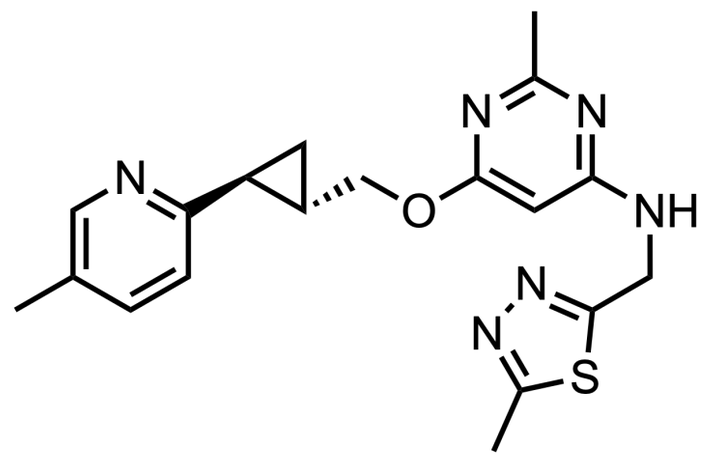


What is it? MK-8189 is a Ph. II clinical candidate for schizophrenia targeting PDE10A, a long-pursued but uncracked target for psychosis. With potentially better target engagement over prior molecules, it could become the first in the class to demonstrate efficacy in this challenging therapeutic area. The discovery and medicinal chemistry campaigns are also notable, featuring a nearly million-fold potency improvement from a fragment, while securing properties that enable durable CNS target engagement. Remarkably, the final candidate, MK-8189 is highly lipophilically efficient (LLE = 7.8) and has a similar overall ligand-binding efficiency to its starting fragment (LBE = 0.54 vs. LBE = 0.57 for 1).

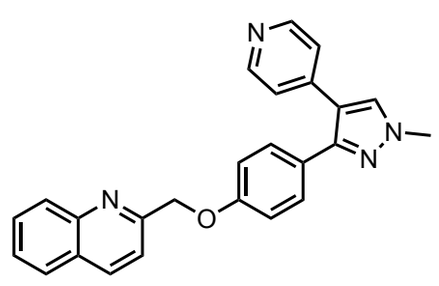
Toward a non-dopaminergic treatment for schizophrenia. Schizophrenia is a serious mental illness with a lifetime prevalence of 0.3-0.5% worldwide, characterized by positive symptoms (e.g. hallucinations, delusions, etc.), negative symptoms (withdrawal, flattening of effect, etc.), and cognitive impairment. The current standard of care is atypical antipsychotics like risperidone and olanzapine, for which dopamine D2 receptor antagonism is core to the mechanisms of action. However, D2 receptor antagonists also induce adverse effects such as cognitive impairment, weight gain, extrapyramidal symptoms, and increased prolactin release and sedation - all of which can lead to significant patient non-compliance (~42%). As a result, alternative approaches to D2 antagonism have been thoroughly explored, with at least 14 mechanisms of action clinically tested in attempts to recapitulate the antipsychotic efficacy of D2 agonists. These include targets such as D1, D4, 5-HT2A, CB-1, NK3, neurotensin, AMPA, M1/M4, 5-HT2C, mGluR2/3, PDE10A, GlyT1, H3, and α7R, with only M1/M4 agonism seeming comparably efficacious so far (see Dec. '22 Molecule of the Month, emraclidine).

PDE10A: a long-pursued, but uncracked target for psychosis. Another such alternative D2 target is PDE10A, a phospodiesterase downstream of the D2 receptor that catalyzes the hydrolysis of secondary messengers cAMP and cGMP within the nervous system. PDE10A is highly localized to the mammalian striatum of the CNS and the testes, and has been implicated as a target for schizophrenia due to the role that striatal dysfunction is believed to play in psychosis and related symptoms. PDE10A triggered industry excitement as a potential novel target for schizophrenia in 1999, especially given the success in targeting phosphodiesterases (PDEs) for other indications, such as PDE3/milrinone, PDE4/apremilast, PDE5/sildenafil. Now, more than 15 companies have pursued PDE10A as a target resulting in >150 patents, 12 clinical candidates, and 4 clinically validated PDE10A PET ligands. Despite the potent activity of these molecules in well-established animal models such as conditioned avoidance response, none of the most advanced, Ph. II PDE10A inhibitors from Pfizer (PF-02545920), Takeda (TAK-063), or Lundbeck (Lu AF11167) have met their primary endpoints for the treatment of schizophrenia. The structure of Lu AF11167 is not disclosed, but we have shown another Lunbeck PDE10A inhibitor, Lu AE90074, for structure and potency comparison.

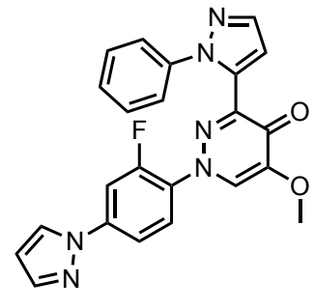
Could MK-8189 perform better than the previous PDE10Ai clinical candidates? While the clinical PDE10A inhibitors tested so far have generally been safe, well-tolerated, and psychoactive, inducing a state of "awake sedation," it is still possible that greater PDE10A target engagement could result in greater efficacy. In PET imaging with healthy volunteers, PF-02545920 demonstrated 14-27% enzyme occupancy at the 10 mg dose, and 45-63% occupancy at 20 mg, but no improvement in PANSS was observed at 5 or 15 mg Q12H. For TAK-063, modeling from a PET study suggests that the 20 mg dose, which did not show PANSS improvement, would have only hit ~30% PDE10A enzyme occupancy. Merck has now seen initial clinical proof-of-concept in patients with acute schizophrenia episodes, indicating that the greater, sustained PDE10A occupancy drives more efficient target engagement through this mechanism than observed with prior inhibitors. Tritiated MK-8189 achieves 50% target occupancy at 52 nM in rodents, with full occupancy achieved at higher concentrations. As of Feb. 26, '22, Merck still has MK-8189 listed in its pipeline and Ph. II trial NCT04624243 is still recruiting.

Chemical matter start from a proprietary pyrimidine fragment screen. The chemical matter for MK-8189 originated from a micromolar hit "compound 5" ("compound 1" in *J. Med. Chem.* **2023**) from fragment screening in a PDE10A biochemical assay with a proprietary library of 1600 soluble, low MW compounds at high concentration (200 μM) back in 2015. Hits were then confirmed in a second tier, by X-ray crystal structures obtained following soaking and co-crystallization of the promising biochemically active hits with the PDE10A catalytic domain. The starting fragment was chosen for its very high ligand efficiency (0.59), synthetic tractability of the privileged pyrimidine scaffold, and binding mode.

A ~1,000,000X potency boost from two substitutions on an activated pyrimidine core. A library array synthesized in parallel via SNAr of the corresponding pyrimidinyl chloride with 350 amines was screened for activity, leading to the identification of "compound 9s." This hit had a remarkable ~1,500x increase in potency from the starting fragment, likely attributable to π-stacking of the aminothiazole between the two aromatic rings of Phe686 and Tyr514 within the "S-pocket" (PDB: 5C2A). An analogous array of ethers prepared via SNAr of the corresponding pyrimidinyl methyl sulfone with ~600 alcohols resulted in the identification of picomolar-active "compound 15h" ("compound 2" in *J. Med. Chem.* **2023**) with another remarkable >500x boost in potency (PDE10A K_i = 0.0082 nM, PDB = 5C2H). The quinoline moiety of 15h engages in hydrogen bonding with the phenol of Tyr683, and overall displays greatly improved PDE selectivity, with a >5000x selectivity ratio over all other PDEs tested.

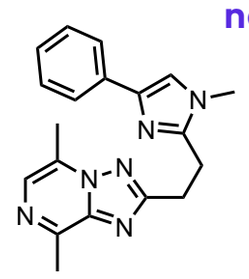


PF-02545920
mardepodect, MP-10
Pfizer
PDE10A IC_{50} = 0.42 nM



TAK-063
balipodect
Takeda
PDE10A IC_{50} = 0.1 nM

***Lu AF11167 structure not disclosed**



Lu AE90074
Lundbeck
PDE10A IC_{50} = 12 nM

January 2023

MK-8189

PDE10A

oral PDE10A inhibitor
Ph. IIb for schizophrenia
from fragment screen + SBDD
J. Med. Chem., January 10, 2023
MERCK, WEST POINT, PA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01521

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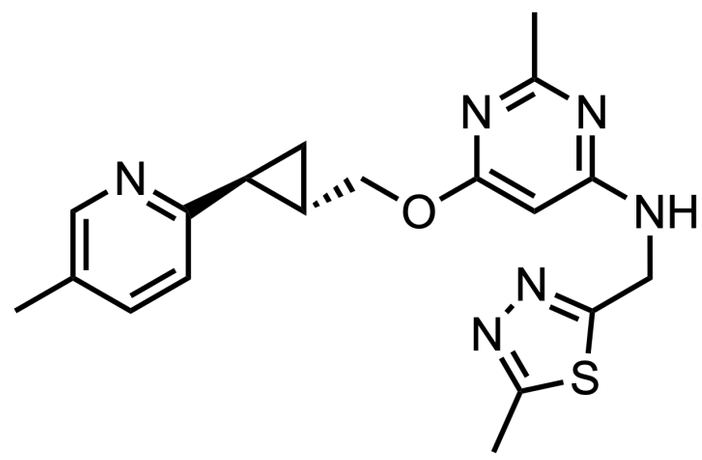
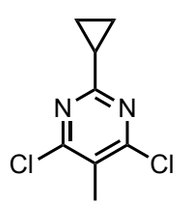
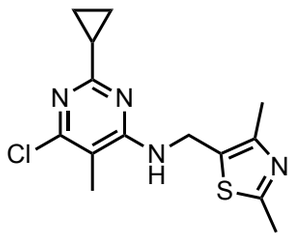
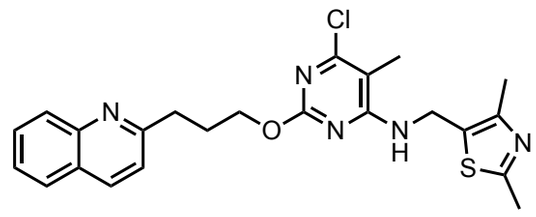




compound 1
(PDB: 5C1W)
PDE10A K_i =8700nM



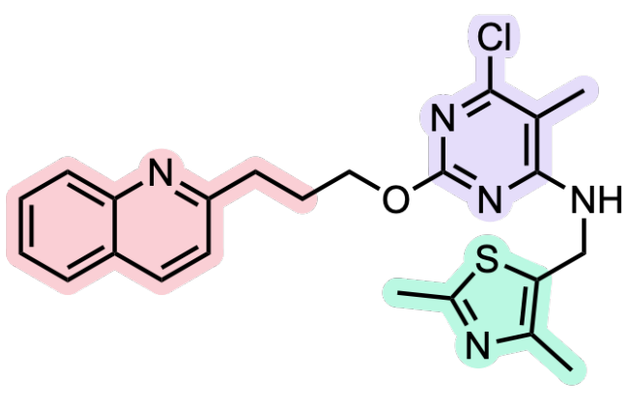
compound 9s
(PDB: 5C2A)
PDE10A K_i = 4.8 nM



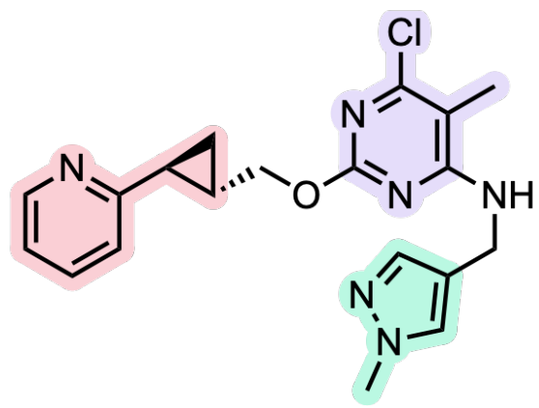
compound 15h
(PDB: 5C2H)
PDE10A K_i = 0.0082 nM

Trading potency for better PK properties. Despite the 10,000-fold boost in potency, "compound 15h" ("compound 2" in *J. Med. Chem.* 2023) unfortunately exhibited poor PK with low bioavailability, high unbound clearance, high hERG activity, inhibition of CYP2C9 and 3A4, and activation of CYP3A4 PXR. Reducing overall lipophilicity, number of rotatable bonds, and replacing the thiazole with a more stable pyrazole led to "compound 3," with a 55-fold drop in potency, but with significantly lower clearance, greater oral bioavailability, and reduced off-target activity. The optically-active *trans*-cyclopropylpyridine linker, resolved by chiral supercritical fluid chromatography (SFC) at an intermediate stage, led to improved metabolic stability as compared to the corresponding propyl linker. Replacing the core with a bicyclic pyrazolopyrimidine of "Pyp-1" further improved the PK properties, with a high passive permeability and low P-gp efflux, which are critical for blood-brain barrier penetration.

