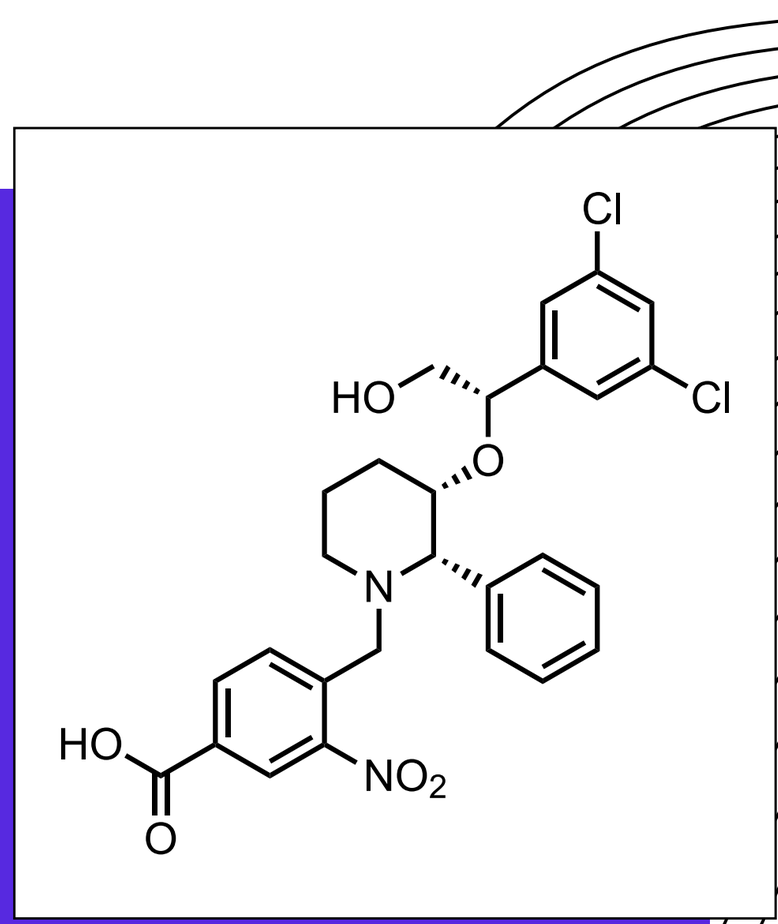
Small Molecules of the Month

July 2022

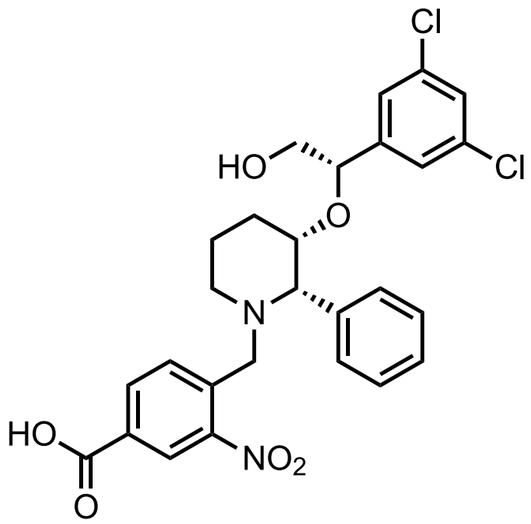


drug hunter

01	Compound 33	PKG1α	Merck
02	RP-6306	PKMYT1	Repare Therapeutics
03	BLU-945	$EGFR^{T790M/C797S}$	Blueprint Medicines
04	SAGE-718	NMDAR	Sage Therapeutics
05	BI-4142	HER2 exon 20 insertion	Boehringer Ingelheim
06	CHF-6366	MABA	Chiesi Farmaceutici
07	Compound 3f	FXIa	Janssen
08	MRTX0902	$SOS1:KRAS^{G12C}$ PPI	Mirati Therapeutics
09	Compound 14	NNMT	Shionogi
10	CC-99677	MK2	Bristol Myers Squibb

Compound 33

PKG1α



intravenous PKG1α activator

satisfactory in vivo rat PK profile

from 2.9 M compounds HTS, SBDD and opt

J. Med. Chem.

Merck & Co., Inc., South San Francisco, CA

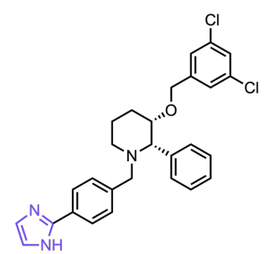
featured article: doi.org/10.1021/acs.jmedchem.1c02109

Context. "Compound 33" (Merck) is a cGMP-dependent protein kinase G1α (PKG1α) activator cGMP modulation is considered one of the most promising approaches for cardiovascular drug discovery with >30 agents either approved for use or in various stages of clinical development. Although PKGs are the main effector of cGMP, cardiovascular drug discovery efforts focusing on this target have not yielded similar success as those observed for guanylate cyclases and phosphodiesterases (PDEs). This is likely attributable to the complexity of the PKG architecture and an incomplete understanding of the mechanisms underlying PKG activation. Current preclinical agents are either cGMP analogs or synthetic peptides. The tool "compound 33" was developed based on the hypothesis that allosteric activation of PKG by a small molecule non-cGMP analog may reduce the off-target effects associated with broader cGMP modulation, such as cross-activation of other cyclic nucleotide-binding proteins and modulation of PDE activity. The compound presents a proof-of-concept for allosteric activation of PKG in a manner consistent with cGMP-induced activation.

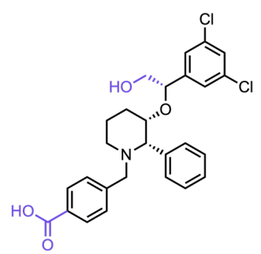
Target. PKG, the primary cGMP effector, is the key enzyme that mediates vasodilatation in various vessel types. cGMP-induced activation of the kinase results in induction of smooth muscle relaxation in blood vessels, lowering of pulmonary pressure, prevention of platelet aggregation, and overall protection against cardiac stress. The PKG1 family is primarily expressed on smooth muscles and comprises the PKG1α and PKG1β isoforms. Although PKG1α has high sequence homology with the 1β isoform, it is more sensitive to cGMP levels and is expressed at greater levels in the heart and lungs. PKG1 activators are typically cGMP analogs, although synthetic peptides have also been described. Past approaches include activation of guanylate cyclases (generating more cGMP) or inhibition of phosphodiesterases (preventing the degradation of cGMP).

Mechanism of Action. PKG is natively activated by cGMP, and direct activation by small molecule therapeutics has been mainly through cyclic nucleotide-binding domain (CNBD) binding by cGMP mimetics. The small molecule PKG1α activator "compound 33" activates a PKG1 substrate, vasodilator-stimulated phosphoprotein (VASP), which is implicated in several downstream effects the cGMP-PKG pathway regulates. The compound also exhibited antiproliferative effects in human pulmonary arterial smooth muscle cells. Structural and biochemical studies revealed that the molecule binds to an allosteric pocket in the kinase and exhibited a mechanism of action consistent with cGMP-induced activation. Merck scientists hypothesize that such an allosteric binding mode may reduce the potential for off-target effects expected of a compound targeting cGMP signaling.

Hit-Finding Strategy. Biochemical potency was evaluated using a pair of complementary activation assays: one in the absence of cGMP (basal activation) and the other with EC_{20} cGMP (partial activation). A prior high-throughput screen had identified "compound 1", as well as the potency-enhanced "compound 2". Biophysical studies of these compounds indicated they bind to an allosteric site yet still have a similar effect as endogenous cGMP on the kinetic binding parameters of PG1α.



compound 1
PKG1α basal EC_{50} = >200 μM
PKG1α partial EC_{50} = 47 μM



compound 2
PKG1α basal EC_{50} = 77 μM
PKG1α partial EC_{50} = 11 μM

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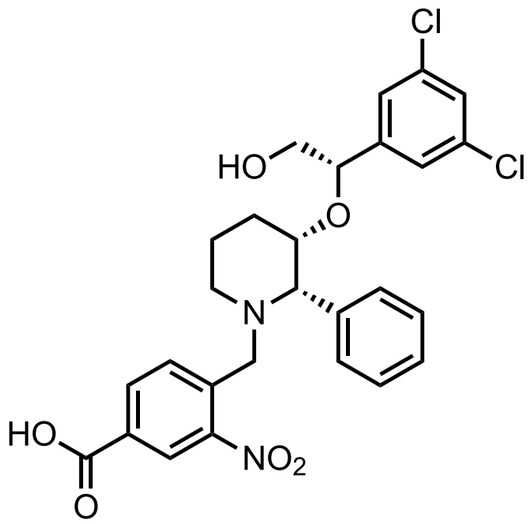
Compound 33

PKG1α



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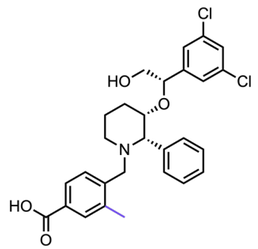
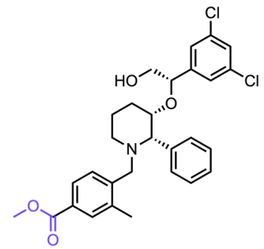
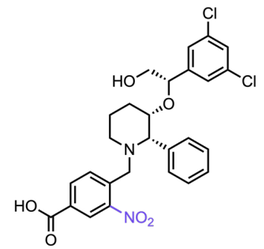
Lead Optimization. Virtual docking of "compound 2" into a known structure of the PKG1α regulatory domain revealed that the potency-boosting alcohol in "compound 2" made no significant interactions in the docking model, although Merck scientists hypothesized that it could hydrogen bond with nearby residues. Initial attempts at optimization focused on the role of this alcohol, with concurrent studies finding that addition of a methyl group at the *ortho position of the N*-benzyl moiety enhanced potency ("compound 3"). However, steric limitations within the binding pocket precluded any further manipulation. The docking model also indicated that the 2-phenyl and dichlorophenyl moieties occupied hydrophobic pockets, but again manipulation of these groups did not lead to substantial improvements in potency. Finally, optimization of the *N*-benzyl moiety led to significant potency improvements. While the methyl ester in "compound 30" maintained sub-micromolar potency in the partial state, all activity in the basal state was lost, indicating the requirement of a free carboxylic acid for basal state activity. Modifications to the benzene ring itself showed relatively tolerant SAR and led to several potent analogs, including "compound 33".



compound 1
PKG1α basal EC_{50} = 95 μM
PKG1α partial EC_{50} = 1 μM



compound 2
PKG1α basal EC_{50} = >200 μM
PKG1α partial EC_{50} = 0.9 μM



compound 2
PKG1α basal EC_{50} = 13 μM
PKG1α partial EC_{50} = 0.52 μM

Binding mode. X-ray co-crystal structure of "compound 33" complexed with PKG1α regulatory domain (**PDB:7SSB**) shows the occupation of a region called "nest", which is allosteric to the cyclic nucleotide-binding domains. The piperidine core is found in the chair conformation, directing the 3,5-dichlorophenyl and phenyl moieties in a hydrophobic pocket, highlighting the (2S, 3S) stereochemistry role in the binding mode. Hydrophobic van der Waals interactions between the 3,5-dichlorophenyl moiety and Phe321/Phe222 residues were observed. Another relevant feature is the alcohol substituent acts as a hydrogen bond acceptor to Gln296, explaining the increase in potency observed with the introduction of this group in the piperidine scaffold.

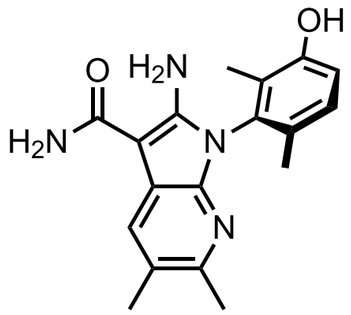
Preclinical Pharmacology. "Compound 33" induced phosphorylation of VASP (EC_{50} = 13.4 μM) and exhibited desirable selectivity for the PKG1α isoform (EC_{50} = 0.52 μM) over PKG1β (EC_{50} = 6.7 μM). The compound also generally exhibited a favorable PK profile in rats, with clearance of 8.1 mL/min/kg, V_d = 0.45 L/kg, and a mean residual time of 0.9 h.

Clinical Development. Preclinical compound

Patent. No publicly available records as of 20 August 2022.

RP-6306

PKMYT1



oral PKMYT1 inhibitor

three Ph. I studies currently ongoing

initial screen of 560 known kinase inhibitors

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Repare Therapeutics, Inc., Ville St- Laurent, CA

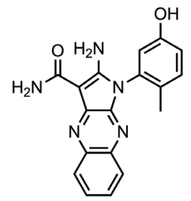
featured article: doi.org/10.1021/acs.jmedchem.2c00552

Context. RP-6306 is an oral protein kinase, membrane-associated tyrosine/threonine 1 (PKMYT1) Inhibitor being developed for various solid tumors. PKMYT1, together with WEE1, make up the WEE family of serine/threonine kinases, which have been implicated in the progression of several cancers, especially solid tumors. At least 4 WEE1 inhibitors have entered clinical development; notable among them is Zentalis' ZN-c3, which we previously featured, and AstraZeneca's adavosertib (AZD1775; formerly MK1775), which is the leading candidate and has been evaluated in >60 trials. AstraZeneca recently pulled the plug on adavosertib, likely due to bone marrow and gut-related toxicities, raising questions on whether the other WEE1 inhibitors may meet a similar fate. The potential first-in-class agent RP-6306 was found in preclinical studies to be highly selective for PKMYT1 vs WEE1, which could potentially insulate the compound against the toxic liabilities observed with adavosertib. How well this theory holds up may be known in the not-too-distant future as RP-6306 is currently in clinical early clinical development.

Target. PKMYT1 is a member of the WEE family of serine/threonine kinases which act to regulate the cell cycle. PKMYT1 mediates the inhibitory phosphorylation of Thr14 and Tyr15 of cell cycle-associated protein CDK1, precluding the latter's ability to trigger mitosis. Poor PKMYT1 activity in genetically vulnerable tumors, such as those with CCNE1 amplification, was associated with loss of major checkpoint regulation in tumor cells resulting in CDK1 hyperactivity, uncontrolled mitosis, and heavy DNA damage, ultimately causing cell death. Unsurprisingly, PKMYT1 has been implicated in an increasing number of cancers, and its overexpression often correlates with poor prognosis and disease progression.

Mechanism of Action. Tumors with CCNE1 amplification are genetically vulnerable and depend on PKMYT1 for checkpoint regulation. It is no surprise then, that CCNE1 amplification had been found to be synthetic lethal with the inhibition of PKMYT1. Inhibition of PKMYT1 activity results in mitotic catastrophe, signified by override of the G2/M cell cycle checkpoint, premature entry into mitosis, and cell death. RP-6306 binds to the PKMYT1 kinase domain and makes interactions in the binding pocket that dictate the compound's potency and observed selectivity against the highly homologous WEE family member WEE1.

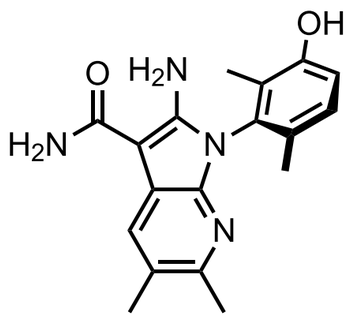
Hit-Finding Strategy. A collection of 560 known kinase inhibitors were initially screened using a fluorescence-polarization assay in which competitive displacement of a commercially available probe was measured. "Compound 1" was identified as an early hit due to its >50-fold selectivity over the homologous WEE1 kinase; it was previously reported to be a non-specific ephrin inhibitor.



compound 1
PKMYT1 cell assay
IC_{50} = >3 µM

RP-6306

PKMYT1



oral PKMYT1 inhibitor

three Ph. I studies currently ongoing

initial screen of 560 known kinase inhibitors

J. Med. Chem.

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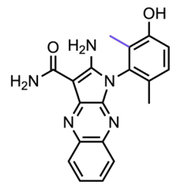
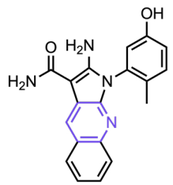
featured article: doi.org/10.1021/acs.jmedchem.2c00552

drug hunter

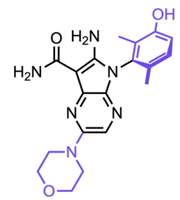
Lead Optimization. SAR was initially built around the phenol in "compound 1", with the best activity coming from the addition of a second methyl group in the 6-position ("compound 6"), suggesting that a large dihedral angle with the tricyclic ring system was important. Further studies indicated that removing a nitrogen on the carboxamide side of the tricyclic scaffold had a favorable impact ("compound 25"). Although these compounds were highly potent, their ADME/PK properties were less than ideal. However, removing the fused aryl ring from the tricyclic core generally led to compounds with more desirable profiles. An impact on permeability was noticed when polar groups were introduced, and a single atropisomer was used, e.g., "compound 51"; however, the best overall balance was found with the bismethyl derivative, RP-6306.



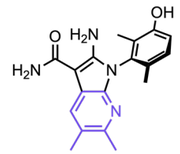
compound 6
PKMYT1 cell assay
IC_{50} = 0.13 µM



compound 25
PKMYT1 cell assay
IC_{50} = 0.36 µM



compound 51
PKMYT1 cell assay
IC_{50} = 0.025 µM



RP-6306
PKMYT1 cell assay
IC_{50} = 0.014 µM

Binding mode. The co-crystal structure of RP-6306 bound to the kinase domain of PKMYT1 (**PDB:8D6E**) shows water-mediated hydrogen bonds between the backbone carbonyl of Gly191 and the amine of the carboxamide, as well as the Lys139 side chain and pyridine nitrogen. Furthermore, a hydrogen bond between the primary amine and the hydroxyl group of the gatekeeper residue, Thr187, is evident. This is likely responsible for the selectivity seen over the highly homologous WEE1, which has an Asn376 at this position, the only difference in its active site.

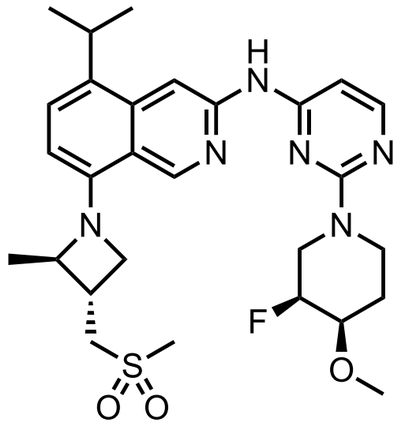
Preclinical Pharmacology. The compound showed strong potency against PKMYT1 (IC_{50} = 0.002 µM) and was found to be selective when screened against a panel of 274 kinases (20x-4150x), including WEE1 (2050x). In vivo efficacy and safety experiments were done in a *CCNE1*-amplified ovarian xenograft mice, with oral dosing in chow at 3, 10, and 60 mg/kg. Statistically significant, and dose-dependent reduction in tumor growth was observed at all doses (P<0.0001). In vivo PK/PD studies demonstrated a strong correlation between efficacy of RP-6306 and its free plasma levels, suggesting that sustained PKMYT1 inhibition is required for efficacy.

Clinical Development. Repare Therapeutics is recruiting volunteers with solid tumors to participate in one of three RP-6306 Ph. I studies currently ongoing. In the MYTHIC study (NCT04855656) the safety and tolerability of RP-6306 alone and in combination with RP-3500 will be assessed, as well as the maximum tolerated dose and preliminary antitumor activity. In the MINOTAUR study (NCT05147350), the safety, tolerability, maximum tolerated dose, recommended Ph. II dose, and preferred schedule of RP-6306 with FOLFIRI will be studied. Another trial (NCT05147272) will study RP-6306 in combination with gemcitabine in patients with eligible advanced solid tumors. There is not much information regarding the trial dose regimen, however, in the EU Clinical Trials Register, RP6306 is listed to be used at 2.5 and 20 mg oral doses in the MYTHIC study.

Patent. The patent WO2021195782A1 filled by Repare Therapeutics methods utilizing inhibitors of PKMYT1 to the treatment of a disease or conditions as cancer, which depend on the activity of enzyme. Pharmaceutical compositions, and methods of preparing compounds and of their use are disclosed in a separated patent WO2021195781A1.

BLU-945

EGFR$^{T790M/C797S}$



oral EGFR mutant inhibitor

Ph. I/II candidate oncology

>25k compound library screening and opt

J. Med. Chem.

Blueprint Medicines, Cambridge, MA

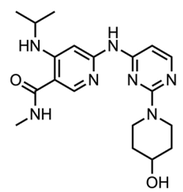
featured article: doi.org/10.1021/acs.jmedchem.2c00704

Context. BLU-945 (Blueprint Medicines) is an oral EGFR mutant inhibitor being developed for treatment-resistant non-small-cell lung cancer (NSCLC). Although three generations of EGFR TKIs are available for *EGFR*-mutant NSCLC, drug resistance and associated disease relapse are still highly prevalent. The widely used third-generation agent osimertinib demonstrates activity against the highly prevalent T790M resistance mutation and the exon 19 deletion (ex19del) and L858R sensitizing mutations. However, it's inactive against the C797S acquired resistance mutation. Consequently, no approved therapies exist for patients with disease progression following treatment with third-generation agents. Another key challenge in EGFR TKI development is selectivity against wildtype (WT) EGFR, with current agents being associated with dermatological and other toxicities due to their concomitant WT-targeting properties. BLU-945, which we recently featured, addresses both of these unmet needs in the EGFR TKI space. The potential best-in-class reversible inhibitor showed strong activity for EGFR+/T790M and EGFR+/T790M/C797S resistant NSCLC mutants while sparing WT EGFR. These are the most common acquired mutations, with 60% of patients developing a resistance-conferring mutation during the course of treatment. The compound was studied in an intracranial implantation model, suggesting brain penetration, and was found to have a favorable in vivo safety profile. It is currently being evaluated in a Ph. I/II study.

Target. As an extensively validated cancer target, especially for NSCLC, *EGFR* mutations that mediate resistance to currently approved 3 generations of TKIs are well characterized. Common resistance mutations include the ex19del and the L858R mutation, both activating mutations; acquired resistance to first- and second-generation TKIs are typically through the T790M mutation (up to 70% of cases), while resistance to the third-generation agent osimertinib is mainly through the C797S mutation. BLU-945 was developed based on the rationale that WT-sparing TKIs that selectively target EGFR+/T790M/C797S–mutant tumors may be associated with a better safety profile vs currently approved TKIs.

Mechanism of Action. Currently approved EGFR TKIs are ATP-competitive inhibitors and either bind reversibly (first-generation agents) or irreversibly (second and third-generation agents) to the kinase. BLU-945 is a reversible inhibitor with >1000x selectively against WT EGFR, which may reduce typical toxicities such as rashes and gastroenterological side effects associated with EGFR TKIs.