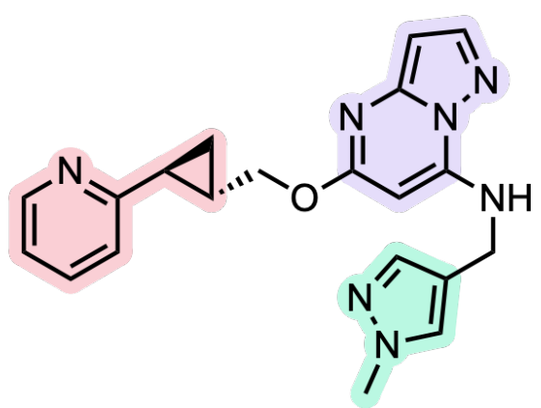
Potency restored with a methyl group and a thiadiazole. In one series, replacement of the *N*-methylpyrazole with the 1,3,4-thiadiazole led to "compound 12" with an 8-fold increase in potency and slightly better solubility without compromising other properties. A survey of heterocyclic cores led to the identification of the isomeric methylpyrimidine core of "compound 13", which had favorable properties overall despite its lower potency (0.6 nM). Some of this potency was restored with the introduction of a methyl group at the meta-position of the pyridine in MK-8189 ("compound 18"), which led to a >10x improvement in potency while conserving other properties.



compound 2



compound 3



Pyp-1

PDB	5C2H		
PDE10A K_i	0.008 nM	0.44 nM	0.23 nM
PDE selectivity	> 5,000x	> 2,500x	> 5,800x
pH 7 solubility	7.0 µM	162 µM	134 µM
hERG MK499 IC_{50}	4.0 µM	> 60 µM	> 60 µM
PXR EC_{50}	2.0 µM	6 µM	23 µM
CYP2C9 IC_{50}	0.3 µM	5 µM	15 µM
CYP3A4 IC_{50}	0.9 µM	4 µM	> 50 µM
Rat CL_u	12,000 mL/min/kg	2,900 mL/min/kg	870 mL/min/kg
oral F	4% (rat)	65% (rat)	81% (rat)

PDB: 5C2H

January 2023

MK-8189

PDE10A

oral PDE10A inhibitor
Ph. IIb for schizophrenia
from fragment screen + SBDD
J. Med. Chem., January 10, 2023
MERCK, WEST POINT, PA
paper DOI: https://doi.org/10.1021/acs.jmedchem.2c01521

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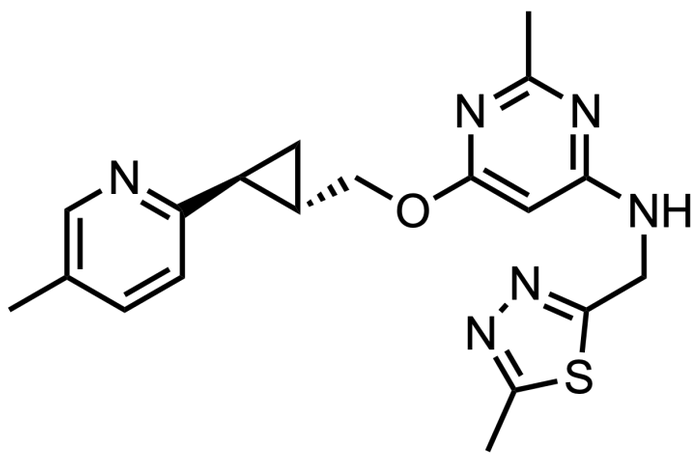
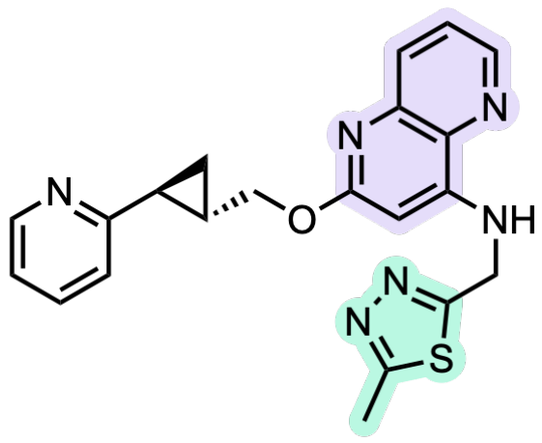


Preclinical data summary. MK-8189 displayed moderate plasma clearance and a relatively low V_D in rats and rhesus monkeys, with a half-life of 4.2 hours and 41% oral bioavailability in monkeys. The compound also had a promising *in vitro* safety and off-target profile (CYP 2C9 and 3A4 IC50 > 50 mM, PXR EC50 > 30 mM, Panlabs panel, 5-strain Ames test for mutagenicity). Transporter studies showed high permeability and that MK-8189 is not a good P-gp substrate. Notably, the candidate achieved 50% enzyme occupancy in rat striatal tissue at plasma levels of 52 nM after oral administration (0.1-10 mg/kg, PO), with full target engagement observed at higher concentrations. The drug also displayed dose-dependent decreases in a psychostimulant-induced hyperlocomotion model after oral dosing in rats at plasma levels, which corresponded to >25% enzyme occupancy. The authors also report that circulating prolactin levels were unaffected and weight gain in rats was minimal. MK-8981 also had a larger therapeutic window between antipsychotic effects and catalepsy (muscle rigidity and fixed posture) than other atypical antipsychotics.

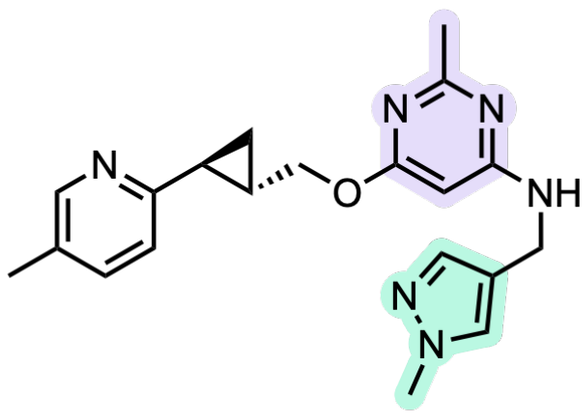
Ph. I/IIa studies are promising and the critical Ph. IIb is underway. MK-8981 was found to be generally well-tolerated in Ph. I trials (n = 14-75; NCT02181803, NCT03565068, NCT04676425, NCT04506905, NCT05227118). A Ph. I study in patients with schizophrenia is currently ongoing (NCT05406440, n = 54, dose = 48, 60, or 80 mg QD) and a Ph. IIa trial is underway (NCT03055338, n = 224). Patients received either MK-8189 (12 mg controlled release) or risperidone (6 mg) and efficacy was assessed by the PANSS total score. The results showed a trend towards improvement versus placebo after 4 weeks and narrowly missed the superiority criteria (difference = -4.7, p = 0.074). The drug showed a more pronounced effect on the PANNS positive subscale score (difference from placebo = -2.2, p < 0.05). In contrast to many existing antipsychotics, weight gain was not observed and discontinuation of treatment due to an adverse event was low (< 10%).

A Phase IIb trial is currently in progress to evaluate the drug at three doses (8 mg, 16 mg, and 24 mg QD, NCT04624243, n = 500). Patients will be treated for a total of 12 weeks in an acute treatment period (week 1-6) and an extension treatment period (week 7-12) versus risperidone (6 mg QD) or placebo. The primary outcome measurements are changes from baseline in the PANSS score, number of adverse events, and number of patients who discontinue treatment. Secondary outcomes include baseline changes from the PANSS positive subscale score and the CGI-S score, along with changes in weight at week 6 and 12. The study is currently recruiting patients and estimated completion date is June 19, 2024.

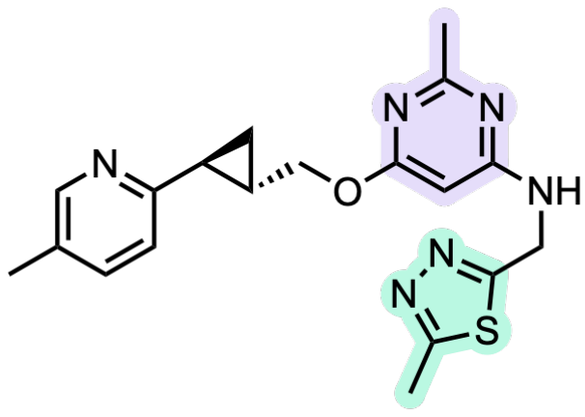
Patents. Merck owns patents for PDE10A inhibitors based on pyrimidines (WO2012044561A2, WO2013028590A1), pyrazolopyrimidines (WO2012044562A2), and a separate series of isoindolinones (WO2012058133A1).



compound 12



compound 13



MK-8189

PDB			8DI4
PDE10A K_i	0.11 nM	0.06 nM	0.029 nM
PDE selectivity	> 3,600x	> 67,000x	> 500,000x
LogD	2.6	1.6	2.1
pH 7 solubility	120 µM	180 µM	167 µM
hERG MK499/Ikr IC_{50}	25 µM	> 60 µM	33 µM
PXR EC_{50}	27 µM	> 30 µM	> 30 µM
CYP2C9 IC_{50}	24 µM	> 50 µM	> 50 µM
CYP3A4 IC_{50}	37 µM	> 50 µM	> 50 µM
Rat CL_u	388 mL/min/kg	91 mL/min/kg	870 mL/min/kg
oral F	48% (rat)	113% (rat)	46% (rat), 41% (rhesus monkey)

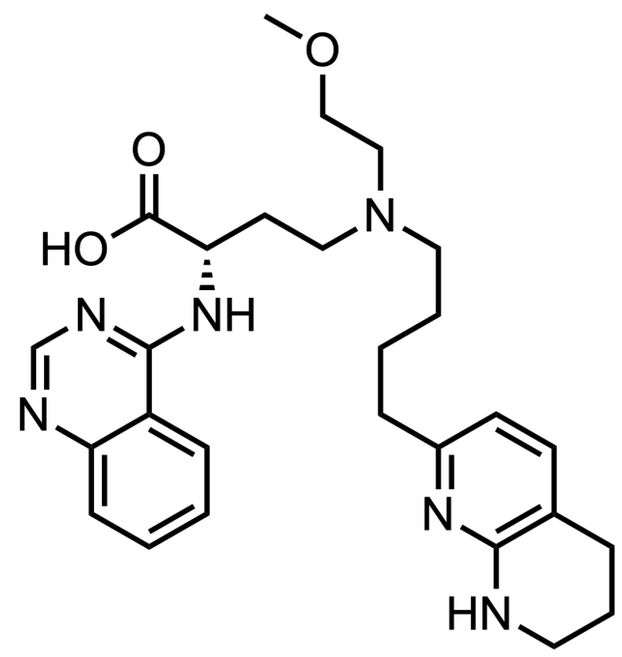
PDB: 8DI4

January 2023

bexotegrast

αvβ6/αvβ1

oral, dual-selective αvβ6/αvβ1 integrin inhibitor
Ph. II for IPF + PSC
related to GSK integrin inhibitors
Press release, January 22, 2023
PLIANT THERAPEUTICS, SAN FRANCISCO, CA
press release: https://ir.pliantrx.com/news-releases/news-release-details/pliant-therapeutics-announces-positive-data-integris-ipf-phase



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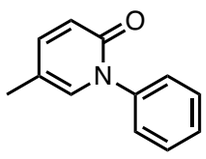
A surprisingly safe and possibly efficacious dual-selective $\alpha_v\beta_6$-$\alpha_v\beta_1$ inhibitor, providing therapeutic entry to the notoriously challenging TGF-β pathway. Integrins are cell adhesion proteins that help cells bind to the extracellular matrix and have a range of signaling functions. The arginyl-glycinyl-aspartic acid (RGD) subfamily of α_v integrins have been studied for decades in a variety of indications including cancer and idiopathic pulmonary fibrosis (IPF). Targeting α_v integrins has been attractive yet challenging as they are upstream of transforming growth factor-β (TGF-β). TGF-β is an extensively studied target with established roles across therapeutic areas, but the systemic blockade of its pathway comes with well-known safety risks. The risks identified in genetic and animal studies include inflammatory defects, hemorrhagic lesions in heart valves, and risk of developing cancers. Any upstream target of TGF-β, including α_v integrins, could therefore carry the same therapeutic risks, but these can be justified by addressing a significant unmet medical need and potentially minimized if the upstream mechanism has an improved therapeutic window and/or less severe toxicities. Pliant recently reported data showing orally bioavailable, dual-selective $\alpha_v\beta_6$ and $\alpha_v\beta_1$ inhibitor, bexotegrast (PLN-74809), has early signs of efficacy and surprisingly positive safety in Ph. II trials for IPF, which caused Pliant's stock to increase 50% to $35 per share and allowing the company to announce their plan of raising $175M in stock sales to support further R&D.

Effective treatments for idiopathic pulmonary fibrosis (IPF) and pulmonary sclerosing cholangitis (PSC) remains a significant unmet medical need. IPF is a progressive lung disease that affects 7-17 patients per 100,000 people per year in the US and results in excessive accumulation of collagen that ultimately destroys tissue function over time. As the primary mediator in fibrosis, TGF-β is activated by α_v integrins, which results in increased collagen production that injures lung or liver tissues. The RGD receptor family integrins are upregulated in pulmonary tissue in IPF and liver tissue in PSC patients, where $\alpha_v\beta_6$ overexpression occurs within epithelial cells and $\alpha_v\beta_1$ overexpression occurs within fibroblasts. Currently, there are two FDA approved drugs to treat IPF, multi-targeted kinase inhibitor nintedanib (Ofev) and pirfenidone (Esbriet), with unclear MOAs. Where these drugs aren't target-based, they only modestly impact disease progression. Furthermore, there are no approved therapies for PSC. The FDA has granted bexotegrast Fast Track Designation for IPF and Orphan Drug Designation for both IPF and PSC. The EMA has also granted Orphan Drug Designation for IPF and PSC. Given the unmet medical need, pursuing integrin inhibition in these indications is therefore a high-risk, high-reward endeavor.

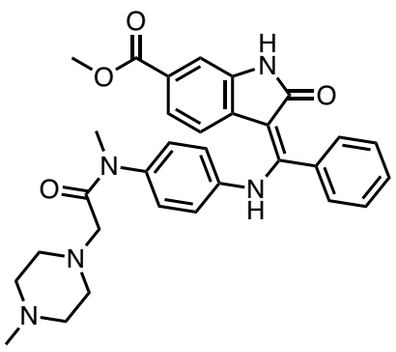
Elucidating the safety risks of $\alpha_v\beta_6$ integrin inhibition. The safety data reported by Pliant was especially surprising given that other $\alpha_v\beta_6$ inhibitors had been terminated for safety concerns, including Biogen's anti-$\alpha_v\beta_6$ antibody (BG00011). In particular, Morphic Therapeutics recently reported preclinical safety issues with its oral, selective $\alpha_v\beta_6$ integrin inhibitor, MORF-627, that led to the termination of its development. The structure of the molecule has yet to be disclosed, but induced urinary bladder tumors in monkeys, which was likely related to AbbVie and J&J halting their licensing deal with Morphic. Morphic's study suggested that the tumor development was on-target and based on prior observations with TGFβR1 kinase inhibitors, and various $\alpha_v\beta_6$ integrin inhibitors of varying selectivities and modalities (e.g., conformation-independent). Even further, hyperplasia induction is a well-understood consequence of TGF-β blockade biology. Concerningly, the finding appears to be relevant to humans in vitro, as MORF-627 induced epithelial proliferation in cultured primary human bladder cells.

MORF-627 vs. GSK3008348 vs. bexotegrast. The released data on MORF-627 is relevant given that it has several significant structural similarities to bexotegrast. Although the structure and discovery story of MORF-627 is undisclosed, both molecules likely derive from GSK's $\alpha_v\beta_6$ integrin program and inhibitor, compound 1 (GSK3008348), a molecule we have previously covered. All molecules have the tetrahydronapthryridine group (important for $\alpha_v\beta_6$ potency), a butanoic acid (necessary for coordination of the metal in the metal-ion dependent adhesion site (MIDAS) on the β domain), a basic amine in the linker, and an aromatic group (highlighted in red). Moieties similar to these are present in a representative structure from Morphic Therapeutics' 2018 patent, example 14. Given that Morphic is no longer working on the target, this patent likely covers the $\alpha_v\beta_6$ integrin inhibitor MORF-627 (WO2018160521A2).