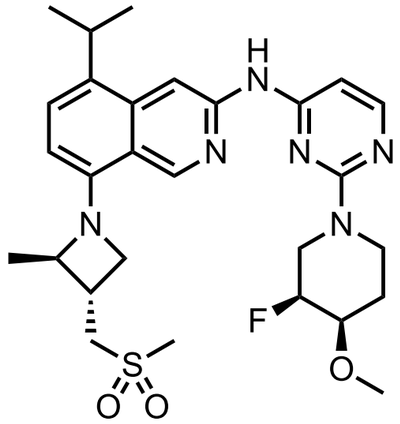
Hit-Finding Strategy. The initial hit that gave rise to BLU-945, "compound 4", was originally discovered from a screen of Blueprint Medicine's proprietary library consisting of over 25,000 compounds with known kinase inhibitory activity. A majority of this library had been pre-screened against >400 human kinases using KINOME*Scan*, allowing them to rapidly identify a starting point from a diverse set of scaffolds. Analysis of the screening data identified "compound 4" as having moderate potency against mutant EGFRs and excellent selectivity over the wild-type protein.



compound 4
Enz EGFR LR/TM: IC_{50} = 914 nM
Enz EGFR WT: IC_{50} = >10,000 nM

BLU-945

EGFR$^{T790M/C797S}$



oral EGFR mutant inhibitor

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>25k compound library screening and opt

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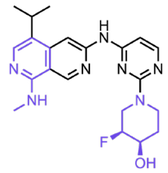
drug hunter

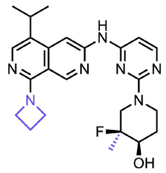
Lead Optimization. Initial optimization focused on the piperidinol ring, where it was found that installing a fluorine adjacent to the alcohol led to a 2-fold enhancement in potency. Moving to the aminopyridine ring system of "compound 4", it was hypothesized that conformational restriction of the amide carbonyl and NH of the aminopyridine would mimic the proposed intramolecular hydrogen bond between the two and lock in the position of the isopropyl moiety ("compound 6"). Although "compound 6" had excellent selectivity over WT EGFR, its overall kinome selectivity was not acceptable. Additional modification of the piperidinol by adding a methyl group to the fluorine-containing carbon and replacement of the methyl amine with an azetidine improved kinome selectivity while still maintaining potency ("compound 9"). Modification of the azetidine ring by the addition of stereogenic methylsulfonyl and methyl moieties ("compound 27") further enhanced selectivity and potency but created issues with Ph. II metabolism. Finally, methylation of the hydroxyl, to block glucuronidation, and removal of the methyl moiety on the piperidine ring, to decrease lipophilicity, provided BLU-945.



compound 6

Enz EGFR LR/TM: IC_{50} = 67 nM

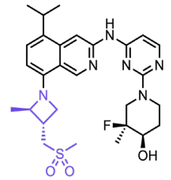
Enz EGFR WT: IC_{50} = >10,000 nM



compound 9

Enz EGFR LR/TM: IC_{50} = 2.1 nM

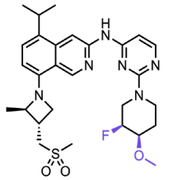
Enz EGFR WT: IC_{50} = >10,000 nM



compound 27

Enz EGFR LR/TM: IC_{50} = 0.3 nM

Enz EGFR WT: IC_{50} = 505 nM



BLU-945

Enz EGFR LR/TM: IC_{50} = 0.4 nM

Enz EGFR WT: IC_{50} = 683 nM

Binding mode: The binding mode of a BLU-945 analog molecule, "compound 24" (structure not shown), in the kinase domain of EGFR L858R/T790M (**PDB: 8D76**) highlights interactions of the sulfone substituent with Lys716 and Lys728 residues. These interactions explain the increased potency of the previous hits without the sulfone group (**PDB: 8D73**).

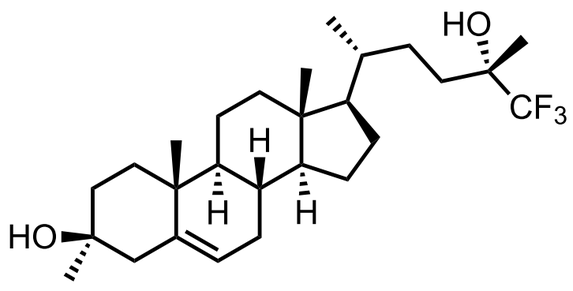
Preclinical Pharmacology. In enzymatic assays, BLU-945 demonstrated sub-nanomolar activity against the EGFR+/T790M, and EGFR+/T790M/C797S acquired mutations and the *EGFR*-activating mutations ex19del and L858R. In an *EGFR* L858R/T790M-driven mouse model, the compound exhibited strong potency (unbound IC_{50} = 0.6 nM). In xenograft mice, BLU-945 was associated with stable disease at 30 mg/kg and tumor regression over 14 days at 100 mg/kg BID comparable to osimertinib dosed at 25 mg/kg. In two osimertinib-resistant models (Ba/F3 EGFR L858R/T790M/C797S, Ba/F3 ex19del/T790M/C797S), a 100 mg/kg dose of the compound was associated with strong tumor regression, with osimertinib demonstrating no efficacy in these animals. Studies in an intracranial implantation model with patient-derived cells suggested that the molecule may be brain-penetrant.

Clinical Development. BLU-945 is currently being evaluated in the SYMPHONY study (NCT04862780). This study aims to assess the safety, drug PK/PD, MTD, RP2D, and preliminary anticancer activity of BLU-945 alone (in the Ph. I portion) and in combination with osimertinib (in the Ph. II portion). Early results published by Blueprint showed that BLU-945 was well tolerated, and a partial response in one patient treated with 400 mg PO QD was reported.

Patent. Blueprint Medicines has filed a patent, WO2021133809A1, describing the invention of new EGFR+/T790M and EGFR+/T790M/C797S mutant inhibitors to treat cancer.

SAGE-718

NMDAR



oral NMDAR positive allosteric modulator

Ph. II candidate in neurology

from previously disclosed PAM and opt

J. Med. Chem.

Sage Therapeutics, Inc., Cambridge, MA

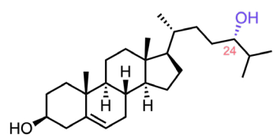
featured article: doi.org/10.1021/acs.jmedchem.2c00313

Context. SAGE-718 (Sage Therapeutics) is an oral *N*-methyl-D-aspartate receptor (NMDAR) Positive Allosteric Modulator (PAM) being developed for cognitive impairment in Huntington's disease. Aberrant NMDAR signaling has been implicated in several neurological and psychiatric disorders, making the receptor an attractive drug target. Although hyperactivation of the receptor has been considered to mediate some cardiovascular and neurodegenerative diseases, its hypofunction has also been associated with diseases such as schizophrenia and Huntington's disease. Due to toxicity-related issues associated with increasing NDMAR activity through an agonist therapeutic, one alternative indirect therapeutic approach is using PAMs, with another through GlyT1 inhibition (see iclepertin, which we recently featured). SAGE-718 was developed from a brain cholesterol metabolite, 24(*S*)-hydroxycholesterol [24(*S*)-HC], which was previously identified as a selective PAM and Huntington's disease biomarker. The potential first-in-class agent has been evaluated in several completed Ph. I and II studies, while at least 3 other Ph. II trials are currently ongoing. Recent data provided by Sage Therapeutics from its completed Ph. II LUMINARY study in patients with mild cognitive impairment and mild dementia due to Alzheimer's disease showed improvements in tests of performance and learning/memory with SAGE-718 administration. In another milestone for Sage Therapeutics, SAGE-718 was granted FDA Fast Track Designation for Huntington's disease.

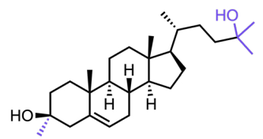
Target. NMDAR is an ionotropic glutamate-gated ion channel family member that is a key regulator of excitatory synaptic function. The receptor has been implicated in various neuropathological and psychiatric diseases. In particular, the protein has been a validated target for learning- and memory-related drug discovery for at least 3 decades, with inhibition of NMDAR action found to be associated with learning impairment and blockage of long-term potentiation. Levels of 24(*S*)-HC were found to be decreased in early Huntington's disease and correlated with cognitive deficiency in a population of patients.

Mechanism of Action. The previously identified 24(*S*)-HC, which was the SAGE-718 starting point, is a potent, direct, and selective NMDAR PAM. 24(*S*)-HC was found to potentiate NMDAR-mediated excitatory postsynaptic currents in rat hippocampal neurons at submicromolar concentrations; however, the metabolite did not similarly modulate other receptors such as the $GABA_A$ receptor. 24(*S*)-HC also played a role in the induction of long-term potentiation and the reversal of hippocampal long-term potentiation deficits induced by the NDMAR antagonist ketamine. Furthermore, Sage Therapeutics has shown a correlation between decreased 24(*S*)-HC levels and cognitive deficits in Huntington's Disease.

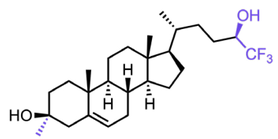
Hit-Finding Strategy. Using the known NMDAR PAM, 24(*S*)-HC, as the primary starting point, structure-activity relationship and PK optimization identified SGE-301 and SGE-550 as potent ligands. However, further improvements were needed to achieve suitable preclinical oral PK profiles and central exposure.



24(*S*)-HC
GluN1/2A
EC_{50} = 150 nM



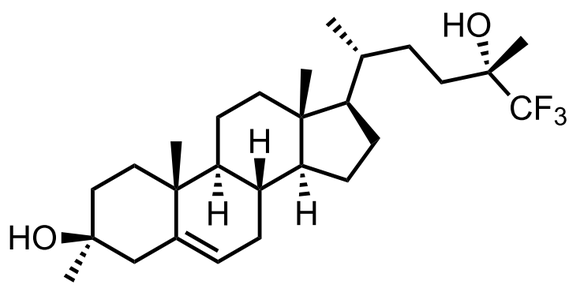
SGE-301
GluN1/2A
EC_{50} = 480 nM



SGE-550
GluN1/2A
EC_{50} = 30 nM

SAGE-718

NMDAR



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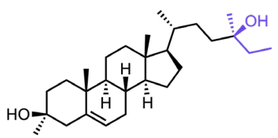
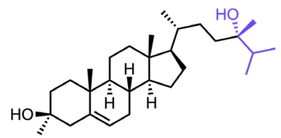
drug hunter

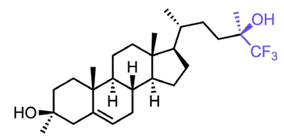
Lead Optimization. Compounds were tested in patch clamp assays against both GluN2A and GluN2B, as the exact subtype responsible for the observed pathology is unknown. As such, compounds were optimized as pan-inhibitors. In early screens, both C-24 hydroxy diastereomers showed activity, so the team at Sage synthesized (*R*) and (*S*) versions for each compound tested. Systemic SAR around the C-24 position indicated that the tertiary alcohol was preferred, leading to "compound 17" and "compound 24". Despite their high potency, these compounds were not advanced because of high clearance rates. This issue was ultimately solved by substituting a trifluoromethyl moiety for one of the C-24 alkyl moieties. "Compound 16" is one resulting diastereomer and maintains activity; the other, SAGE-718, is more than three times as potent against GluN2A and more than twice as potent against GluN2B.



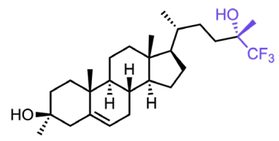
compound 17
GluN2A: EC_{50} = 55 nM
GluN2B: EC_{50} = 100 nM



compound 24
GluN2A: EC_{50} = 60 nM
GluN2B: EC_{50} = 23 nM



compound 16
GluN2A: EC_{50} = 310 nM
GluN2B: EC_{50} = 180 nM



SAGE-718
GluN2A: EC_{50} = 86 nM
GluN2B: EC_{50} = 79 nM

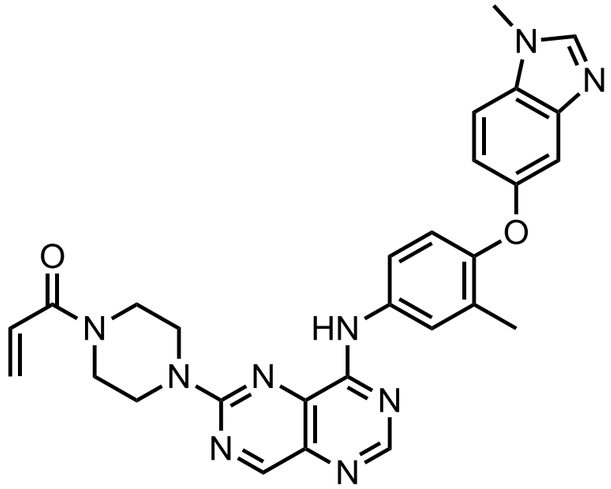
Preclinical Pharmacology. The compound exhibited minimal activity against CYP isoforms (>30 µM) and a cardiac panel comprising key ion channels (>30 µM). Furthermore, no hits were identified in a cross target-selectivity assessment against a broad panel of more than 75 receptors and enzymes. In vivo studies evaluated the NMDAR PAM activity in a 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced hyperlocomotion animal model. Administration of 5-10 mg/kg of SAGE-718 90 min prior to induction of hyperlocomotion led to significant decreases in DOI-induced hyperlocomotion in animals (>2-fold decrease in distance traveled 30 min after DOI administration).

Clinical Development. Currently, nine studies are listed in the clinical trial registry for SAGE-718. The safety, tolerability, and pharmacologic properties of SAGE-718 in healthy subjects have been assessed in three Ph. I studies with healthy subjects. A Ph. I study with a small cohort of six patients with mild to moderate Huntington's disease reported improved cognitive functions. Data from the Ph. II PARADIGM study (NCT04476017) showed that 3 mg PO QD of SAGE-718 was associated with improved performance on tests of learning and memory in participants with Parkinson's disease with mild cognitive impairment. The statistical difference from baseline was verified on day 14, and sustained effects and improving trends were seen out to day 28. Following the Ph. II LUMINARY study (NCT04602624), in patients with mild cognitive impairment (MCI) or mild dementia due to Alzheimer's disease, Sage Therapeutics reported that 3 mg PO QD over 14 days led to a statistically significant 2.3-point improvement in Montreal Cognitive Assessment scores at day 28, compared with baseline. Sage is now recruiting for three Ph. II studies. The DIMENSION study (NCT05107128) is a placebo-controlled trial in patients with early Huntington's disease. The dose regimen will be 1.2 mg PO QD from days 1 to 27 and 0.9 mg PO QD from days 28 to 84. The SURVEYOR study (NCT05358821) intends to assess the magnitude of the baseline difference between participants with early Huntington's disease and healthy participants with respect to measures of cognitive performance. The dose regimen will be 1.2 mg PO QD. The PRECEDENT study (NCT05318937) plans to evaluate the safety and the effect of 1.2 mg PO QD of SAGE-718 on cognitive performance in participants with Parkinson's disease associated with mild cognitive impairment.

Patent. Sage Therapeutics filled patent WO2017007836A1 describing their oxysterol-based *N*-methyl-D-aspartate receptors positive allosteric modulator series. The US patent US10201550B2 is valid until July 2036.

BI-4142

HER2 exon 20 insertion



oral HER2 exon 20 mutants selective inhibitor

tumor regression in the HER2^{YVMA} xenograft model

from 12k compound in-house library screening

and opt

Nat. Cancer

Boehringer Ingelheim RCV, Vienna, AT

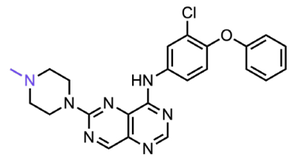
featured article: doi.org/10.1038/s43018-022-00412-y

Context. BI-4142 (Boehringer Ingelheim) is a HER2 exon 20 insertion mutant inhibitor being developed for non-small cell lung cancer (NSCLC). Despite the general advances made in HER2-targeted therapy and the existence of several agents targeting HER2-positive breast cancer, a high unmet need exists for patients with HER2 mutant NSCLC characterized by oncogenic mutations in exon 20. Approved agents include current EGFR TKIs as well as pan-HER2 TKIs, which are limited in their efficacy due to a lack of selectivity vs. EGFR and EGFR-WT–mediated toxicities which limit effective dosing. In September 2021, the FDA granted accelerated approval to Takeda's mobocertinib (Exkivity®, a February 2021 MOTM), which targets the related EGFR exon 20 insertion positive NSCLC. BI-4142 was developed as a selective EGFR-WT sparing inhibitor and presents an interesting example of developing a kinase inhibitor that is selective between an oncogenic variant and a WT family member. The compound demonstrated promising preclinical activity and is currently being evaluated in a Ph. I study, according to Boehringer Ingelheim. In the meantime, Spectrum Pharmaceuticals' poziotinib appears to be leading the way in the HER2 exon 20 insertion mutant inhibitor space, with the FDA recently accepting an NDA application for the drug based on promising data from the ZENITH20 trial.

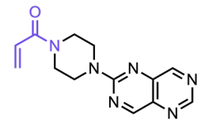
Target. Mutations in HER2, a member of the erbB receptor tyrosine kinase family that mediates development, are found in up to 4% of patients with NSCLC. Exon 20 insertional mutations in *HER2* cause kinase hyperactivity and have been found in animal experiments to drive oncogenic growth. The most common HER2 exon 20 mutation in NSCLC is a 12-base pair in-frame insertion that causes the duplication of four amino acids YVMA (HER2^{YVMA}). Scientists at Boehringer Ingelheim were interested in a compound that not only had potent HER2 exon 20 activity but was also selective enough (>50x) to have EGFR-WT sparing activity. Such a compound would be expected to reduce the EGFR-WT–mediated toxicities associated with current erbB TKIs with pan activity.

Mechanism of Action. BI-4142 is a covalent ATP-competitive inhibitor and exerts its effects through binding to a cysteine residue in the kinase domain. Selective inhibition of HER2^{YVMA} was found to preclude tumor growth. In contrast, EGFR-WT inhibition in these HER2 mutant tumors had a negligible therapeutic effect, suggesting that EGFR-WT inhibition is not required to achieve full efficacy. Following the observation that HER2^{YVMA} exerts its oncogenic effects partly through hyperactivation of the MAPK pathway, Boehringer Ingelheim found that a combination of BI-4142 with a SOS1-KRAS inhibitor was associated with a more pronounced antitumor activity.

Hit-Finding Strategy. Initial hits were discovered by using a HEK293 cell-based screen of a ~12,000 compound library which measured HER2^{YVMA} phosphorylation. The library was narrowed down to 900 compounds, which were rescreened for a dose-response in cells overexpressing HER2^{YVMA} and EGFR^{WT}. Thirty-five of these compounds were found to have greater than 5x selectivity for HER2 phosphorylation over EGFR phosphorylation, with "compound 1" becoming the initial hit. Reasoning that covalent modification would increase potency led to a series of acrylamide electrophiles, e.g., "compound 2".



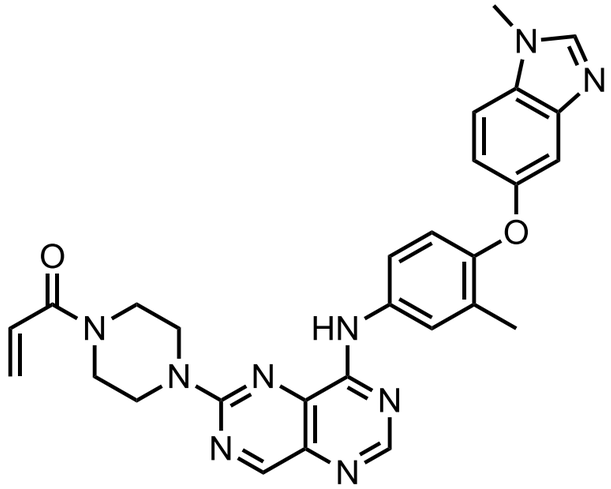
compound 1
HEK HER2^{YVMA}: IC_{50} = 4,400 nM
HEK EGFR^{WT} : IC_{50} = >10 µM



compound 2
Ba/F3 HER2^{YVMA}: IC_{50} = 16 nM
Ba/F3 EGFR^{WT} : IC_{50} = 55 nM

BI-4142

HER2 exon 20 insertion



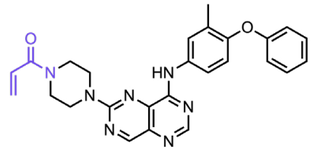
oral HER2 exon 20 mutants selective inhibitor tumor regression in the HER2^{YVMA} xenograft model from 12k compound in-house library screening and opt

Nat. Cancer

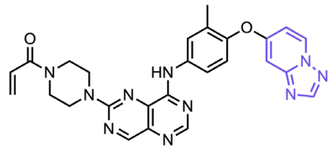
Boehringer Ingelheim RCV, Vienna, AT

featured article: [doi.org/10.1038/s43018-022-00412-y](doi.org/10.1038/s43018-022-00412-y)

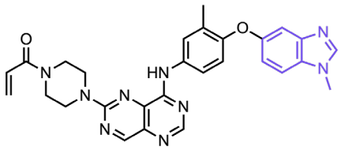
Lead Optimization. A number of the compounds identified in the initial screen contained a pyrimido[5,4-*d*]pyrimidines core, so this was held constant while the SAR of the sidechains was evaluated. Initial optimization focused on the aniline, adding in a small lipophilic substituent to the 3-position ("compound 3"). Further optimization focused on replacement of the phenyl ring with bicyclic heteroaromatic rings to drive selectivity for HER2^{YVMA} over EGFR^{WT}. When a [1,2,4] triazolo[1,5-*a*]pyridine moiety was used, HER2^{YVMA} potency remained while selectivity over EGFR^{WT} improved to ~50-fold (BI-1622). Ultimately, BI-4142 was found by removal of the nitrogen at position 1 of the [1,2,4] triazolo[1,5-*a*]pyridine and addition of a methyl group to the nitrogen at position 2.



compound 3
HEK HER2^{YVMA}: IC_{50} = 1.3 nM
HEK EGFR^{WT}: IC_{50} = 25 nM



BI-1622
HEK HER2^{YVMA}: IC_{50} = 3 nM
HEK EGFR^{WT} : IC_{50} = 150 nM



BI-4142
HEK HER2^{YVMA}: IC_{50} = 10 nM
HEK EGFR^{WT} : IC_{50} = 270 nM

Binding mode. BI-4142 is a Type II kinase inhibitor. X-ray crystal structure of a close analog (**PDB:7PCD**) in the ATP binding site of wild-type HER2 (used as a substitute for HER2^{YVMA}, whose structure was not reported) was employed to generate some insights into ligand-protein interaction. Some relevant interactions include the acrylamide covalent bond with Cys805 residue in the front pocket, interactions of pyrimido[5,4-*d*]pyrimidine core with Met803 and Leu726, and the nitrogen interaction with Ser783 in the hydrophobic back pocket, crucial to the selectivity over wild type EGFR.