No significant safety concerns for bexotegrast in preclinical + clinical studies to-date. Despite the apparent on-target effects observed with MORF-627, bexotegrast safety data appears to be remarkably clean. Pliant reports no evidence of toxicity in monkeys with 3 month sub-chronic and 9 month chronic dosing, including no pulmonary infiltrates, no bladder cancer, no respiratory or CV effects in telemetered NHPs, and an NOAEL level at the highest dose tested in NHPs. Additionally, there have been no adverse effects reported in over 600 humans dosed (640 mg sd/320 mg md) to-date. Preclinical study data shared by Pliant indicated no significant concerns for genotoxicity, hERG liability, or impact on reproductive toxicity in mouse or rabbit.



nintedanib
150 mg BID



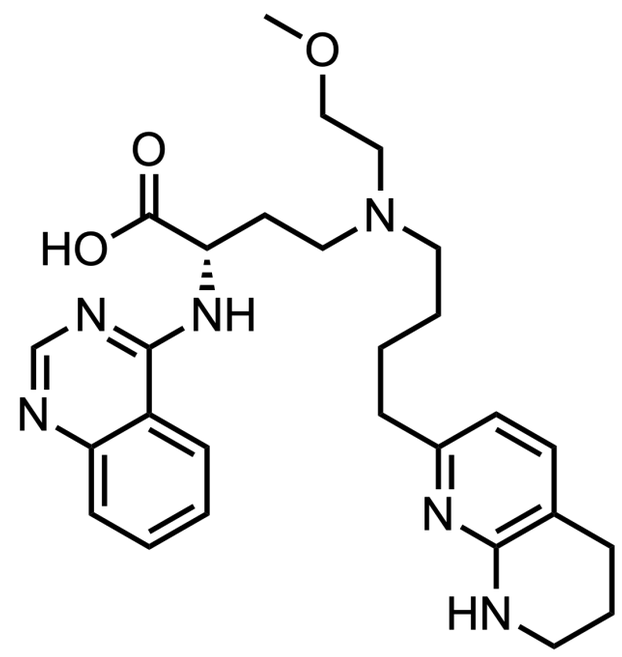
pirfenidone
801 mg TID

January 2023

bexotegrast

αvβ6/αvβ1

oral, dual-selective αvβ6/αvβ1 integrin inhibitor
Ph. II for IPF + PSC
related to GSK integrin inhibitors
Press release, January 22, 2023
PLIANT THERAPEUTICS, SAN FRANCISCO, CA
press release: https://ir.pliantrx.com/news-releases/news-release-details/pliant-therapeutics-announces-positive-data-integris-ipf-phase



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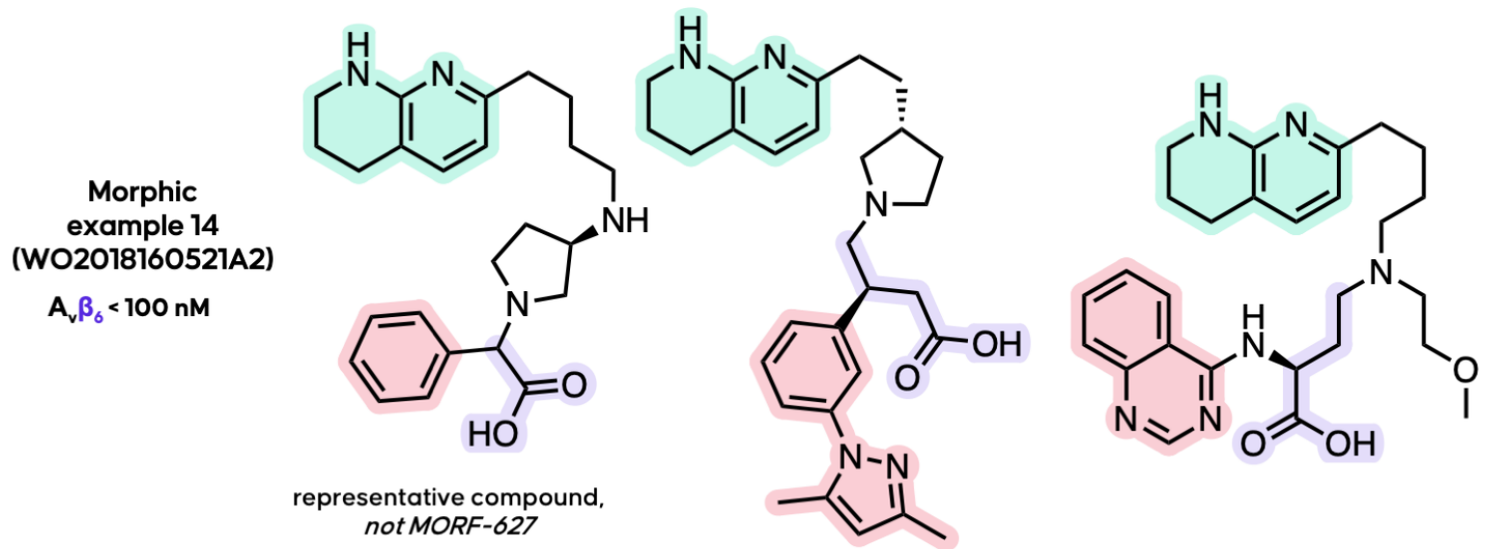
Bexotegrast exhibits nanomolar activity and complete target engagement at the tested doses. From recently reported Ph. IIa data (NCT04396756) encompassing a 12-week treatment period, bexotegrast was found to be safe and tolerable with once-daily doses in patients with IPF (n = 119) ranging from 40 mg to 320 mg. The corresponding treatment-related adverse events were rated as only mild or moderate. Currently, bexotegrast is in Ph. II studies for IPF (NCT05621252, NCT04396756) and PSC (40, 80, and 160 mg doses, NCT04480840). A PET study revealed all patients (n= 5) exhibited >50% $\alpha_v\beta_6$ target engagement, with >90% target engagement in the lungs at the highest doses (i.e., 240 and 320 mg, NCT04072315). Administration of bexotegrast in healthy volunteers showed a favorable pharmacokinetic profiles with steady state concentrations reached within 5-7 days and an average half-life of 40 h observed (10-40 mg QD for 14 days). At 12-weeks, patients treated with bexotegrast (n= 67) had an 80% improvement in forced vital capacity (FVC) when compared to placebo (n= 23) (-15.1 mL vs -74.1 mL, pooled doses of bexotegrast vs control, respectively). When dosed with 320 mg of bexotegrast, patients in this cohort exhibited a significant increase in FVC at all study timepoints (i.e., at 4, 8, and 12 weeks). Finally, no patients treated with bexotegrast at any dose exhibited disease progression (i.e., FVC percent predicted decline ≥ 10%). Preclinically, bexotegrast acts potently via the expected mechanism - using a classic bleomycin mouse model for IPF, bexotegrast exhibited dose-dependent inhibition of Smad3 phosphorylation and targeted blockade of TGF-β in fibrotic tissue. In tissue cultures from IPF patients, only a 2 nM concentration of bexotegrast was required to significantly reduce collagen gene expression by 50%, compared to micro- and millimolar effective concentrations required for nintedanib and pirfenidone, respectively.

In contrast with single-integrin targeting compounds, bexotegrast's nanomolar inhibition of two integrins simultaneously produces an additive effect, reducing collagen in ex vivo IPF lung tissue cultures (IC_{50} $\alpha_v\beta_6$ = 5.7 nM, $\alpha_v\beta_1$ = 3.4 nM). This also suggests that the $\alpha_v\beta_1$ integrin is important for fibrosis progression . One can speculate that this dual-targeting strategy may contribute to greater efficacy within lung tissue, operating within a larger window to systemically suppress TGF-β signaling.

So why is bexotegrast different? Based on the history of $\alpha_v\beta_6$ inhibitors, the safety observed for bexotegrast is remarkable. Without additional data, one can only speculate on the reasons. One possibility is that bexotegrast may have greater distribution to the lungs rather than to other organs (such as bladder and liver) than other integrin inhibitors. Another possibility is that a MOA involving $\alpha_v\beta_6$ binding that leads to integrin internalization and subsequent lysosomal degradation, which may result in different downstream effects. Past studies with GSK's molecule revealed that molecular binding to the RGD domain of $\alpha_v\beta_6$ triggered integrin endocytosis and subsequent lysosomal degradation, which may also be a predominant mechanism for bexotegrast or MORF-627, though there is no data disclosed on this. This mechanism indicates that sustained target engagement might be achieved with one of the molecules (for better or worse) at C_{max} without the requirement of prolonged target coverage. Although GSK's molecule had a promising safety and tolerability profile as an inhaled therapeutic in a Ph. I study (1-3000 µg), it was strategically terminated, presumably to focus on their oncology pipeline. As a result, specifics around the degradation mechanism in humans remains poorly understood. Regardless, bexotegrast and α_v integrin biology will likely continue to be scrutinized for any hint of an explanation.

Patents. Pliant currently holds 3 patents: "Amino acid compounds and methods of use" (WO2019173653A1, 2019), "Dosage forms and regimens for amino acid compounds" (WO2020210404A1, 2020), and "Treatment of respiratory diseases with amino acid compounds" (WO2021225912A1, 2021).



$\alpha_v\beta_x$ integrin binding	MORF-627 (not disclosed)	GSK3008348	bexotegrast
$\alpha_v\beta_6$	0.9	3.4	5.7
$\alpha_v\beta_1$	486	4.0	3.4
$\alpha_v\beta_3$	>200,000	299	>10,000
$\alpha_v\beta_5$	>200,000	23.5	6989
$\alpha_v\beta_8$	330	6.8	2539

Binding of inhibitors to $\alpha_v\beta_x$ integrin, IC_{50} values (nM)

January 2023

BDM2

LEDGF-IN

oral HIV-1 IN-LEDGF/p75 allosteric inhibitor
Ph. I for FIH safety
from cell screen of INLAI library
BioRxiv, January 28, 2022
BIODIM, ROMAINVILLE, FR
paper DOI: https://doi.org/10.1101/2023.01.28.523533

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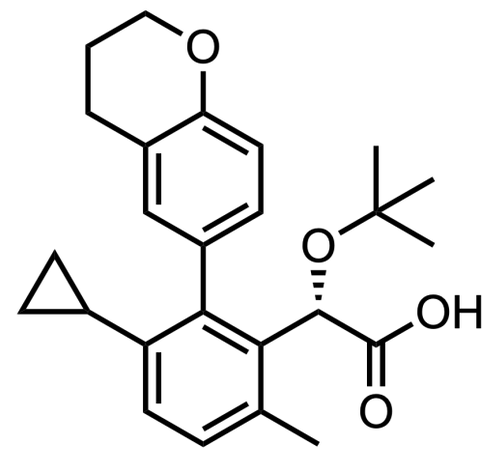


Newly disclosed HIV-1 antiretroviral is the first integrase-LEDGF/p75 allosteric HIV-1 inhibitor to clear Ph. I safety trials. Biodim has recently disclosed a small molecule candidate, BDM-2, that demonstrates nanomolar activity against HIV-1 replication in vitro and showed promising safety in healthy subjects in a 2020 Ph. I clinical trial (NCT03634085). BDM-2 is an allosteric HIV-1 integrase (IN) inhibitor (ALLINI), a class of compounds discovered prior to 2009 by Boehringer Ingelheim (WO2009/062285A1) that have a distinct mechanism of action from other HIV antiretroviral therapies, potentially offering a route to countering HIV mutants that are resistant to existing therapies. The molecule is highly potent, with an EC_{50} of 8.7 nM for HIV-1 NL4-3 inhibition in MT4 cells (via HTFR), exceeding that of raltegravir, the first FDA-approved INSTI. Furthermore, the molecule is reported to have activity against an HIV strain that is resistant to all other known classes of HIV drugs, and does not antagonize the activity of any known classes of drugs.

How does it work? HIV integrase is a well-established target, now with five-approved HIV integrase strand transfer inhibitors (INSTIs). In contrast to INSTIs, which block the catalytic site of HIV-1 IN that facilitates DNA strand transfer activity, this novel ALLINI class of antiretrovirals inhibits HIV-1 replication by targeting a different HIV integrase site, the cellular LEDGF/p75 (LEDGF) protein binding site on HIV-1 IN. LEDGF binds within a cleft in IN multimers and acts as a tether to support integration into gene-dense regions of the host cell's genome. Inhibitor binding at this site disrupts integration by interfering with the IN-LEDGF interaction and promoting IN multimerization that inhibits IN-mediated 3' end processing and DNA strand transfer. Virions produced in the presence of ALLINIs are also defective for reverse transcription and are non-infectious, likely due to this aberrant IN multimerization during assembly of the viral particles.

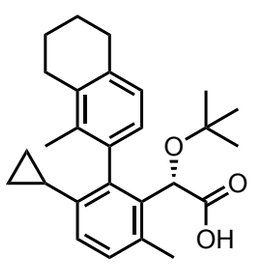
Another opportunity to address HIV drug resistance. Though there are currently five INSTIs approved by the FDA for HIV-1 treatment, all five are typically prescribed in combination with other antiretroviral therapies due to resistance mechanisms arising from genetic HIV-1 mutations. BDM-2 maintained activity against HIV-1 mutants resistant to other FDA-approved antiretrovirals and showed no antagonism with other antiretrovirals in combination studies in MT4 cells, signaling that combination therapies involving BDM-2 would continue to be effective in combination with existing HIV-1 treatments. While BDM-2 is not immune to resistance, the HIV-1 mutants resistant to BDM-2 have orthogonal resistance mechanisms to other drug classes, with the most detrimental mutation T174I arising within the LEDGF/p75 binding pocket.

Early trials show an acceptable safety profile. Repeat dose toxicity studies in mice (1000 mg/kg/day) and dogs (300 mg/kg/day) over a two-week study period identified the liver as the target organ. Though increased liver weight was observed in both species and elevated liver enzymes were observed in dogs along with some vomiting, these effects were reversible. In a FIH Ph. I trial (NCT03634085) that concluded June 2020, no serious adverse effects were observed in a cohort of 16 healthy male subjects dosed at 50-3600 mg (oral) of BDM-2. Mild adverse effects included a few instances of diarrhea, dizziness, and nausea. No PK data has been reported from the trial and no other clinical trials with BDM-2 have been announced.

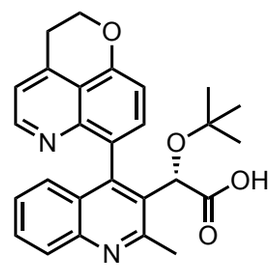
What else is out there? The safety data is valuable given that the first ALLINI to enter the clinic, BI-224436 from Boehringer Ingelheim, had its Ph. I trial interrupted for an unknown reason (NCT01276990), presumably because of toxicity issues. Gilead similarly reported a potent ALLINI compound, GS-9822, which triggered renal and urinary bladder toxicity. BMS has also reported a potent tetrahydronapthyridine series, but the new owner VIIV has not entered the clinic with them. In addition to BDM-2, Ph. I results for another ALLINI, pirmitegravir/STP0404, were shared in late 2022 and a Ph. IIa trial is anticipated.

A signature α-tert-butoxy benzylic carboxylic acid motif. While the discovery of BDM-2 is not described, it is structurally related to previously disclosed molecules in the class. The first ALLINI series discovered by Boehringer Ingelheim consisted of quinoline or thiazolopyridine cores with a pendant carboxylic acid, and those with nanomolar potency for HIV-1 replication in cell-based assays contained a bulky α-*tert*-butoxy group. The most potent IN-LEDGF/p75 allosteric inhibitors all contain an α-*tert*-butoxy benzylic carboxylic acid motif, a signature of the molecule class. Replacement of this group with methoxy, propyl, or -H leads to diminished potency. Co-crystal structures of potent ALLINIs with HIV-1 IN suggest there are H-bond interactions between the carboxylic acid and *tert*-butoxy group with Glu170, His171 and Thr174 residues in LEDGF/p57 binding pocket.

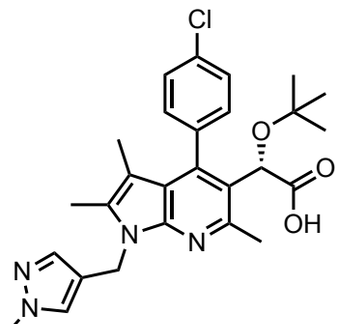
Patents. "Inhibitors of viral replication, their process of preparation and their therapeutical uses": WO2015001125A1 (2014).



MUT871
Biodim



BI-224436
Boehringer Ingelheim



STP0404
ST Pharm, Co.

January 2023

GB0139

galectin-3

inhaled galectin-3 inhibitor
Ph. II for IPF + Ph. Ib/IIa for COVID-19 pneumonitis
synthetic galactoside derivative
medRxiv, January 10, 2023
GALECTO, INC., COPENHAGEN, DE
paper DOI: https://doi.org/10.1101/2021.12.21.21267983

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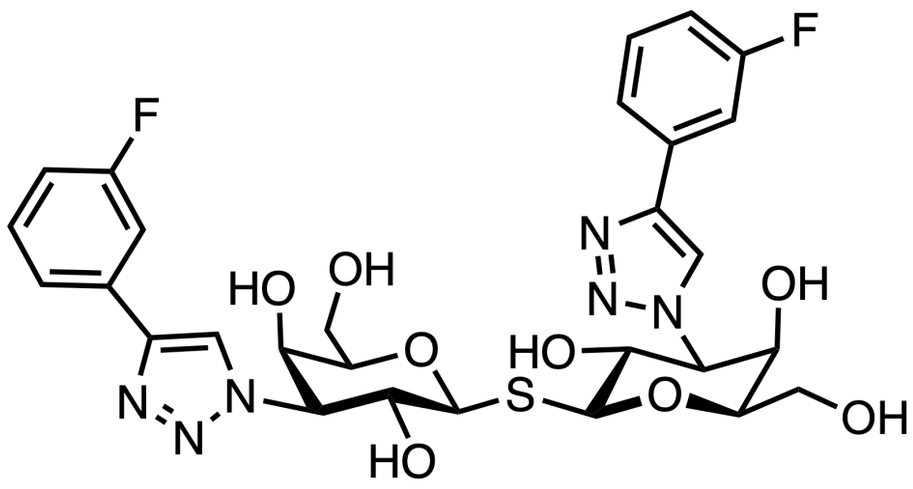


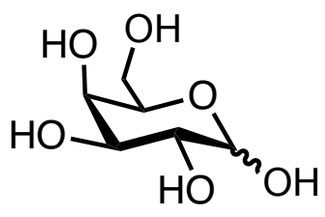
What is it? GB0139 is a selective, inhaled inhibitor of galectin-3 and galectin-1 in Ph. II for IPF and COVID-19 pneumonitis, and is Galecto's ($GLTO) lead asset. Galecto was founded in 2011 with Lund University academic galectin research leaders Ulf Nilsson and Hakon Leffler, and has had a research focus on galectin-3 modulators to treat fibrotic diseases. We recently covered another Galecto development compound, GB1211, a related oral galectin-3 inhibitor, Ph. I/II candidate, and September 2022 Molecule of the Month. With the 2022 Nobel Prize in Chemistry awarded for "click chemistry," this molecule is an interesting example of a development candidate derived from an azide-alkyne cycloaddition.

Galectin-3 as a target. Galectins are a relatively underexplored target class, despite academic research suggesting potential roles in a variety of difficult-to-treat diseases, including idiopathic pulmonary fibrosis (IPF). As the name suggests, galectins are small (~30 kDa) lectins, or carbohydrate-binding proteins, that bind to β-D-galactopyranoside-containing glycoproteins and modulate glycoprotein localization, transport and residence times in both cellular compartments and on cell surfaces. Galectin-3, associated with macrophages in particular, appears to be an important regulator of chronic inflammation in the liver, lungs, kidneys and within tumors, as Gal-3-secreting macrophages promote myofibroblast differentiation and scarring. Galectin-3 expression rises sharply in the human serum of lung fibrosis patients during acute exacerbations, and high levels of galectin-3 in tumors correlate with worse prognosis.

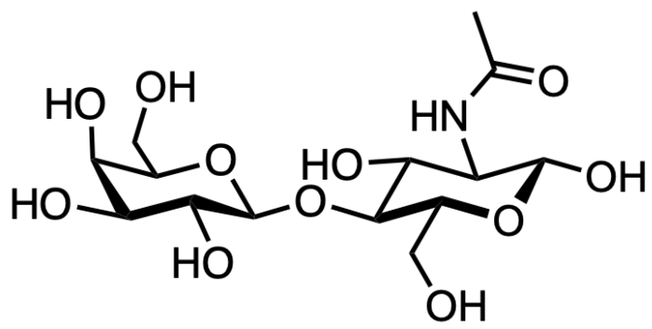
Why do we care? As two of the most clinically advanced galectin-3-targeting drugs, GB0139 and GB1211 offer opportunities to validate galectin-3 biology in humans with first-in-class drug potential in challenging disease settings. Preclinical studies with GB0139 have shown that inhibition of galectin-3 acts downstream of TGF-β to mitigate progression of lung fibrosis, suggesting that galectin-3 inhibitors could have antifibrotic activity without the systemic toxicities related to TGF-β inhibition (e.g., see 'bexotegrast' this month). GB0139 and GB1211 have so far been well-tolerated in humans and have demonstrated significant dose-dependent effects in humans on various serum biomarkers, including reducing Gal-3 levels in macrophages, as well as lowering of biomarkers associated with IPF disease severity and progression. One such biomarker is YKL-40, a prognostic for disease progression in the Ph. III trials of the IPF drug pirfenidone. GB0139 will next need to demonstrate efficacy on Forced Vital Capacity (FVC) as a standard key endpoint for IPF in its Ph. II trial (NCT03832946, 3 mg by inhalation QD), which is due to complete in June 2023.

How was it discovered? Given the natural affinity of galectins for D-galactopyranosides, this class of molecules made a logical starting point. In 2005, a fluorescence polarization assay was used to demonstrate that the more hydrolytically stable thiodigalactoside (TDG) had better affinity for galectin-3 as compared to *N*-acetyllactosamine (LacNAc), the best-known, naturally occurring disaccharide ligand for galectins at that time. Aryltriazole-containing thiodigalactosides derived from "click chemistry" with azidosugars were investigated, leading to GB0139 ("compound 4", Gal-3 K_d = 14 nM, Gal-1 K_d = 12 nM, >8x selectivity over others). The X-ray crystal structure suggests that the significant increase in affinity relative to thiodigalactoside is in part due to the cation-π interactions of the triazole and fluorophenyl groups with Arg144 and Arg186 (**PDB: 5E89**).

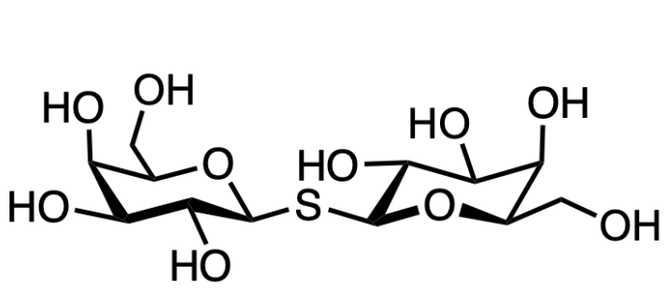
Patents. "Galactoside inhibitor of galectin-3 and its use for treating pulmonary fibrosis," US9580456B2 (**2017**). "Galactoside inhibitor of galectins,"US9243021B2 (**2016**). "Galactoside inhibitor of galectins," US9688713B2 (**2017**). "Idiopathic pulmonary fibrosis-detection, monitoring, prediction methods," US20210221836A1 (**2021**).



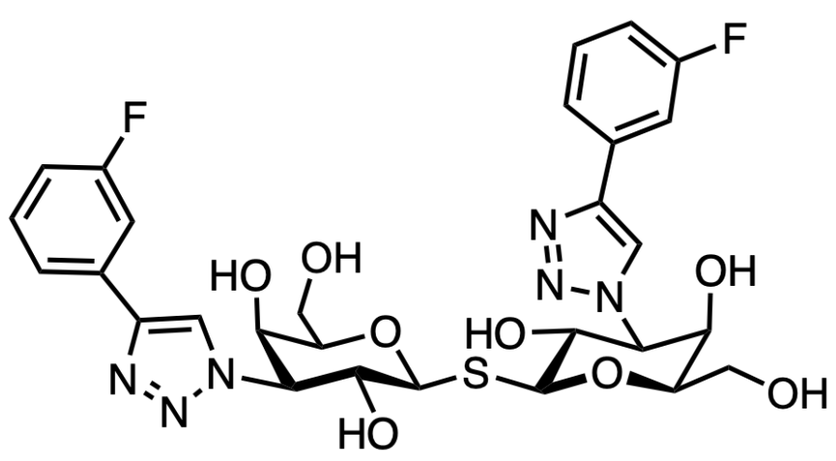
D-galactopyranoside



LacNAc
N-acetyllactosamine
Gal-3 K_d = 0.2-1 mM



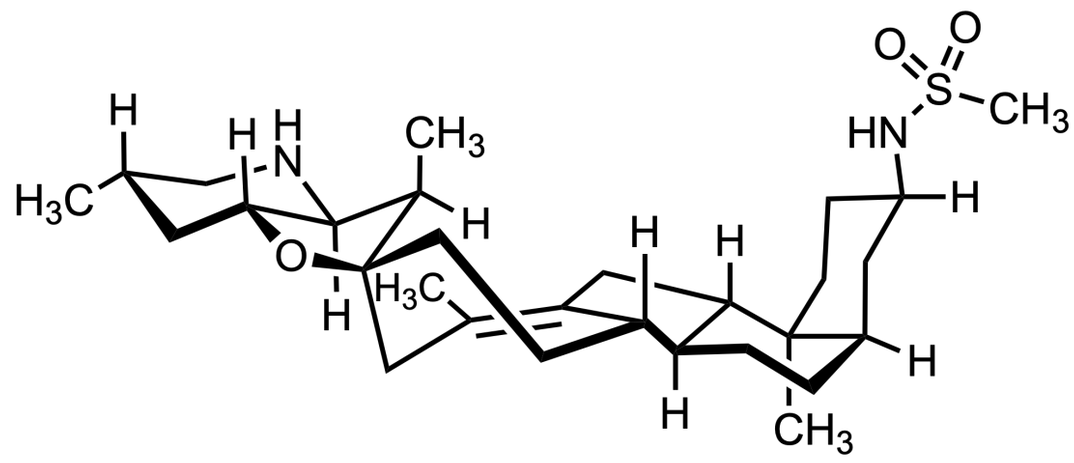
TDG
thiodigalactoside
Gal-3 K_d = 49 µM



GB0139
compound 4
Gal-3 K_d = 2.3 nM

January 2023

saridegib



SMO

oral SMO inhibitor
Ph. III for Gorlin syndrome
from SAR of in-house library hit
Press release, January 27, 2023
INFINITY PHARMACEUTICALS, CAMBRIDGE, MA (SOL-GEL)
press release: https://ir.sol-gel.com/news-releases/news-release-details/sol-gel-acquires-patidegib-phase-3-fda-breakthrough-designated

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What is it? Saridegib (IPI-926, patidegib) is a Smoothened receptor (SMO) antagonist and hedgehog pathway inhibitor currently in Ph. III trials for Gorlin syndrome. The molecule is a semisynthetic analog of the naturally-occuring SMO antagonist, cyclopamine, and has a long history and originates from Infinity Pharmaceuticals. After a clinical disappointment as an oral therapy for myelofibrosis and chondrosarcoma in 2012, Infinity out-licensed the molecule to PellePharm in 2013, a company later backed by BridgeBio. Recently, it again changed hands from BridgeBio/PellePharm to Sol-Gel Technologies in a ~$75M transaction. Sol-Gel plans to continue development of the molecule for Gorlin syndrome and basal cell carcinomas as a topical treatment to avoid systemic toxicities, giving this older molecule hope of a new life. This case study is a relatively rare modern example of natural product-inspired drug discovery.