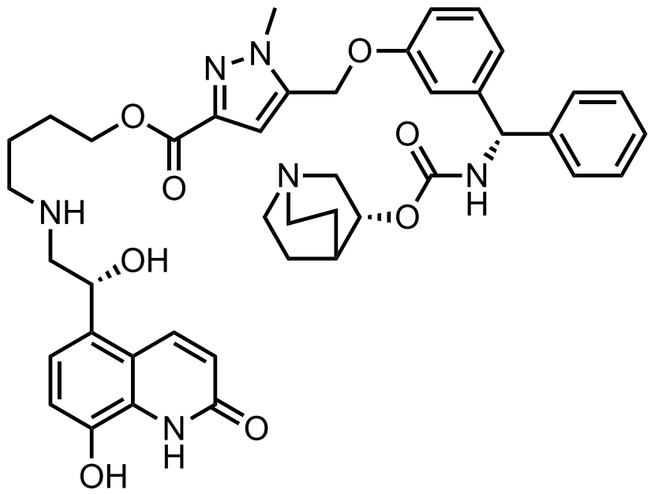
Preclinical Pharmacology. BI-4142 demonstrated strong potency for HER2 (IC_{50} = 10 nM) and showed >25-fold selectivity vs EGFR. In various HER2 mutated cell lines, including those with alternate exon 20 mutations, IC_{50}s were <100 nM, while activity against EGFR^{WT} was poor as desired (IC_{50} >5 µM). In vivo experiments were done in animals harboring PC-9 HER2^{YVMA} tumors. BI-4142 given orally at 3, 10, 30, and 100 mg/kg BID resulted in tumor growth inhibitions of 113%, 126%, 153%, and 166%, respectively.

Clinical Development. Preclinical compound

Patent. Boehringer Ingelheim disclosed its series of [1,3]diazine[5,4-*d*]pyrimidines in patent WO2021213800A1 and WO2021156178A1. US patent US20210332054A1 is pending as of August 2022.

CHF-6366

mAChrs and ADRB2



inhaled mACHrs and ADRB2 agonist

Ph. I/II candidate in Asthma and COPD discontinued

soft drug design from previously disclosed lead MABA

J. Med. Chem.

Chiesi Farmaceutici S.p.A, Parma, IT

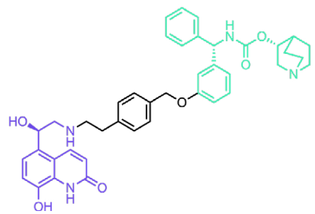
featured article: doi.org/10.1021/acs.jmedchem.2c00609

Context. CHF-6366 (Chiesi Farmaceutici S.p.A) is a muscarinic antagonist and β2 agonist (MABA) being developed as an inhaled treatment for respiratory diseases. Muscarinic antagonists and β2 agonists both act to inhibit bronchoconstriction, but through different mechanisms, making the combination of both agents a desirable strategy for achieving enhanced efficacy in treating pulmonary diseases. Although long-acting formulations of muscarinic antagonists and β2 agonists have been combined and used actively in the last 10 years (e.g., Anoro Ellipta and Stiolto Respimat), various drawbacks associated with such multicomponent inhalers have led to efforts to develop bifunctional agents with dual activity on either receptor. Such MABAs are expected to be single molecular entities comprising two covalently-linked pharmacophores. At least 5 MABA agents are currently in preclinical/clinical development, the front runners of which are GSK's batefenterol (GSK961081) and AstraZeneca's navafenterol (AZD8871). CHF-6366 was designed to circumvent a key challenge that may be encountered by existing agents under development; according to Chiesi Farmaceutici scientists, they sought an agent that displayed comparable efficacy and duration of action at each individual receptor. The approach used is an interesting example of relying on soft drug design to develop a heterobifunctional agent which is stable in the desired organ(s) but undergoes rapid systematic clearance. A Ph. I/II study evaluating the compound in chronic obstructive pulmonary disease (COPD) has been completed.

Target. ⊠2 adrenoreceptors (ADRB2s) and muscarinic acetylcholine receptors (mAChRs) are widely expressed in the respiratory tract and are key to pulmonary function. Airway smooth muscle diameter and thus resistance to airflow is dictated by the cross-talk between both receptors: activation of muscarinic receptors under conditions of lung inflammation results in airway smooth muscle contraction and hence increased resistance to airflow, while β2 receptors antagonize airway smooth muscle contraction. Therefore, the most frequently prescribed bronchodilators for pulmonary diseases such as asthma and COPD are either muscarinic antagonists or β2 agonists. In developing CHF-6366, Chiesi Farmaceutici scientists desired an inhalable compound with bifunctional comparable activity on muscarinic and β2 receptors.

Mechanism of Action. β2-agonists act to relax the smooth muscles in the airway, regardless of the cause of bronchoconstriction, while muscarinic antagonists act to achieve a similar effect by limiting the actions of acetylcholine released from parasympathetic nerves within the lungs.

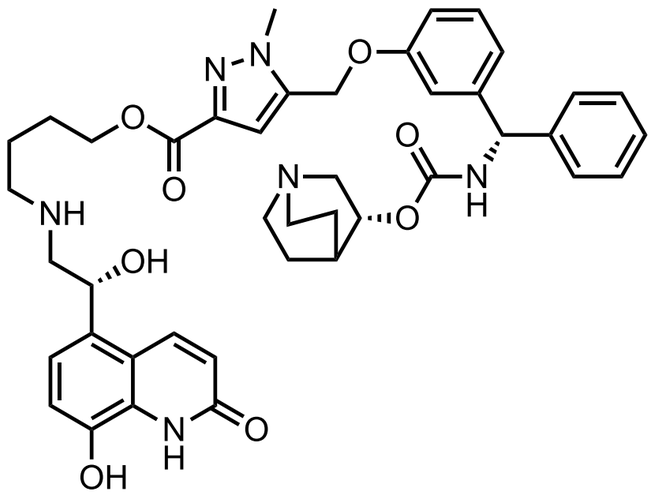
Hit-Finding Strategy. The initial hit, "compound 13", was designed by linking a β_2 pharmacophore and a quinuclidinyl M_3 chemotype. Linker properties optimized included length, tPSA, and clogP. The assays used to evaluate activity were human cloned M_3 receptor using [^{3}H]-*N*-methylscopolamine competition and human cloned β_2 receptor using ^{125}I-cyanopindolol competition.



compound 13
M_3: pK_i = 9.9
β_2: pK_i = 9.6

CHF-6366

mAChrs and ADRB2



inhaled mAChrs and ADRB2 agonist

Ph. I/II candidate in Asthma and COPD discontinued

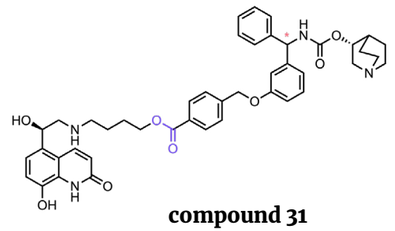
soft drug design from previously disclosed lead MABA

J. Med. Chem.

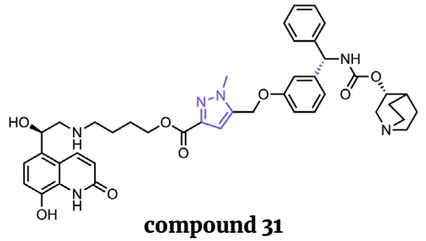
Chiesi Farmaceutici S.p.A, Parma, IT

featured article: doi.org/10.1021/acs.jmedchem.2c00609

Lead Optimization. Although "compound 13" delivered the desired affinity balance, it proved to have limited metabolic instability, a trait generally welcomed by drug hunters. In this case, though, Chiesi Farmaceutici scientists wanted instability in their molecule so that the individual M_3 and β_2 pieces would separate once they reached their intended target. Optimization of the linked pharmacophores primarily involved fine-tuning the linker properties, including tPSA and clogP, with the racemic "compound 31" coming out of these initial optimizations. Although stability was reduced, there was now a marked difference between the M_3 and β_2 affinities. Further optimization was done, including studies to define the best M_3 diastereomer. The *S*-benzylcarbamate was found to have slightly better activity and, when combined with the optimized linker, provided CHF-6366 as the clinical candidate.



compound 31
M_3: pK_i = 9.5
β_2: pK_i = 10.4



compound 31
M_3: pK_i = 10.2
β_2: pK_i = 11.4

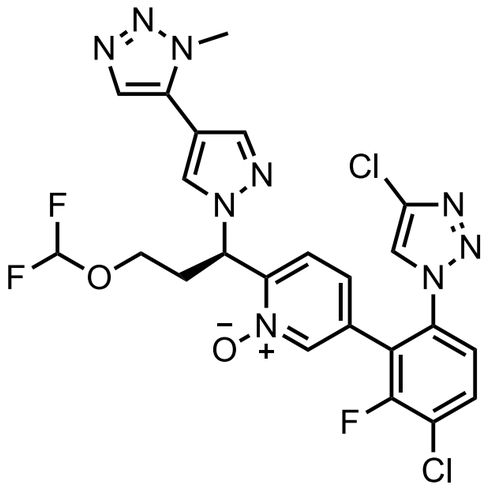
Preclinical Pharmacology. In vitro PK studies revealed that the compound exhibited the desired poor stability in rat ($t_{1/2}$ <5 min) and human plasma ($t_{1/2}$ = 20 min), the rationale being to limit potential cardiotoxicity from long blood exposure. As desired, the compound had high stability in human lungs ($t_{1/2}$ >220 min). An in vivo guinea pig model of bronchoconstriction based on the Konzett-Roessler methodology was used for efficacy studies. At a dose of 0.3 nmol/kg, the compound exhibited balanced activity on each individual receptor as measured by % inhibition of bronchoconstriction (29% [muscarinic] vs 28% [β2]), while an additive effect was observed when the MABA activity was measured. The compound was well tolerated in safety experiments, with only minor cardiovascular events observed that were mitigated following a care dose escalation phase.

Clinical Development. Chiesi Farmaceutici S.p.A. completed a Ph. I/II (NCT03378648) trial. The researchers employed single ascending doses in healthy male volunteers and patients with asthma, and single-dose regimens in male patients with COPD. In healthy male volunteers, inhaled doses of 5, 10, 20, 40, 80, 160, and 360 mcg were assessed. The results showed that CHF 6366 was safe with no clinically significant abnormalities observed in laboratory evaluations, vital signs, ECG, or physical examinations and well tolerated at all doses with negligible systemic exposure. In early 2022, CHF-6366 was withdrawn from the Chiesi Farmaceutici pipeline, however no explanation was provided.

Patent. Chiesi Farmaceutici filled the patent WO2014086924A1 describing compounds that act both as muscarinic receptor antagonists and β_2 adrenergic as CHF-6366. The patent was granted with an expiration date of 2033-12-06 (US8987299B2).

Compound 3f

FXIa



oral Factor XIa inhibitor

IV efficacy in rabbit AV shunt model

from literature starting point and SBDD

J. Med. Chem.

Janssen Research & Development, L.L.C.,

Spring House, PA

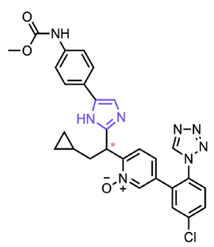
featured article: doi.org/10.1021/acs.jmedchem.2c00442

Context. "Compound 3f" (Janssen) is an oral Factor XIa (FXIa) inhibitor being developed for thromboembolic diseases. The suitability of FXIa as a target in the development of anticoagulation agents stems from observations that the coagulation factor contributes significantly to pathological blood clotting (thrombosis) but plays a negligible role in physiological blood clotting (hemostasis). Unsurprisingly, >100 patent applications covering various categories of FXIa inhibitors, from small molecules to oligonucleotides, have been filed since the 1990s (see our previously featured FXIa inhibitors asundexian, milvexian, and "compound 23"). The development of pyridine-*N*-oxide–based "compound 3f" presents an example of utilizing nonclassical active site interactions to modulate potency and bioavailability. The compound demonstrated strong selectivity over other coagulation proteases and had an acceptable PK, with concomitant robust activity in animal studies.

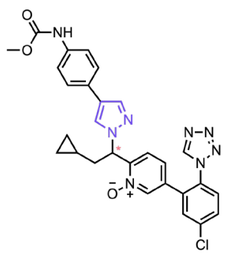
Target. FXIa is a plasma coagulation serine protease that contributes to the generation of thrombin and is one of the enzymes of the coagulation cascade. The protein is a well-validated target for anticoagulation agents. Patients with a deficiency in Factor XI (FXI), the zymogen of FXIa, have been found to have a lower risk of thromboembolic diseases, whereas increased FXIa activity has been associated with increased risk for thromboembolic disorders.