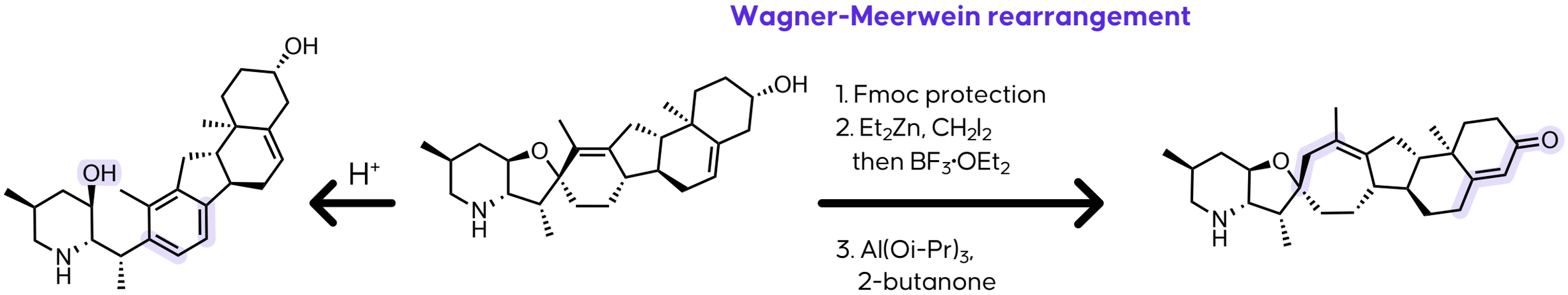
Why do we care? Hedgehog (Hh) pathway inhibitors have been of long-standing interest in cancer research. Activation of the Smoothened receptor (SMO) promotes cell growth, and overactivation is cancerous. The first human connection between the Hh pathway and cancer came from the rare genetic disorder, Gorlin's syndrome, which predisposes individuals to basal cell carcinomas and other tumors. Gorlin's syndrome results from genetic inactivation of PTCH, a suppressor of SMO, and has no disease-modifying treatments. Hence, SMO antagonists have been approved for basal cell carcinoma treatment and may be an effective approach to treating this genetic disease.

How was it discovered? Saridegib is derived from the natural product cyclopamine, a steroid alkaloid that can be isolated from *Veratrum californicum* (corn lily). The name "cyclopamine" comes from the lore of the one-eyed cyclops monster, as ingestion of high amounts of the plant causes birth defects in lambs that include holoprosencephaly, when the embryonic brain fails to divide into two hemispheres, resulting in lambs with only one eye. This property was later tied to cyclopamine's SMO antagonism, as the hedgehog pathway is essential to axial patterning during embryonic development. Despite significant topical efficacy on psoriatic skin lesions and BCC, cyclopamine itself was a poor oral drug candidate due preclinical side effects including dehydration and death, and poor oral bioavailability owing to its poor solubility and acid instability.

Improved acid stability in semisynthetic 7-membered ring analogs. Infinity scientists started with isolated cyclopamine to produce semisynthetic derivatives with improved properties. Homologation of the unstable 6-membered D-ring to a 7-membered ring via cyclopropanation and acid-catalyzed rearrangement resulted in potent molecules with acid stability. An additional Oppenauer oxidation afforded IPI-269609, which served as a valuable in vivo tool compound with xenograft activity, but suffered from relatively low potency and insufficient metabolic stability.

Improved PK and surprising potency increases through reduction of a metabolic hotspot. The enone moiety of IPI-269609 in particular was readily metabolized via reduction and glucuronidation in monkeys. Reduction of the enone to a cis-decalone ("compound 6") led to a remarkable >23x increase in potency. As the ketone could still be metabolized by reduction and cleared via glucuronidation of the corresponding alcohol, alternative polar groups were evaluated, as a non-polar group in this position rendered compounds that were only weakly active. The (*R*)-sulfonamido derivative of saridegib ("compound 28") had comparable potency to "compound 6", with significantly greater cellular activity than cyclopamine (IC_{50} = 1.4 nM vs. 114 nM for cyclopamine in C3H10T1/2 cells) and greatly improved PK properties relative to the enone starting point. Saridegib demonstrated tumor regression in an allograft model of Hh-pathway-dependent medulloblastoma at 40 mpk PO QD, whereas "compound 2" showed no activity in the same model at its maximum tolerated dose.

Simmons-Smith cyclopropanation
Wagner-Meerwein rearrangement



veratramine

cyclopamine
Hh EC_{50} = 170 nM
Aq. solubility = 5 µg/mL
acid-labile

Oppenauer oxidation

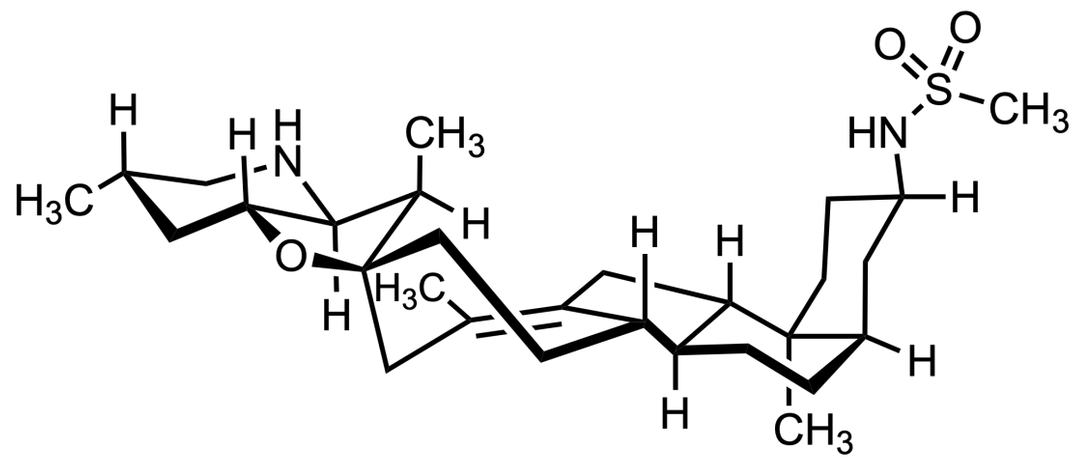
IPI-269609 (compound 2)
Hh EC_{50} = 200 nM
Aq. solubility = 100 µg/mL
acid-stable

January 2023

saridegib

SMO

oral SMO inhibitor
Ph. III for Gorlin syndrome
from SAR of in-house library hit
Press release, January 27, 2023
INFINITY PHARMACEUTICALS, CAMBRIDGE, MA (SOL-GEL)
press release: https://ir.sol-gel.com/news-releases/news-release-details/sol-gel-acquires-patidegib-phase-3-fda-breakthrough-designated



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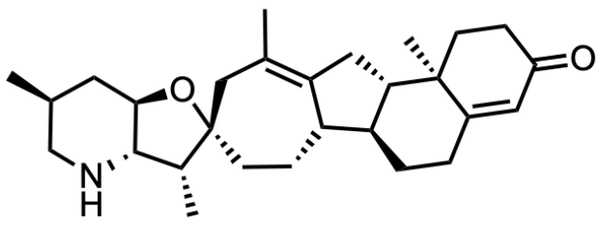
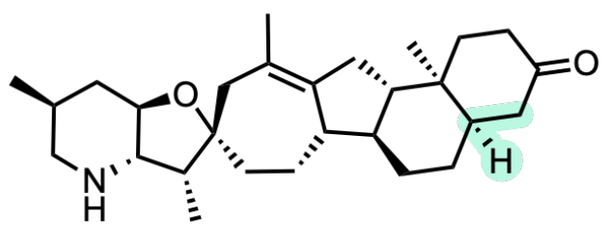
What else is out there? Unfortunately for the Infinity oncology program, but fortunately for patients, Genentech's much simpler but efficacious oral SMO inhibitor, vismodegib (GDC-0449), was first to demonstrate clinical activity in basal cell carcinoma and was approved in 2012 (150 mg PO QD). Novartis's sonidegib (NVP-LDE-225, Sun Pharma) followed in 2015 for locally advanced BCC (200 mg PO QD).

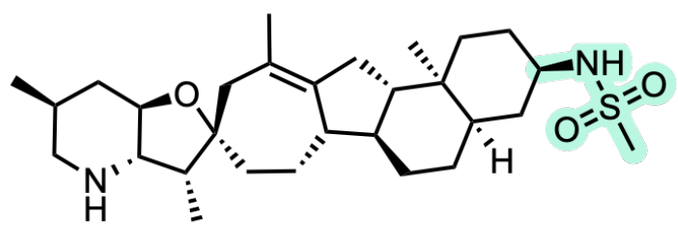
Patents. For "compound 2" (IPI-269609) by Infinity Pharmaceuticals Inc: US20110034498A1, WO2007123511. For IPI-926 by Infinity Pharmaceuticals Inc: US8227509B2. For saridegib/patidegib by PellePharm: US10695344B2, US20170231968A1.



IPI-269609
(compound 2)

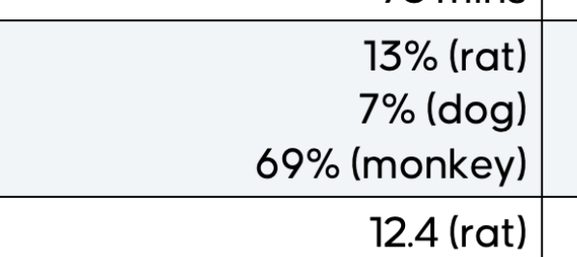


compound 6

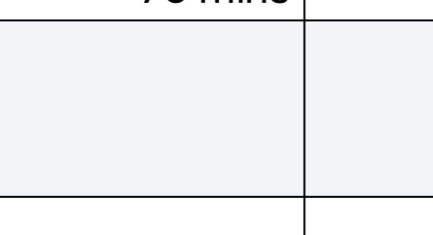


saridegib
(compound 28)

Hh EC_{50} (cell)	0.3 µM	0.013 µM	0.007 µM
$t_{1/2}$ (cell)	75 mins	70 mins	85 mins
oral F	13% (rat) 7% (dog) 69% (monkey)		100% (rat) 50% (dog) 74% (monkey)
oral Cl (L/hr/kg)	12.4 (rat) 4.7 (dog) 6.2 (monkey)		0.21 (rat) 0.66 (dog) 0.90 (monkey)
oral V_d (L/kg)	28 (rat) 13.9 (dog) 21.3 (monkey)		30 (rat) 15.3 (dog) 9.5 (monkey)
oral $t_{1/2}$ (hr)	1.7 (rat) 2.2 (dog) 2.4 (monkey)		>24 (rat) ~15.5 (dog) 8.2 (monkey)



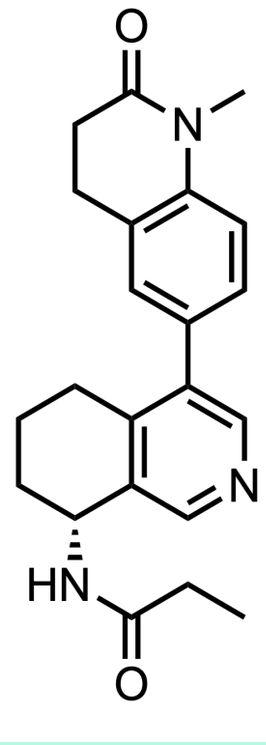
sonidegib



vismodegib

January 2023

baxdrostat



aldosterone synthase

oral aldosterone synthase (CYP11B2) inhibitor
Ph. I/II for HTN, CKD, primary aldosteronism
from in-house screen of aldosterone synthase inhibitors
Press release, January 9, 2023
ROCHE, BASEL, CH (ASTRAZENECA)
press release: https://www.astrazeneca.com/media-centre/press-releases/2023/astrazeneca-acquire-cincor-for-cardiorenal-asset.html

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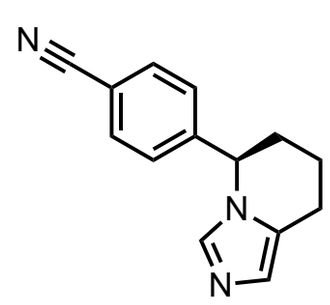
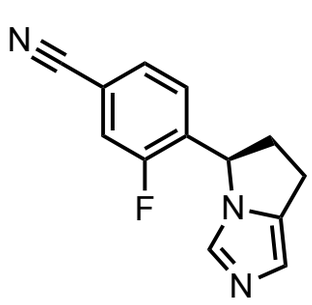
What is it? Baxdrostat (RO6836191, CIN-107) is an oral aldosterone synthase (CYP11B2) inhibitor in several Ph. II trials (0.5-2 mg QD) for hypertension, CKD, and primary aldosteronism. The molecule is highly selective (>100x) against a cortisol synthesis enzyme, CYP11B1, allowing it to avoid negative effects due to cortisol reduction in the clinic, a longstanding challenge for clinical application of aldosterone synthase inhibitors. The molecule demonstrated dose-dependent PK/PD and overwhelming, trial-stopping efficacy in terms of reductions in systolic and diastolic blood pressure in hypertensive patients, contributing to the value of its recent $1.8B acquisition by AstraZeneca via CinCor. Originally discovered by Roche, it was out-licensed to a Cincinnati company with a 2022 IPO, CinCor, after Roche exited CV drug development, and serves as another nice example of high-quality, out-licensed pharma molecules receiving a second life in biotech.

Why do we care? While the potential of aldosterone synthase inhibition for treating hypertension has been recognized for at least three decades, its reduction to practice has been hampered by a lack of selectivity for CYP11B2 over CYP11B1 until now, due to the 93% aa sequence similarity between the two enzymes. This potential new approach to treat hypertension is significant given the sheer magnitude of hypertension prevalence worldwide. As much as 20% of the global population suffers from hypertension, including nearly half of all adults in the US. Despite many existing treatment options, as many as 13-15M patients in the US suffer from treatment-resistant hypertension (rHTN) after three or more medications, and as many as 30-35M US patients have uncontrolled hypertension (uHTN) after one or two therapies. Untreated hypertension is a high-risk state that leads to well-established increased risks of cardiovascular disease, stroke, and all-cause mortality. In 2017, the American College of Cardiology/American Heart Association (ACC/AHA) hypertension guideline lowered the definition of hypertension and treatment target from 140/90 mmHg to 130/80 mmHg based on these risks. Baxdrostat so far appears to significantly lower blood pressure by double-digit mmHg units in patients with resistant hypertension, making it a potentially timely new tool to tackle this growing problem.

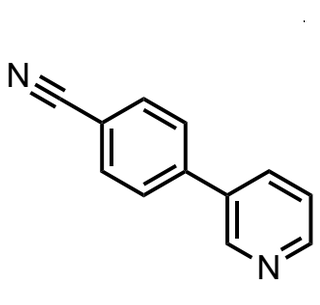
How does it work? Excess aldosterone can raise blood pressure by increasing sodium retention, and is a key component of the renin-angiotensin-aldosterone system (RAAS) that many successful blood pressure medications act on, including mineralocorticoid receptor (MR) antagonists like spironolactone (1959), angiotensin-converting enzyme (ACE) inhibitors like captopril (1980), and angiotensin receptor blockers (ARBs) like valsartan (1996). By preventing aldosterone synthesis, aldosterone synthase inhibitors act downstream of angiotensin but upstream of MR antagonists, which prevent the aldosterone from engaging its nuclear receptor to moduate gene expression. This allows aldosterone synthase inhibitors like baxdrostat to have rapid action due to the non-genomic effects of aldosterone mediated by GPCRs such as GPER1, address mechanisms of hypertension resistant to other treatments, and potentially have a better safety profile since MR engages with ligands other than aldosterone. Like most CYP inhibitors, baxdrostat binds to the heme center of CYP11B2 through its pyridine moiety (K_i = 13 nM), preventing hydroxylation of the aldosterone precursor, but sparing cortisol synthesis due to its >100x selectivity over CYP11B1. In humans, maximum aldosterone suppression is observed at a single dose of 10 mg, with no impact on cortisol up to 360 mg.



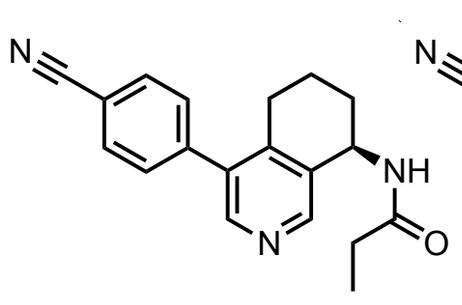
Fadrazole
(FAD286)



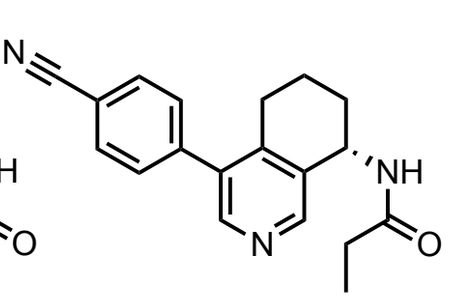
LCI699



compound 18



(*R*)-compound 6



(*S*)-compound 6

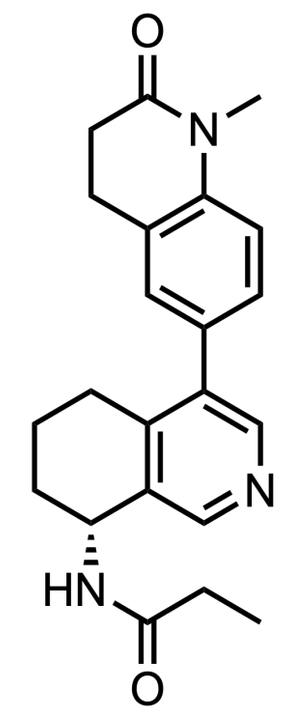
CYP11B2 IC_{50} (h)	6 nM	3 nM	40 nM	9 nM	942 nM
selectivity factor (h)	12	6	8	22	9

January 2023

baxdrostat

aldosterone synthase

oral aldosterone synthase (CYP11B2) inhibitor
Ph. I/II for HTN, CKD, primary aldosteronism
from in-house screen of aldosterone synthase inhibitors
Press release, January 9, 2023
ROCHE, BASEL, CH (ASTRAZENECA)
press release: https://www.astrazeneca.com/media-centre/press-releases/2023/astrazeneca-acquire-cincor-for-cardiorenal-asset.html



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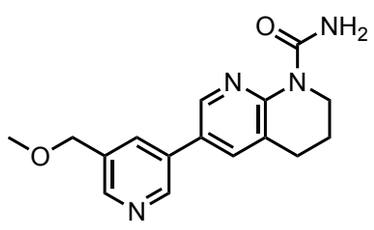
How was it discovered? Research into selective aldosterone synthase inhibitors was inspired by Novartis's primary aldosteronism clinical data on LCI699, a CYP11B2 inhibitor with 3.6-fold selectivity over CYP11B1. While CYP11B2 was safely and effectively inhibited in PA patients, reducing aldosterone levels, increasing potassium levels, and reducing blood pressure, cortisol inhibition still was observed. Roche's program started with a library of imidazole isosteres lacking substitution next to the basic nitrogen atom, resulting in potent but weakly selective pyridine **18**. Homology models using bovine CYP11A1 as a template (PDB: 3MZS) suggested selectivity could be achieved with substitution at the 4,5-positions of the pyridine. Indeed, 4,5-disubstituted tetrahydroisoquinoline compounds such as (*R*)-**6** possessed greater selectivity across CYP enzymes while maintaining other properties. Details of the progression from **6** to baxdrostat are limited, but >100x cellular selectivity of human CYP11B2 over human CYP11B1 was achieved with replacement of the nitrile with a lactam ring.

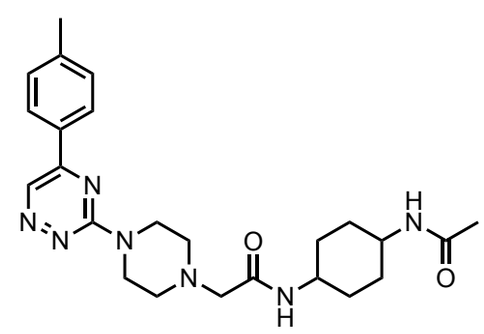
What else is out there? The efficacy and safety profile of baxdrostat, with a remarkably low dose at 2 mg QD, will make it a difficult-to-displace, best-in-class molecule should it continue to prove safe and efficacious. The molecule does not appear to impact cortisol levels or function, does not induce significant hyperkalemia (0.2% of measurements in 1.5% of subjects), and has a relatively flat PK profile with a low (25 ng/mL) trough concentration. Recently, Mineralys, a company opportunistically founded by VC firm Catalys Pacific in 2019, raised nearly $200M in an IPO to advance a competitive aldosterone synthase inhibitor, lorundrostat. Lorundrostat, licensed from Mitsubishi Tanabe, is structurally quite different. While lorundrostat also appears to demonstrate blood pressure reduction in a Ph. II study with no cortisol suppression (374:1 selectivity ratio for B2 vs. B1), the molecule is given at much higher 50-100 mg QD daily doses. Unless baxdrostat experiences major unexpected safety issues during development, which seems less likely given its remarkably low dose, it is not clear how lorundrostat will differentiate on its own. Other companies including Boehringer Ingelheim (BI689648) and PhaseBio (PB6440) appear to have selective aldosterone synthase inhibitors in or approaching development, but given PhaseBio's bankruptcy, baxdrostat seems to have a significant lead in the field. The potential first-in-class and best-in-class positioning of baxdrostat is a remarkable coup for the small company CinCor, given how many well-established drug discovery groups, including BI, Roche, Merck, and others, have been active in the space.

What's next? With positive results in rHTN reported from the BrigHTN trial (NCT04519658), which was stopped early for efficacy, CinCor/AstraZeneca next intended to demonstrate activity in the bigger uHTN population with the HALO trial. Surprisingly, the endpoints were missed in the HALO trial, which CinCor attributed primarily to non-adherence in the 53% of patients who were Hispanic/Latino. Indeed, surprisingly, of the 20/54 patients completing the 2 mg study that were non-adherent, 19/20 patients were in the Hispanic/Latino subgroup. Biologically, renin increases appeared to be lower in Hispanic adherent patients as well relative to non-Hispanic adherent patients, suggesting drivers of hypertension may be different in the Hispanic group as well. It is likely that with study modifications to address these issues, efficacy can be observed in Ph. III, especially since robust efficacy was observed in Ph. II in other subgroups including African-Americans. Given the heterogeneity of CYP enzymes within humans leading to human PK variability, it is not inconceivable that drug activity could be different within the patient subgroups. The surprising trial result is a nice example illustrating how important it is to factor human diversity into drug development.

Patents. "New phenyl-dihydropyridine derivatives as aldosterone synthase inhibitors", Roche, WO2015055604A1 (**2015**). "New bicyclic dihydroquinoline-2-one derivatives", Roche, WO2013041591A1 (**2013**). - covers baxdrostat. "New phenyl-tetrahydroisoquinoline derivatives", Roche, WO2013156423A1 (**2013**). "New dihydroquinoline-2-one derivatives as aldosterone synthase (cyp1 1 b2 or cyp1 1 b1) inhibitors", Roche, WO2014135561A1 (**2014**).



BI 689648



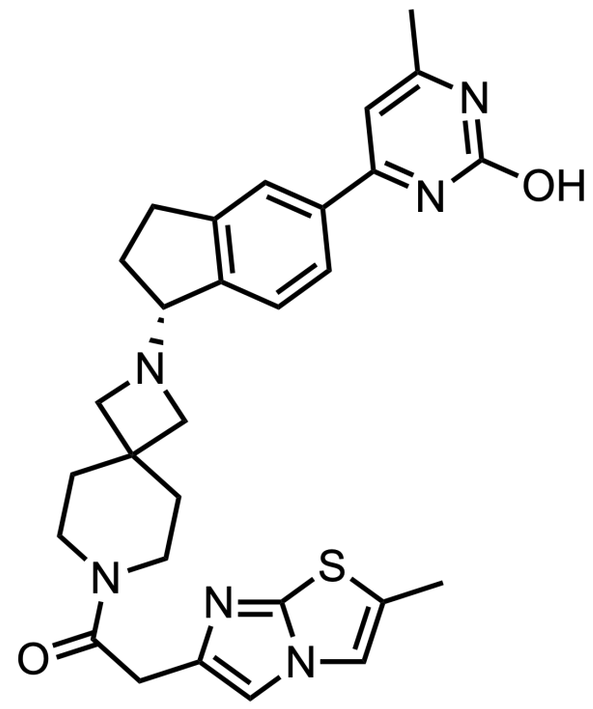
lorundrostat

January 2023

PF-6870961

GHSR1a

GHSR1a inverse agonist
(metabolite of oral molecule)
Ph. Ia for heavy alcohol drinking
major hydroxy metabolite of PF-5190457
JPET, January 11, 2023
NIDA/NIAAA, NIH (PFIZER)
paper DOI: https://doi.org/10.1124/jpet.122.001393



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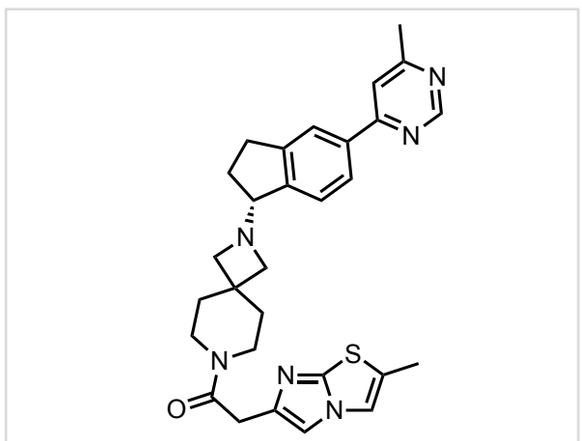
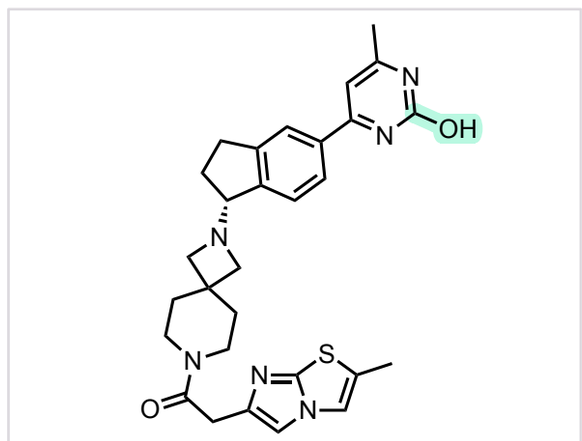
A metabolite and its parent: same target inhibition, different mechanisms. PF-6870961 is a growth hormone secretagogue receptor 1a (GHSR1a) inverse agonist biased for inhibition of ***β***-arrestin signaling, in contrast to its parent compound, PF-5190457, an unbiased GHSR1a inverse agonist. PF-6870961 was discovered as a major metabolite of PF-5190457 by Pfizer during the analysis of sera from patients in Ph. Ib for heavy alcohol drinking (NCT02039349). Then, in preclinical rodent models and initial human studies, the metabolite was found to have an effect on food intake and craving. This case study serves as an interesting example of a metabolite with comparable potency to its parent, but acts through a different mechanism of action of ***β***-arrestin-biased inverse agonism. Metabolite PF-6870961 more potently inhibits ***β***-arrestin signaling by the GPCR than its parent, while its the binding and inhibition of inositol phosphate accumulation is reduced as compared to parent, PF-5190457.



PF-5190457
parent
higher binding affinity + inhibition potency of GHSR1a-induced **inositol phosphate accumulation**



PF-6870961
hydroxy metabolite
higher inhibition potency of GHSR1a-induced **β-arrestin recruitment**

Why do we care? Alcohol use disorder (AUD) contributes to millions of deaths worldwide, but there are very few treatment options available for AUD. The pharmacological agents available do not reduce the urge to consume alcohol; rather they induce negative effects upon drinking (disulfiram, oral), remove the positive effects typically obtained from drinking (naltrexone, oral or depot injection), or reduce symptoms of withdrawal (e.g., acamprosate, various off-label drugs like baclofen). Preclinical studies have provided evidence for the role of ghrelin hormone in AUD, including alcohol seeking, craving, and reward. Ghrelin binds the growth hormone secretagog receptor (GHSR), rendering a GHSR inhibitor cas potentially viable treatment option for AUD.

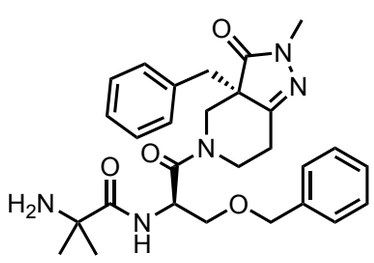
PF-5190457 was the first and, to our knowledge, only oral GHSR inverse agonist to reach the clinic. The identification of a biased inverse agonist metabolite, PF-6870961 is interesting as many GPCR modulators suffer from tachyphylaxis (reduced efficacy over time) due to internalization of the target GPCR receptor following beta-arrestin signaling. Since the food-related behavioral effects appear to be due to the downstream effects of ***β***-arrestin signaling, this could also suggest a path forward for a GHSR inverse agonist with more durable effects. The compound pair could be a useful tool for understanding the biology of GHSR modulation, as well as the pharmacology of biased inverse agonism in general.

What is the current therapeutic landscape for GHSR as a target? Currently, there are no commercially available treatments targeting GHSR for human use in any indication, though there is a GHSR agonist-based FDA-approved Adult Growth Hormone Deficiency oral diagnostic test, that uses macimorelin from Aeterna Zentaris. In the veterinary space, Pfizer's GHSR agonist capromorelin is used for weight loss in dogs and cats.