Mechanism of Action. FXI activation to FXIa results in the generation of another coagulation factor, FIXa, ultimately leading to the generation of thrombin and the formation of fibrin. "Compound 3f", a pyridine *N*-oxide-containing inhibitor, makes nonclassical interactions at the FXIa active site, which Janssen scientists speculate are key to its enhanced potency and improved cell permeability.

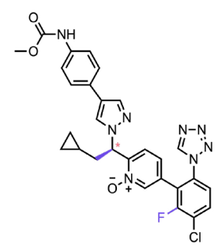
Hit-Finding Strategy. Starting from the known pyridine *N*-oxide-based FXIa inhibitor "compound 1", molecular modeling suggested that the metabolically liable imidazole could be replaced with a pyrazole, leading to "compound 2a", although there was a slight drop in potency. This was improved by selecting the more active enantiomer and adding fluorine at the 2-position of the benzene ring ("compound 2d"). Although early PK studies showed that "compound 2d" had reasonable anticoagulant activity in an activated partial thromboplastin time (aPTT) assay ($EC_{1.5}$ – 4.02 µM), good permeability, and excellent oral bioavailability in rats, improvements were still necessary as inhibition of clot weight plateaued at 41%, despite increasing doses.



compound 1
FXIa: K_i = 0.25 nM



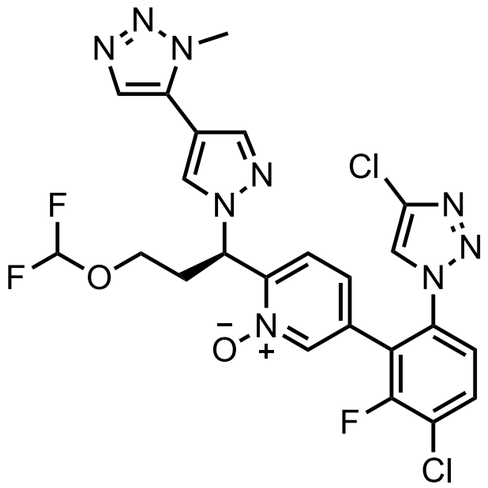
compound 2a
FXIa: K_i = 7.8 nM



compound 2d
FXIa: K_i = 1.3 nM

Compound 3f

FXIa



oral Factor XIa inhibitor

IV efficacy in rabbit AV shunt model

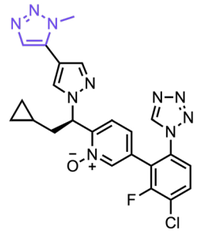
from literature starting point and SBDD

J. Med. Chem.

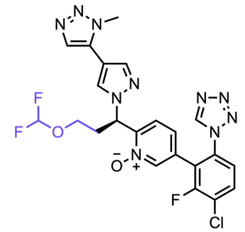
Janssen Research & Development, L.L.C., Spring House, PA

featured article: doi.org/10.1021/acs.jmedchem.2c00442

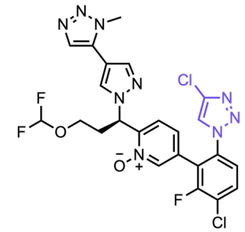
Lead Optimization. Initial lead optimization focused on improving contacts within the S2' pocket, as such interactions have been shown to improve binding affinity. Several heteroaromatic derivatives were tried, with the methylated triazole in "compound 2l" showing the greatest promise. Although "compound 2l" had high potency, its cellular permeability and oral bioavailability needed improvement. Optimization next centered on the S1' pocket, a small, shallow pocket surrounded by a hydrophobic side chain (Leu415), a disulfide (Cys416/Cys432), and a polar side chain (His431). A non-classical F_2C–H···O hydrogen bonding interaction with Leu415 was employed ("compound 3c"), which led to both improved potency and metabolic stability but did not impart the desired cellular permeability. This was achieved through modification of the tetrazole to become more lipophilic, with "compound 3f" having the best combination of FXIa binding activity, anticoagulant activity, and PK profile.



compound 2l
FXIa: K_i = 0.2 nM



compound 3c
FXIa: K_i = 0.12 nM



compound 3c
FXIa: K_i = 0.12 nM

Binding mode. The X-ray cocrystal structure of 3f bound to FXIa (**PDB 7V18**) shows several crucial hydrogen bond interactions inside the oxyanion hole. An interesting nonclassical F_2C–H···O hydrogen bond with Leu415 residue improves the "compound 3f" potency and metabolic stability regarding other analogs without this interaction.

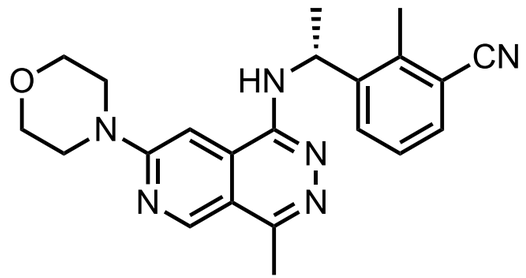
Preclinical Pharmacology. Selectivity of the compound against a panel of human proteases showed activity for only FXIa and plasma kallikrein (IC_{50} = 23 nM), another coagulation factor whose activation ultimately results in generation of FXIa. In vivo efficacy studies to evaluate antithrombotic activity were done in a rabbit AV shunt thrombosis model. Dosed with a bolus + infusion doses of 1.7 mg/kg + 2.0 mg/kg/h and 8.5 mg/kg + 10 mg/kg/h, the percent reductions in thrombus weight were 36.5% and 62.2%, respectively. Clotting time at 20 min after both doses increased by ~2 fold (1.7 mg/kg + 2.0 mg/kg/h dose) and ~3 fold (8.5 mg/kg + 10 mg/kg/h); after 60 min, these were ~1.5 fold and ~2.7 fold, respectively. No changes in prothrombin time were seen, suggesting that physiological blood clotting was unaffected by the activity of the compound.

Clinical Development. Preclinical compound

Patent. No publicly available records as of 20 August 2022.

MRTX0902

SOS1:KRAS^{G12C} PPI



oral brain-penetrant SOS1:KRAS^{G12C} PPI inhibitor

efficacy in MIA PaCa-2 tumor mouse xenograft model

from literature starting point and opt

J. Med. Chem.

Mirati Therapeutics, San Diego, CA

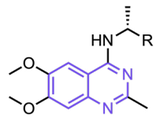
featured article: doi.org/10.1021/acs.jmedchem.2c00741

Context. MRTX0902 (Mirati Therapeutics) is an oral brain penetrating SOS1:KRAS protein-protein interaction inhibitor. Since the first report of the druggability of the KRAS^{G12C} mutant, KRAS has been a hotly pursued oncotarget. Notably, the first-in-class sotorasib (Lumakras; Amgen) was recently approved, while Mirati Therapeutics' highly promising adagrasib (MRTX84) is on track to be approved by regulators. However, emerging data on acquired resistance to adagrasib and sotorasib suggest that additional therapies that enhance the duration of response to KRAS^{G12C} inhibitors may be useful. Considered the "pacemaker" of KRAS due to its key role in switching the GTPase to the active state, SOS may be a desirable target for inhibiting KRAS activity. The SOS1 binder MRTX0902 uniquely disrupts the KRAS:SOS1 protein-protein interaction which, when combined with a KRAS^{G12C} inhibitor, may potentiate the overall antitumor effect. Proof of this concept was confirmed in preclinical studies, with a combination of MRTX0902 and adagrasib resulting in complete tumor regression in animals. MRTX0902 also has a highly brain penetrant properties, making any resulting drug one that might find strong utility for patients who develop brain metastases, a frequent hallmark of KRAS mutant-driven cancer progression. The compound is currently in IND-enabling studies.

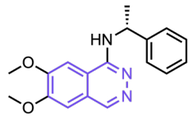
Target. SOS is a guanine nucleotide exchange factor that activates KRAS function. The binding of SOS switches KRAS from the inactive GDP-loaded state to the active GTP-loaded state. Genomic studies have shown that tumors highly dependent on KRAS activation mutations are especially sensitive to the genetic perturbation of SOS1, the dominant SOS protein. Based on SOS1's well-defined binding pocket adjacent to the KRAS:SOS1 interface, Mirati Therapeutics scientists hypothesized that targeting the KRAS:SOS1 protein-protein interaction may be an ideal therapeutic strategy.

Mechanism of Action. MRTX0902 disrupts the KRAS:SOS1 protein-protein interaction, precluding the activation of KRAS by SOS1 and locking the former in the GDP-loaded state. Similarly, adagrasib binds to a region on KRAS^{G12C} that locks the GDP-bound KRAS^{G12C} in the inactive state and prevents reactivation by nucleotide exchange. Mirati scientists speculated that combining adagrasib with the SOS1 binder MRTX0902 may lead to increased levels of GDP-loaded KRAS^{G12C}, leading to an increased response vs. adagrasib alone.

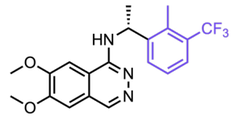
Hit-Finding Strategy. Modeling efforts based on previously reported co-crystal structures of SOS1 led Mirati scientists to transpose the *N*1-quinazoline nitrogen in "compound 4" into a phthalazine ring system found in "compound 6". The potency of the inhibitors was measured with an HTRF displacement assay (K_i) and an In-Cell Western Assay that quantifies phosphorylated ERK1/2 (pERK) in MKN1 cells (IC_{50}). Substitution studies on the phenyl ring identified "compound 10" as the initial hit. It should be noted that other SOS1 binders, such as BAY-293 and BI-3406, share the same quinazoline ring as "compound 4".



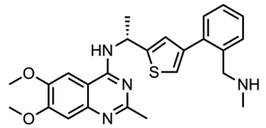
compound 4



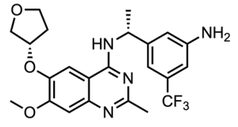
compound 6
K_i = 637 nM
IC_{50} = >10,000 nM



compound 10
K_i = 10 nM
IC_{50} = 378 nM



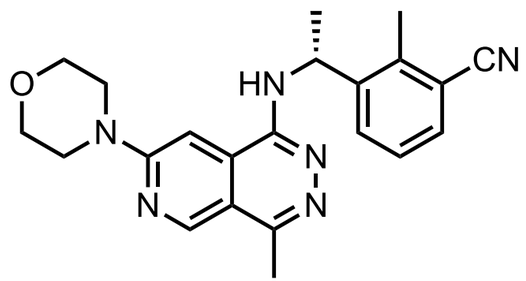
BAY-293
KRAS^{G12C}:SOS1^{cat}
Interaction assay IC_{50} = 21 nM



compound 10
SOS_1 (SPR)
K_D = 3 nM

MRTX0902

SOS1:KRAS^{G12C} PPI



oral brain-penetrant SOS1:KRAS^{G12C} PPI inhibitor

efficacy in MIA PaCa-2 tumor mouse xenograft model

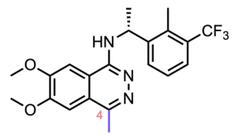
from literature starting point and opt

J. Med. Chem.

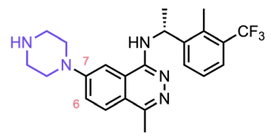
Mirati Therapeutics, San Diego, CA

featured article: doi.org/10.1021/acs.jmedchem.2c00741

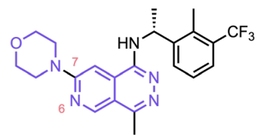
Lead Optimization. In vitro profiling of the early phthalazine-based compounds indicated that the C4-position was highly susceptible to aldehyde oxidase (AO) metabolism; however, this could easily be mitigated by the addition of a methyl group ("compound 13"). Attention then turned to the SAR around the C7-substituent, with the goal of extending the molecule into the KRAS:SOS1 protein-protein interface; modeling suggested modification at C6 would have negligible effect. Substitution of the C7-methoxy group with piperidine led to a ~8-fold increase in binding affinity ("compound 15"). Given its promising profile, "compound 15" was advanced to PK studies, which demonstrated moderate clearance in human liver microsomes, but high IV clearance, as well as low bioavailability (in mice). Clearance was improved by replacing the C-6 carbon with nitrogen, yielding a pyridopyridazine, and the basic piperidine with a neutral morpholine ("compound 24"). Although the clearance issues were now resolved, "compound 24" suffered from CYP3A4 inhibition (IC_{50} = 640 nM). Modification of the C-1 benzyl amine provided the final piece of the puzzle, leading to MRTX0902.