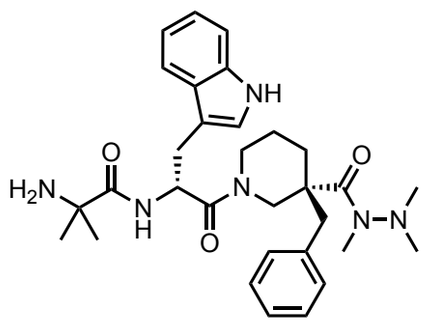
Other GHSR ligands that have progressed to the clinic largely include pseudopeptides and peptidomimetics are primarily agonists derived from enkephalin analogs, substance P, or ghrelin. Novo Nordisk/Helsinn Healthcare's anamorelin, an oral GHSR agonist, exhibits anabolic effects and is currently in Ph. III clinical for cancer cachexia and anorexia (NCT04844970). Ibutamoren (MK-0677), an oral GHSR agonist from Merck, was in development for fibromyalgia, but was discontinued after Ph. II (NCT00116129) and appears to have been repositioned recently for NAFLD and NASH (NCT05364684).

Other reported preclinical GHSR inverse agonists with different base scaffolds include "ligand 29", featuring a 1,2,4-triazole scaffold, from IBMM; "compound 20", featuring a 2-aminoalkyl nicotinamide scaffold, from Asubio Pharma; and "compound 47", which was formed through chimeric design, from Helsinn Healthcare.

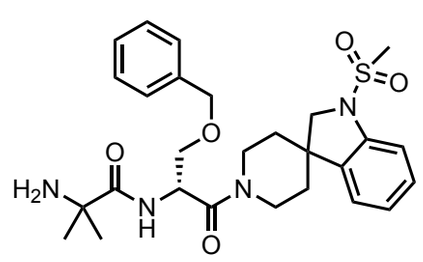
How was the parent compound discovered? Pfizer scientists identified a series of GHSR inverse agonists featuring a spirocyclic piperidine-azetidine scaffold starting from hit "compound 10." The hit was identified using a calcium mobilization FLIPR assay (initial screen) and a GTP-γ-S secondary assay (to identify inverse agonists). Optimization led to the centrally active "compound 11," which demonstrated GHSR inverse agonism. A benefit of the series was that it consistently showed inverse agonism of the ghrelin receptor, rather than switching between antagonism and partial agonism as has been observed in other series. Optimization from "compound 11" proceeded with the goals of lowering logD, clogP, reducing off target binding, and increasing TPSA and LipE, to reduce the risk of adverse events and toxicity, and decreasing CNS penetration, to reduce CNS-based side effects. The resulting candidate, 16h (PF-5190457) had high lipophilic efficiency ($LipE_{elogD}$ = 6.9) with moderate human liver microsomes clearance and lower off-target activity, measured against a CEREP panel. It was later found that PF-5190457 exhibits a distinct binding mode to the ghrelin receptor compared to agonists and neutral antagonists (PDB: 7F83), which may explain the functional consistency.



capromorelin
Pfizer
GHSR agonist



anamorelin
Novo Nordisk/Helsinn Healthcare
oral GHSR agonist



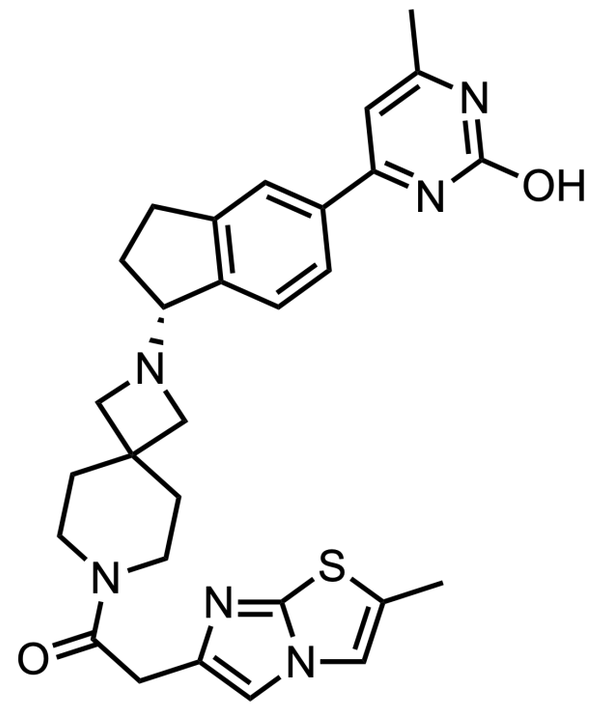
ibutamoren (MK-0677)
Merck
oral GHSR agonist

January 2023

PF-6870961

GHSR1a

GHSR1a inverse agonist
(metabolite of oral molecule)
Ph. Ia for heavy alcohol drinking
major hydroxy metabolite of PF-5190457
JPET, January 11, 2023
NIDA/NIAAA, NIH (PFIZER)
paper DOI: https://doi.org/10.1124/jpet.122.001393



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How was metabolite PF-6870961 identified? After its discovery, Pfizer evaluated PF-5190457 for diabetes, reaching Ph. I (NCT01372163), but eventually discontinued development for "strategic reasons" in 2012. Due to the demonstrated role of ghrelin in preclinical models of AUD, PF-5190457 was investigated by researchers at NIDA/NIAAA for its ability to modulate alcohol taking behaviors. After promising rodent data the drug was taken into a Ph. Ib clinical trial (NCT02039349, n=12) to determine maximum dose and tolerability when combined with heavy alcohol drinking.

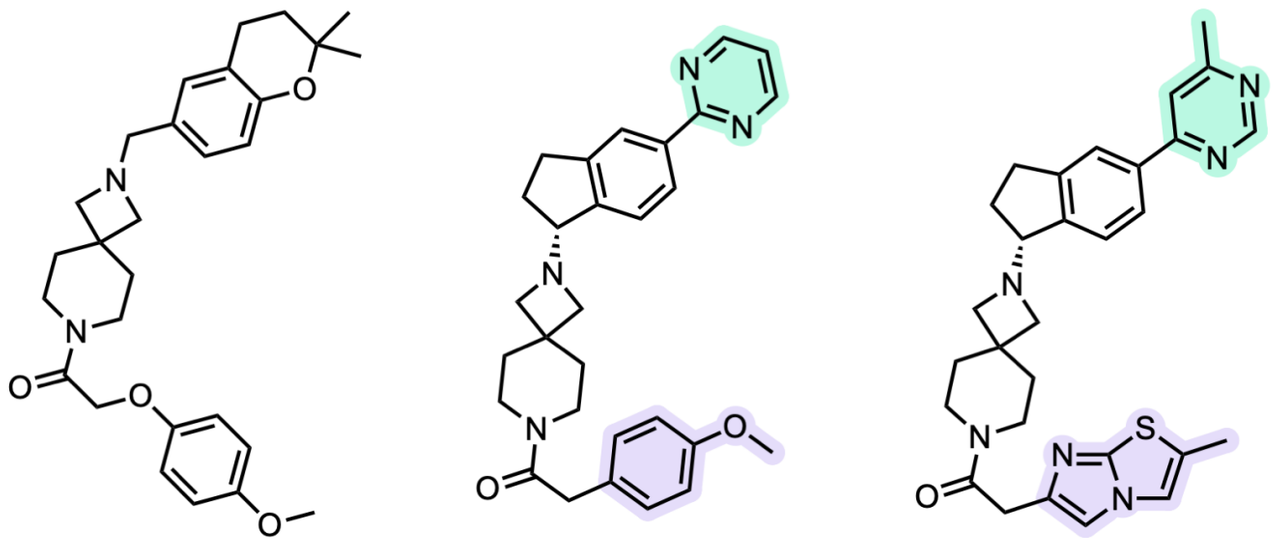
During the PK analysis (healthy controls, NCT01247896), participant sera was pooled and analyzed revealing a hydroxy metabolite, which could be reproduced by incubating PF-5190457 in liver cytosol. The aldehyde oxidase inhibitor raloxifene prevented the formation of PF-6870961 in human liver cytosol, suggesting a central role for this enzyme in the bioformation of PF-6870961. The xanthine oxidase inhibitor febuxostat also affected PF-6870961 formation, although to a lesser extent, suggesting that aldehyde oxidase is primarily responsible for the formation of PF-6870961. PF-6870961 was not identified in any prior screening studies of PF-5190457, thus its discovery in the clinic was unforeseen.

Hydroxy metabolite surprisingly confers bias toward β-arrestin recruitment. PF-6870961 has a lower affinity for GHSR1a relative to its parent compound (PF-5190457 h K_i = 3.0 nM, PF-6870961 h K_i = 74 nM) and subsequent weaker inverse agonism, specifically of inositol 3,4,5 triphosphate (IP) production stimulated by $G\alpha_q$ induction (PF-5190457 IC_{50} = 6.8 nM, PF-6870961 IC_{50} = 300 nM).

However, comparing β-arrestin recruitment, the two compounds had similar potency (PF-5190457 IC_{50} = 3.4 nM, PF-6870961 IC_{50} = 1.1 nM), both of which was stronger than that of the endogenous GHSR1a antagonist LEAP-2 (IC_{50} = 20 nM). This biased inverse agonism of β-arrestin recruitment may be attributed to hydrogen bonding with the metabolite's hydroxyl group forming with residues within the extracellular loop 2. Mutations in charged residues of this region have been shown to have a strong effect on C-terminal flexibility and β-arrestin signaling.

Data from rodent food self-administration experiments suggest that the food related behavioral effects observed from PF-5190457 and PF-6870961 are mediated by the downstream effects of β-arrestin signaling. A short (five-step) synthetic route for the metabolite has since been developed, based on a modified version of the synthesis for the parent compound, PF-5190457, that will allow for further investigation.

Patents. None for PF-6870961. Pfizer has several patents for PF-5190457 (WO-2011114271-A1), including for the treatment of sleep disorders (US-2015119381-A1) and neurodegenerative disorders (US20170121385A1).



compound 10 **compound 11** **PF-5190457**

GHSR pK_i (nM)	6.7	8.2	8.4
logD	3	2.3	1.5
TPSA	51	59	95
HLM $CL_{int,app}$ (mL/min/kg)	15	37	21
P_{app}	-	13	5
hMDR ba/ab ratio	2	2.4	7.2

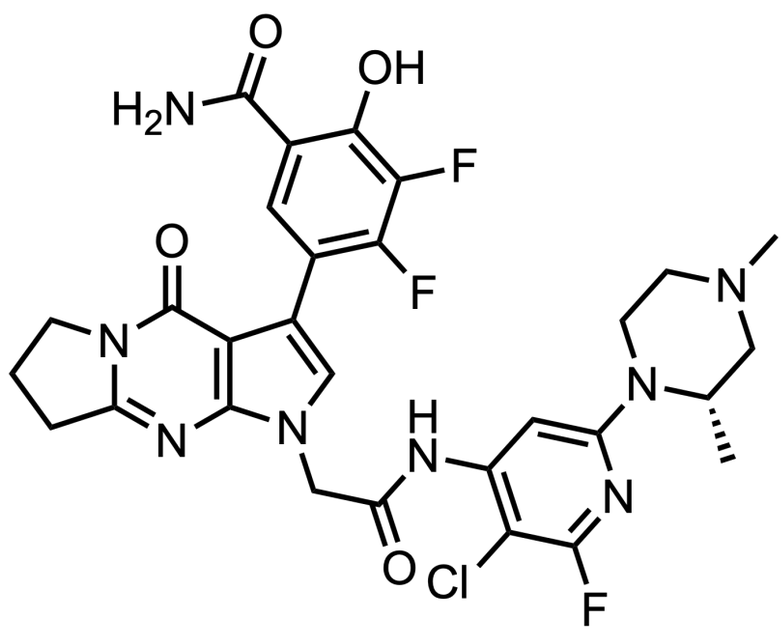
	IC50 (nM)		Ki (nM)
	IP	β-arrestin	hGHSR
LEAP-2 (endogenous antagonist)	4.77	20.5	-
PF-5190457	6.76	3.39	2.96
PF-6870961	301	1.10	73.6

January 2023

JNJ-65234637

BCL6

oral BCL6 BTB inhibitor
promising in vitro safety + in vivo oral PK data
from virtual screen + SBDD
ACS Med. Chem. Lett., January 12, 2023
OICR, TORONTO, CA (JANSSEN)
paper DOI:
https://doi.org/10.1021/acsmedchemlett.2c00502



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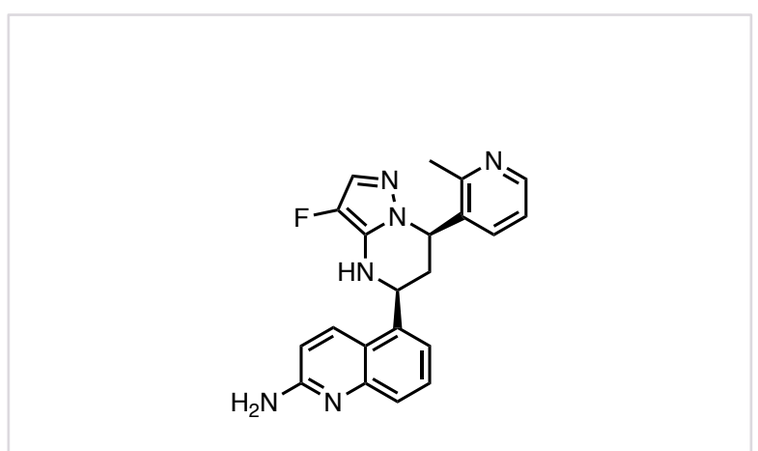
A protein-protein interaction inhibitor for a challenging-to-drug master transcription factor. OICR12694 is an oral protein-protein interaction inhibitor targeting the challenging-to-drug oncogenic transcription factor, B cell lymphoma 6 (BCL-6). As a master regulator of B cell differentiation in germinal centers and hence a common driver of B-cell malignancies like diffuse large B-cell lymphoma (DLBCL), BCL-6 inhibition could be beneficial for treatment of B-cell cancers and B-cell-mediated diseases. In 2015, JNJ signed a deal with Novera and the Ontario Institute for Cancer Research (OICR) to an exclusive worldwide license option for small molecules to treat blood cancers for up to $348M ($450M Canadian), in which the OICR will identify novel candidates from its drug discovery program and JNJ will be responsible for further commercial development.

Why do we care? The current treatment for DLBCL is a chemotherapy regimen that incorporates rituximab (Rituxan), cyclophosphamide, doxorubicin (Hydroxydaunomycin), vincristine (Oncovin), and prednisone (R-CHOP), which is curative in 65% of patients. However, prognosis is poor for patients receiving 2nd and 3rd line treatment. Various BCL-6 inhibitors (GSK137, BI-3802, CCT369260) have recently been identified with different MoAs, including a glue degrader mechanism. Although BI-3802 induces BCL-6 polymerization leading to proteasomal degradation via S1AH1, it is unlikely to have sufficient PK for oral dosing (~43 nM cell IC_{50} vs. 100 mg/kg, C_{max} = 599 nM, AUD = 4650 nM x h) in mice. Currently, BCL-6 inhibitors are still only preclinical, but progress could be beneficial for DLBCL, an aggressive and prevalent disease with limited options after R-CHOP. The medicinal chemistry campaign is also an interesting case study for improving potency and properties against a large, shallow interface using many incremental changes.

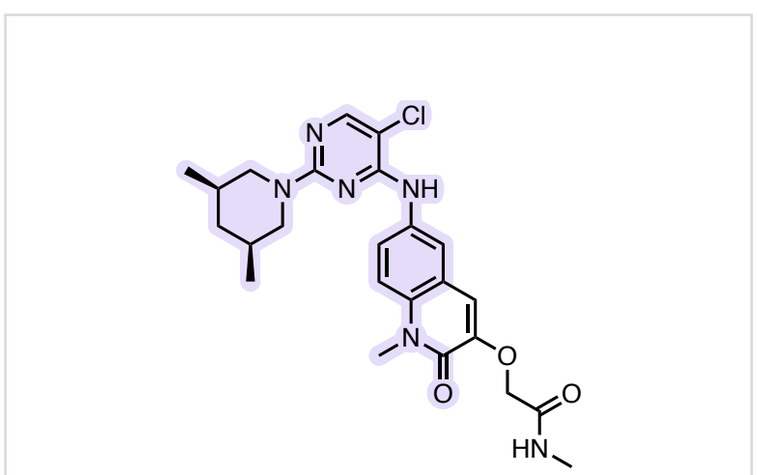
Starting chemical matter for a shallow binding pocket from a virtual screen. BCL-6 inhibitors interrupt protein-protein interactions between the N-terminal (Broad-complex, Tramtrack, and Bric-à-brac) lateral groove of the BTB domain of BCL-6 and co-repressor (NCoR, SMRT, BCOR) recruitment. This lateral groove is composed of a 17-amino acid sequence, which is not conserved among other transcription factors in the BTB family, which may aid in selectivity. A virtual screen using the X-ray crystal structure of the lateral groove of BCL-6 (PDB: 3LBZ) was employed to find suitable starting points with the bioavailability necessary for chronic dosing in this indication. The top scoring compounds were initially filtered via a competitive fluorescence polarization (FP) assay using a fluorescently tagged SMRT-based peptide, followed by further validation using the more sensitive surface plasmon resonance (SPR) technique to measure direct binding affinity to purified BCL-6 BTB dimer. This virtual screen and validation cascade led to confirmation of a weak hit with pyrrolopyridone , "compound 6" (K_D = 282 µM).

>55,000x potency improvement through iterative structure-based design. The key steps in potency optimization are shown in the corresponding figure. Initial SAR studies explored the substitutions in the southern portion of "compound 6," resulting in incremental in potency. Introduction of a nitrogen to the core led to a modest increase in potency ("compound 17"). Addition of *p- or m*-substituted phenyl groups in compounds 22 and 23 didn't exhibit significant potency improvements but, remarkably, a ~400x potency improvement was observed from "compound 17" (K_D = 21 µM) to "compound 24" (K_D = 0.054 µM) with a combined cyano-phenol group. This is a surprising example of how two small groups can impact lead optimization together, even if not independently (i.e., compound 22 and compound 23).

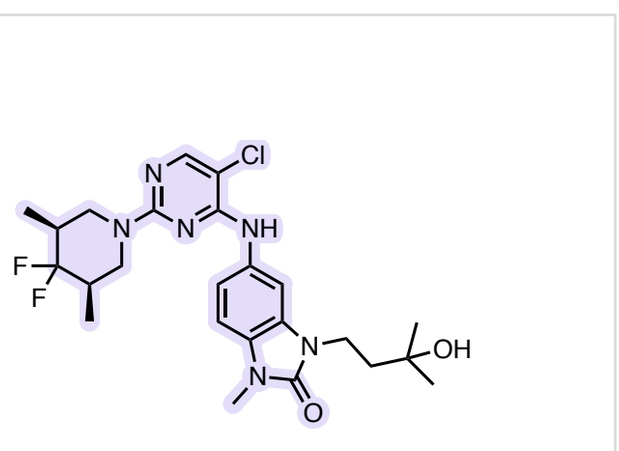
Despite the promising potency of "compound 24," it had poor metabolic stability (MLM = 6% remaining after 1 h, HLM = 39%). Exchange of the nitrile for an amide to enable intramolecular hydrogen bonding in "compound 30" was performed to reduce glucuronidation of the phenolic hydroxyl group by UDP-glucuronosyl transferase. The addition of fluorines for metabolic stability and incorporation of a pentane ring for added potency yielded JNJ-65234637 (compound 58, PDB: 7LWG) with single digit nanomolar potency and promising antiproliferative activity (IC_{50} = 92 nM) in a Karpas-422 cellular assay (BCL-6 dependent cell line).



GSK-137



BI-3802



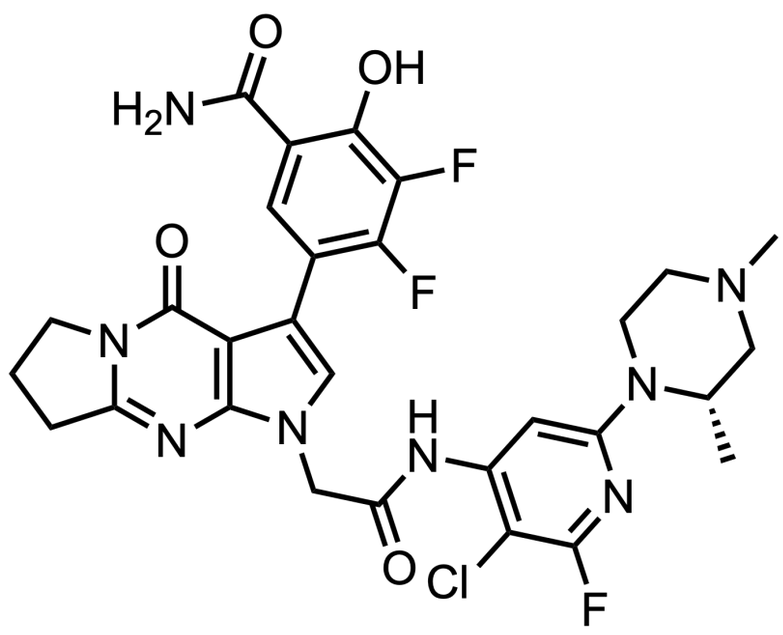
CCT369260

January 2023

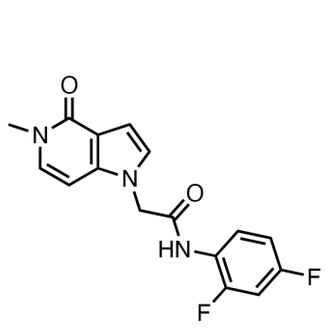
JNJ-65234637

BCL6

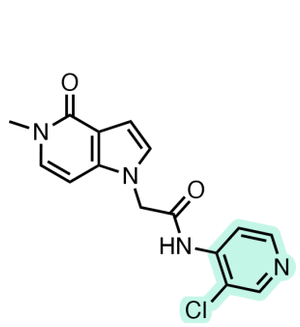
oral BCL6 BTB inhibitor
promising in vitro safety + in vivo oral PK data
from virtual screen + SBDD
ACS Med. Chem. Lett., January 12, 2023
OICR, TORONTO, CA (JANSSEN)
paper DOI:
https://doi.org/10.1021/acsmedchemlett.2c00502



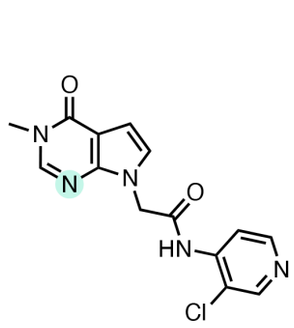
View Online



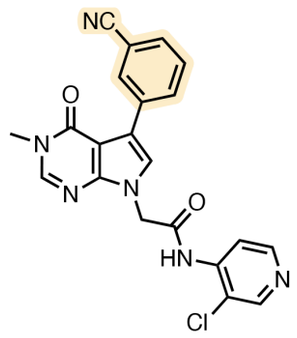
compound 6
K_D (SPR) = 282 μM



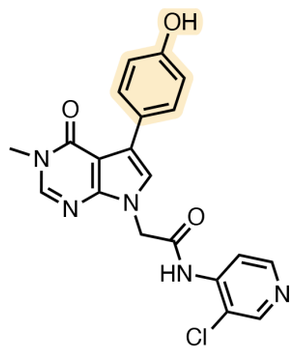
compound 11
K_D (SPR) = 119 μM



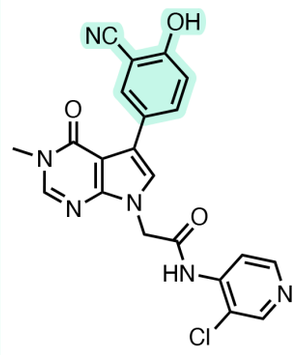
compound 17
K_D (SPR) = 21 μM



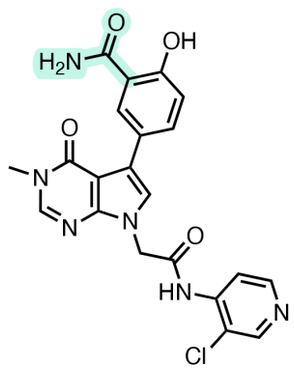
compound 22
K_D (SPR) = 11 μM



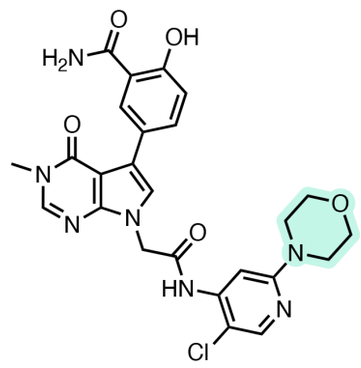
compound 23
K_D (SPR) = 14 μM



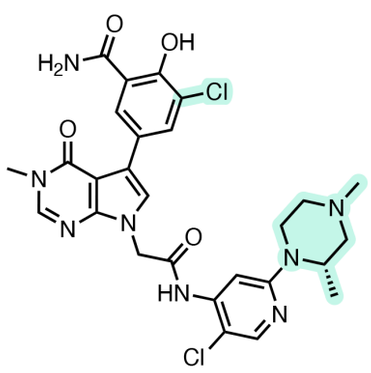
compound 24
K_D (SPR) = 0.054 μM



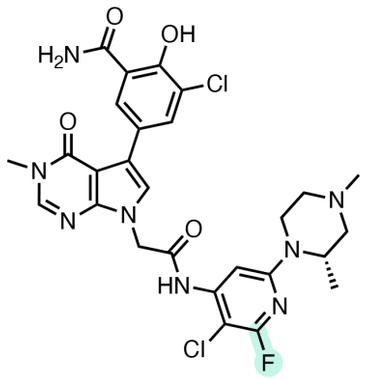
compound 30
K_D (SPR) = 0.120 μM



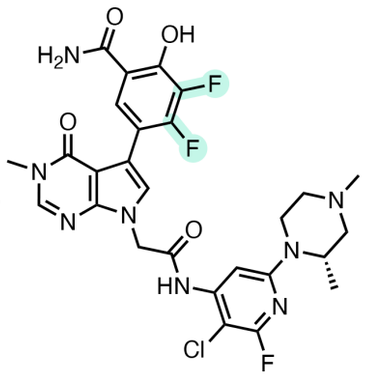
compound 31
K_D (SPR) = 0.038 μM



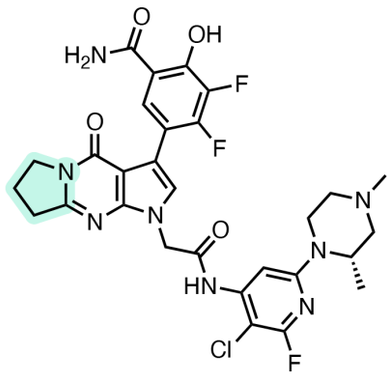
compound 35
K_D (SPR) = 0.011 μM



compound 44
K_D (SPR) = 0.009 μM



compound 45
K_D (SPR) = 0.011 μM



compound 58
K_D (SPR) = 0.005 μM

Pharmacology summary and development status of OICR12694 (JNJ-65234637). JNJ-65234637 exhibited a promising PK profile in dogs after a 5 mg/kg dose with good oral bioavailability (F% = 47) and half-life ($t_{1/2}$ = 6.1 h), coupled with low clearance (CL = 9.1 mL/min/kg). In addition, the molecule had >100-fold binding selectivity against other BTB proteins (i.e., BAZF, MIZ1, PLZF, FAZF, Kaiso, LRF). No CYP inhibition was observed for CYP 1A2, 2C8, 2C9, 2C19, 2D6, and 3A4 at concentrations up to 10 μM. Kinase profiling using the
DiscoverX Kinome scan revealed minimal inhibition of kinases at 1 μM, however a Eurofins safety panel of other potential off-target interactions did show >50% inhibition for 3 of the 53 targets, with BZD (52%), MT1 (66%), and 5-HT3 (86%) at 10 μM. Despite promising PK and in vitro results, JNJ-65234637 is not currently in clinical development. While there is likely room for further improvement in the overall binding efficiency and properties of the final compound, the molecule represents an important proof-of-concept on a difficult target, and will likely be utilized as a tool compound for further study of BCL-6-driven cancers and B-cell-mediated diseases.

Patents. Ontario Institute for Cancer Research holds the patents related to this molecule including, "Tricyclic inhibitors of the BCL6 BTB domain protein-protein interaction and uses thereof" (US11242351B2, 2018) and "Inhibitors of the bcl6 btb domain protein-protein interaction and uses thereof" (WO2019119145A1, 2019).

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07 July	08 August	09 September	10 October	11 November	12 December

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07 July	08 August	09 September	10 October	11 November	12 December

2020

	02 February	03 March	04 April	05 May	06 June
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