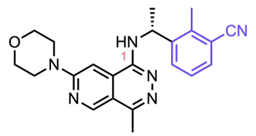
compound 13
K_i = 2.6 nM
IC_{50} = 195 nM



compound 15
K_i = 0.33 nM
IC_{50} = 46 nM



compound 24
K_i = 0.91 nM
IC_{50} = 26 nM



MRTX0902
K_i = 1.9 nM
IC_{50} = 29 nM

Binding mode. The co-crystal structure of MRTX0902 linked to SOS1 (**PDB:7UKR**) reveals the π-stacking interaction of pyridopyridazine core with His905 and an interesting salt bridge with Glu902. The morpholine ring extends out of the binding pocket to the region where protein-protein interaction occurs, leading to disruption of the SOS1:KRAS complex.

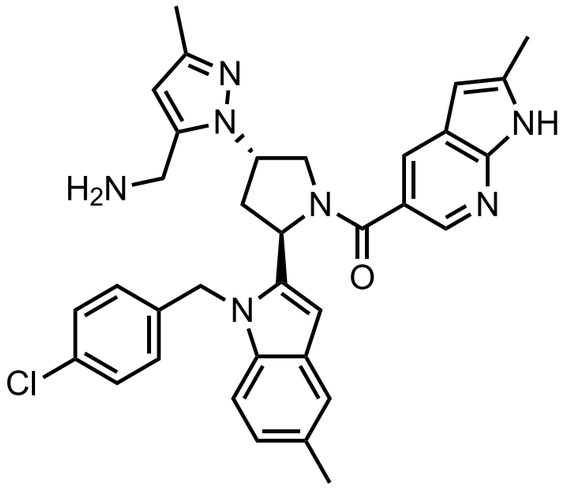
Preclinical Pharmacology. The compound demonstrated favorable PK in mice, rats, and dogs (Cl: 4.4–14.6 mL/min/kg; Vd: 0.28–0.48 L/kg; IV $t_{1/2}$: 0.62-1.3; F: 38-83%). The compound also had a favorable CNS PK profile in mice, with measured concentrations demonstrating full coverage of the determined in vitro IC_{50} of 29 nM. In an MIA PaCa-2 mouse model, 25 and 50 mg/kg oral BID dosing of the compound for 25 days resulted in tumor growth inhibitions of 41% and 53%, respectively. Given as a single agent at 10 mg/kg, adagrasib mediated a tumor growth inhibition of 94%, although no regressions were observed beyond 25 days. When MRTX0902 (25 mg/kg BID) was combined with adagrasib (10 mg/kg QD), tumor regression was −54%. At 50 mg/kg of MRTX0902 BID + 10 mg/kg of QD, near complete regression (−92%) was seen, while two animals were tumor-free.

Clinical Development. According to the Mirati pipeline, an IND application is planned to be submitted this year.

Patent. The selective brain-penetrant SOS1:KRASG12C PPI inhibitors were described in the Mirati Therapeutics patent application WO2021127429A1 (US patent office code US20210188857A1).

Compound 14

NNMT



elective NNMT inhibitor

100-times higher inhibitory activity
than original peptide

peptide library screening, de novo design and SBDD

J. Med. Chem.

Shionogi Pharmaceutical Research Center,
Toyonaka, JP

featured article: doi.org/10.1021/acs.jmedchem.2c00919

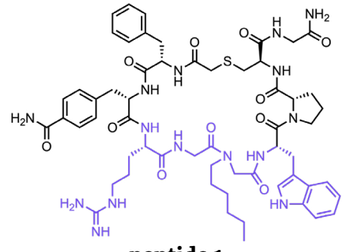
drug hunter

Context. "Compound 14" (Shionogi Pharmaceuticals) is a nicotinamide *N*-methyltransferase (NNMT) inhibitor. For some "difficult to drug" targets such as protein-protein interactions, peptides are attractive therapeutic modalities due to their high binding specificity and synthetic accessibility. Macrocyclization of peptides may help improve upon their typical drawbacks, such as poor stability and cell permeability; however, the therapeutic efficacy of macrocyclic peptides is also limited by their molecular sizes and polar surface areas. To address these issues, Shionogi scientists adopted a novel "Peptide-to-Small Molecule" strategy that was based on structural hopping of macrocyclic peptides into nonpeptide small molecules. To evaluate the strategy, NNMT, a widely expressed epigenetic enzyme that has been associated with several diseases, was used. Starting from a macrocyclic peptide hit identified through an in vitro screen, a potent small molecule was developed through optimization that exhibited sub-micromolar cellular activity (IC_{50} = 0.40 µM). Additional preclinical data, when available, will shine further light on the potential utility of this strategy as a drug discovery tool.

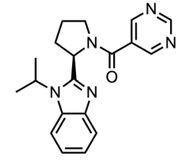
Target. NNMT is a cytosolic enzyme that uses *S*-adenosyl methionine as a cofactor to methylate the pyridyl ring of nicotinamide to give 1-methyl-nicotinamide; it has been implicated in several diseases. It was selected as the target to evaluate the "Peptide-to-Small Molecule" strategy due in part to its non-interaction with other peptides or proteins in its enzymatic function. Further, proof of inhibition of NNMT by macrocyclic peptides has been previously reported.

Mechanism of Action. Overactivity of NNMT, especially in adipose and liver tissues, is implicated in a number of metabolic disorders, including obesity, insulin resistance, and type 2 diabetes. In mice with high fat diet-induced obesity, treatment with an NNMT inhibitor led to reduced body weight, increased insulin sensitivity, and glucose tolerance similar to that of lean control mice. Previously reported NNMT inhibitors target the nicotinamide or *S*-adenosyl methionine binding sites (small molecules) or allosteric sites on the enzyme (cyclic peptides). "Compound 14" mimics the key allosteric interactions of the precursor macrocyclic peptide but shares structural similarities with the peptide. Downstream effects of the inhibition of NNMT by the compound are yet to be reported.

Hit-Finding Strategy. The hit-finding strategy reported here is a novel "peptide-to-small-molecule" approach, which employs a macrocyclic peptide display screen followed by a pharmacophore-based small molecule design. The advantage of this strategy is the combination of the potent activity often afforded by macrocyclic peptides with the metabolic stability and membrane permeability of small molecules. "Peptide 1" was identified as the initial hit peptide using an affinity selection display with macrocyclic peptide libraries against NNMT. A SAR-based pharmacophore characterization of "peptide 1" conducted using alanine scanning revealed that the four amino acids from the arginine to the tryptophan (counterclockwise, bottom of the structure) were critical to binding affinity. A co-crystal structure of "peptide 1" with NNMT enabled in silico screening of 6 million fragments to identify small molecules that possess the identified pharmacophore requirements, ultimately leading to "virtual hit-A".



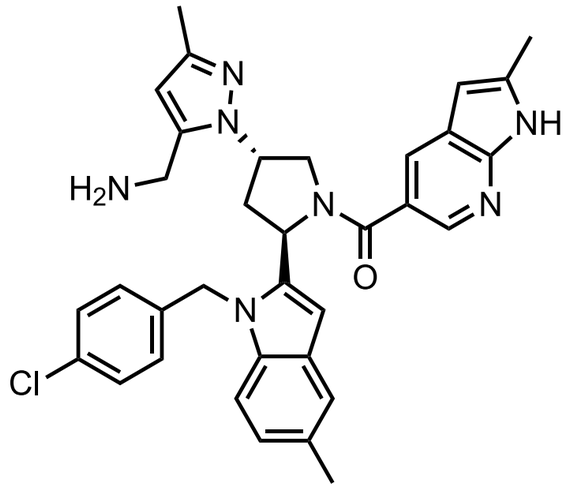
peptide 1
cell-free NNMT: IC_{50}= 0.1 µM
cell-based NNMT: IC_{50}= >100 µM



virtual hit-A
Docking Score:
-8.3 kcal/mol

Compound 14

NNMT



elective NNMT inhibitor

100-times higher inhibitory activity
than original peptide

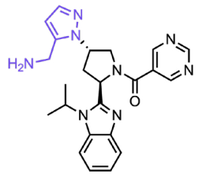
peptide library screening, de novo design and SBDD

J. Med. Chem.

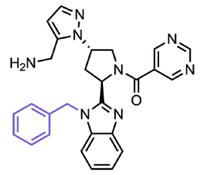
Shionogi Pharmaceutical Research Center,
Toyonaka, JP

featured article: doi.org/10.1021/acs.jmedchem.2c00919

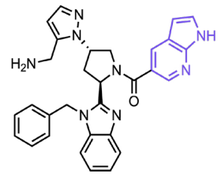
Lead Optimization. Optimization using structure-guided design led to "designed molecule-B", with its primary amine hydrogen bonding with the backbone carbonyl of Thr163, "designed molecule-C", which can participate in a T-shaped π-π interaction with Phe5, and "designed molecule-D", which acts as an HBA with Val143, an HBD with Asp142, and engages in π stacking with Tyr86 (all in silico). "Designed molecule-D" was synthesized ("compound 11") and tested, showing weak inhibitory activity in in vitro assays. Relatively minor SAR optimization involved the addition of a methyl group on each aromatic heterocycle, removal of one nitrogen atom, and addition of chlorine in the 4-position of the benzyl group. "Compound 14" is two orders of magnitude more potent (in vitro) than "peptide 1", with half the molecular weight. Furthermore, it has sub-micromolar activity in cellular assays, whereas the peptide has no inhibitory activity.



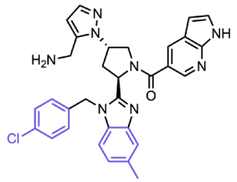
virtual hit-B
Docking Score:
-9.7 kcal/mol



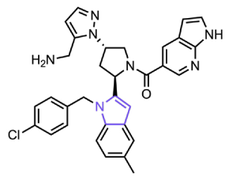
virtual hit-C
Docking Score:
-10.2 kcal/mol



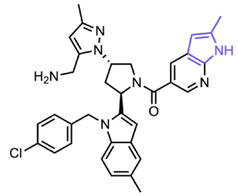
virtual hit-D (11)
Docking Score: -11.5 kcal/mol
cell-free NNMT: IC_{50}= 54 µM
cell-based NNMT: IC_{50}= >100 µM



compound 12
cell-free NNMT: IC_{50}= 0.39 µM
cell-based NNMT: IC_{50}= 42 µM



compound 13
cell-free NNMT: IC_{50}= 0.016 µM
cell-based NNMT: IC_{50}= 5.5 µM



compound 14
cell-free NNMT: IC_{50}= 0.0011 µM
cell-based NNMT: IC_{50}= 0.4 µM

Binding mode. The co-crystal structure of close analog, "compound 13" (**PDB:7WMT**), revealed π-stacking interactions between azaindole moiety and Tyr11 and Tyr86 residues. The hydrophobic cryptic pocket is occupied by the indole and benzyl moieties.

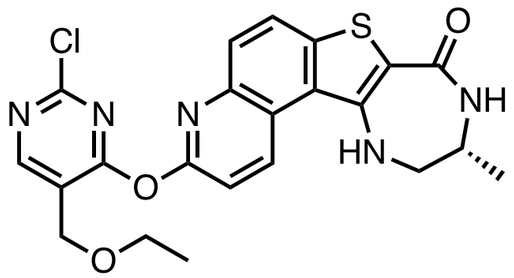
Preclinical Pharmacology. The compound demonstrated strong in vitro potency (cell-free IC_{50} = 1.1 nM; cell-based IC_{50} = 0.40 µM) vs the precursor macrocyclic peptide (cell-free IC_{50} = 0.10 µM). The compound was also selective (IC_{50} >100 µM) against indolethylamine *N*-methyltransferase (INMT), the closest structural homolog of NNMT. Additional preclinical PK/PD data have yet to be reported by Shionogi scientists.

Clinical Development. Preclinical compound

Patent. No publicly available records as of 20 August 2022.

CC-99677

MK2



oral, covalent MK2 inhibitor

Ph. II candidate in ankylosing spondylitis

from previously disclosed MK2 inhibitor and SBDD

Transl Res

Bristol Myers Squibb, Princeton, NJ

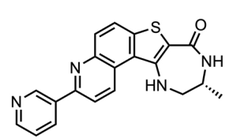
featured article: doi.org/10.1016/j.trsl.2022.06.005

Context. CC-99677 (BMS) is an oral, covalent MAPK–activated protein kinase-2 (MK2) inhibitor being developed for autoimmune diseases. Although p38 is the most heralded member of the p38-MAPK signaling pathway, one that mediates the release of pro-inflammatory cytokines and is implicated in several inflammatory diseases, attempts to directly inhibit the kinase have been largely unsuccessful. Notably, these agents have been associated with a lack of sustained efficacy (tachyphylaxis), potentially stemming from their inhibition of other key proteins involved in feedback regulation of the signaling pathway. Consequently, targeting MK2, a downstream target of p38, is hypothesized as a strategy to circumvent this compensatory feedback mechanism. CC-99677 was found by BMS scientists to mediate a cytokine inhibitory profile different from that of previously described p38 inhibitors. While it is selective and has good efficacy in vivo, it's not necessarily guaranteed that sustained suppression of inflammation will be observed in humans. The compound is currently being evaluated in Ph. I/II studies, and the race, with Aclaris Therapeutics' zunsemetinib (CDD-450), to demonstrate proof-of-concept with an MK2 inhibitor appears to be on.

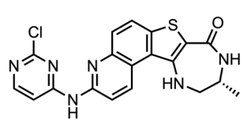
Target. MK2 is a direct downstream target of p38–MAPK, a key regulator of inflammatory cytokine biosynthesis. In mice models, lack of MK2 was associated with resistance to LPS-induced endotoxic shock, while levels of TNFα, IL-6, and interferon-γ were reduced. Such MK2-deficient mice are also known to be resistant to inflammatory diseases such as arthritis.

Mechanism of Action. Activation of MK2 by p38–MAPK overall leads to increased translation and stability of mRNAs of pro-inflammatory cytokines such as IL-6 and TNFα. Typical anti-MK2 agents are ATP-competitive inhibitors that preclude the enzyme's ability to phosphorylate target substrates. The ATP-competitive CC-99677 adopts a novel two-step mechanism of activation involving (1) binding to the ATP site and (2) utilization of a nucleophilic aromatic substitution reaction between an electrophilic chloropyrimidine moiety and cysteine 140, establishing a covalent thioether bond. In contrast to typical p38MAPK inhibitors, CC-99677 was found to minimally inhibit IL-1β but strongly inhibit monocyte chemoattractant protein 1 (MCP-1), which is suspected of playing a role in the feedback loop that dampens the anti-inflammatory effects of p38–MAPK inhibitors.

Hit-Finding Strategy. Inspired by a previously reported MK2 inhibitor, PF-3644022, the focus was to solve the biochemical efficiency (BE) problem previously encountered with orthosteric inhibitors of the kinase. Examination of X-ray crystal structures of inhibitors bound to MK2 helped guide the design of new compounds and to propose new potent ATP-competitive compounds with an electrophilic moiety capable of covalent linkage. "Compound 1" became their initial hit.



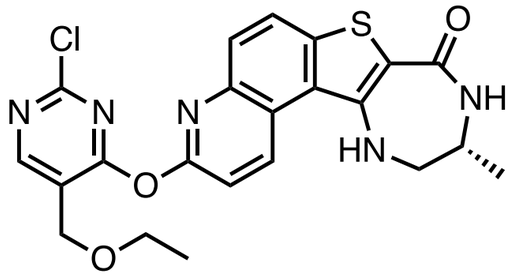
PF-3644022



compound 1

CC-99677

MK2



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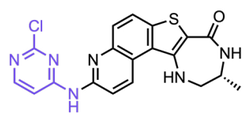
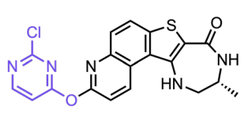
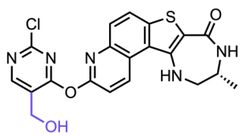
Lead Optimization. Molecular docking studies indicated that an *N*-linked ("compound 1") or *O*-linked chloropyrimidine ("compound 3") would place the reactive C-Cl bond proximal to Cys140. Although "compound 1" was fairly potent, it demonstrated little to no covalent addition in MALDI MS. Furthermore, compounds in the *N*-linked series generally had poor PK properties. Much of this was remedied by switching to the *O*-linked series; however, this improvement came at the cost of decreased microsomal and physical stability. Introduction of the benzylic alcohol in "compound 5" improved metabolic stability, but there was a noted decrease in permeability and cellular potency, most likely due to the polarity of the hydroxyl. Capping the alcohol as an ether provided a good balance among potency, reactivity, and PK properties, leading to CC-99677.



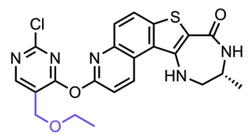
compound 1
MK2: IC_{50} = 35 nM
pHSP27: EC_{50} = 1350 nM
BE: IC_{50}/EC_{50} = 0.03



compound 3
MK2: IC_{50} = 53.9 nM
pHSP27: EC_{50} = 104 nM
BE: IC_{50}/EC_{50} = 0.52



compound 5
MK2: IC_{50} = 115.4 nM
pHSP27: EC_{50} = 610 nM
BE: IC_{50}/EC_{50} = 0.19



CC-99677
MK2: IC_{50} = 156.3 nM
pHSP27: EC_{50} = 89 nM
BE: IC_{50}/EC_{50} = 1.76

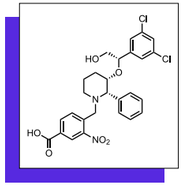
Preclinical Pharmacology. The compound at 1 μM was found to have off-target activity (>80%) against 11 kinases and >50% against 22 kinases in a 364-kinase panel screen. However, in cellular experiments, no functional inhibition of these off-target kinases was observed. In vivo studies were done in a rat model of ankylosing spondylitis and paw swelling. At oral doses of 20 or 100 mg/kg for 35 days, the compound mediated a reduction in paw swelling of 41% and 65%, respectively.

Clinical Development. Celgene completed four Ph. I studies, with two more Ph. I studies expected to be launched soon. In healthy participants (NCT03554993), single ascending oral doses ranging from 3 to 400 mg were demonstrated to be safe without dose-related trends in clinical laboratory results, vital signs, or electrocardiogram results. The adverse events can be summarized by symptoms like mild somnolence and mild nausea at a 100 mg dose level. In 2 cases at the 400 mg dose level, transient rashes without other organ involvement were reported. Celgene is now starting to recruit into a Ph. II study (NCT04947579) with ankylosing spondylitis patients. Dose regimens of 60 and 150 mg PO QD will be employed to assess the efficacy.

Patent. The MAPK-activated protein kinase-2 (MK2) inhibitor CC-99677 was patented by Celgene (WO2016044463A2), with patent rights granted until September 2035 (US9458175B2).

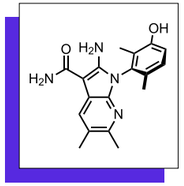
Small Molecules of the Month

July 2022
drughunter.com



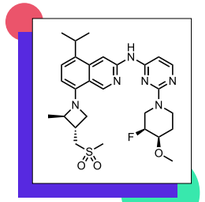
Compound 33 | PKG1α

intravenous PKG1α activator
satisfactory in vivo rat PK profile
from 2.9 M compounds HTS, SBDD and opt
J. Med. Chem.
Merck & Co., Inc., South San Francisco, CA



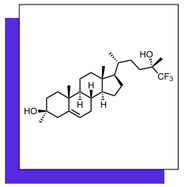
RP-6306 | PKMYT1

oral PKMYT1 inhibitor
three Ph. I studies currently ongoing
initial screen of 560 known kinase inhibitors
J. Med. Chem.
Repare Therapeutics, Inc., Ville St- Laurent, CA



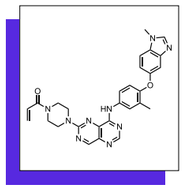
BLU-945 | EGFR$^{T790M/C797S}$

oral EGFR mutant inhibitor
Ph. I/II candidate oncology
>25k compound library screening and opt
J. Med. Chem.
Blueprint Medicines, Cambridge, MA



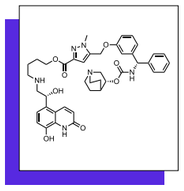
SAGE-718 | NMDAR

oral NMDAR positive allosteric modulator
Ph. II candidate in neurology
from previously disclosed PAM and opt
J. Med. Chem.
Sage Therapeutics, Inc., Cambridge, MA



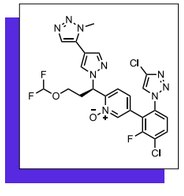
BI-4142 | HER2 exon 20 insertion

oral HER2 exon 20 mutants selective inhibitor
tumor regression in the HER2^{YVMA} xenograft model
from 12k compound in-house library screening and opt
Nat. Cancer
Boehringer Ingelheim RCV, Vienna, AT



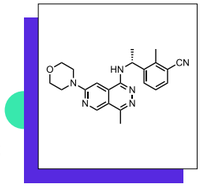
CHF-6366 | mACHrs and ADRB2

inhaled mACHrs and ADRB2 agonist
Ph. I/II candidate in Asthma and COPD discontinued
soft drug design from previously disclosed lead MABA
J. Med. Chem.
Chiesi Farmaceutici S.p.A, Parma, IT



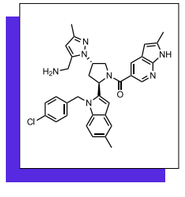
Compound 3f | FXIa

oral Factor XIa inhibitor
IV efficacy in rabbit AV shunt model
from literature starting point and SBDD
J. Med. Chem.
Janssen Research & Development, L.L.C., Spring House, PA



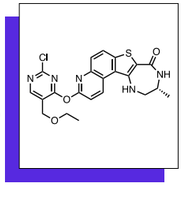
MRTX0902 | SOS1:KRAS^{G12C} PPI

oral brain-penetrant SOS1:KRAS^{G12C} PPI inhibitor
efficacy in MIA PaCa-2 tumor mouse xenograft model
from literature starting point and opt
J. Med. Chem.
Mirati Therapeutics, San Diego, CA



Compound 14 | NNMT

elective NNMT inhibitor
100-times higher inhibitory activity than original peptide
peptide library screening, de novo design and SBDD
J. Med. Chem.
Shionogi Pharmaceutical Research Center, Toyonaka, JP



CC-99677 | MK2

oral, covalent MK2 inhibitor
Ph. II candidate in ankylosing spondylitis
from previously disclosed MK2 inhibitor and SBDD
Transl Res
Bristol Myers Squibb, Princeton, NJ

discover together

drughunter.com
info@drughunter.com