Spiroazetidines in Drug Discovery

Key Points

- Offer more interactions with target proteins
- Provide superior physiochemical properties
- Offer novel intellectual properties

Overview

Spiroazetidines, like all spirocyclic scaffolds, are bestowed with three advantages over their flat counterparts: inherent three-dimensional structures may offer more interactions with target proteins; spiroazetidines may provide superior physiochemical properties thus more drug-like; and novel structures may offer fresh intellectual properties. Meanwhile, although no spiroazetidine-containing drugs are current approved for marketing, at least two of them have been advanced to clinical trials. Their applications in medicinal chemistry are destined to grow, especially since many of them are now commercially available.

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Spirocycles are making more and more frequent appearances in drugs as both core structures and peripheries of drug molecules. They have at least three advantages as drug fragments:

a. Spirocyclic scaffolds are inherently three-dimensional and can project functionalities in all three dimensions to interact more extensively with the protein target of interest and lower off-target effects in comparison to their two-dimensional counterparts;

b. They are sp^3-carbon-rich with high value of *fraction of saturated carbon* (Fsp^3) and sp^3-carbon-rich molecules are correlated with favorable physiochemical properties such as higher aqueous solubility;[1] and

c. Spirocycles often offer new chemical space to create novel intellectual properties, as amply documented by Zheng et al.[2]

With regard to bicyclic spiroazetidines, the other ring may be fused to azetidine at either the α- or β-position of the nitrogen atom. Overwhelming examples in the literature are the β-fused azetidine spirocycles.

β-fused azetidines α-fused azetidines

Spiroazetidine-containing Drugs

Only one spiroazetidine-containing drug is on the market in Japan: delgocitinib (Corectim, **1**). Discovered by Japanese Tobacco, it is a pan-JAK inhibitor approved as a topical treatment of atopic dermatitis.

Janus kinases (JAKs) recruit signal transducers and activators of transcription (STATS) to cytokine receptors, leading to modulation of gene expression. They are intracellular tyrosine kinases that mediate the signaling of numerous cytokines and growth factors involved in the regulation of immunity, inflammation, and hematopoiesis. There are four members of the Janus kinase family: JAK1, JAK2, JAK3, and TYK2.

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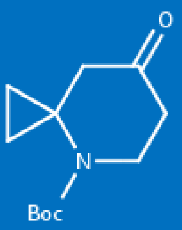
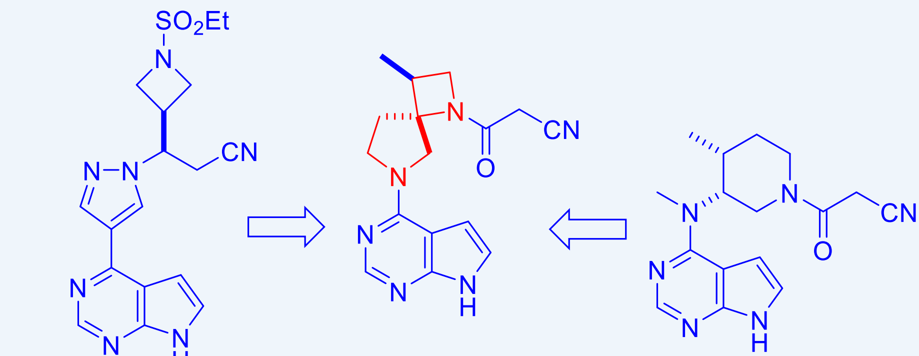


PBZS2138

Incyte′s JAK1/2 dual inhibitor baricitinib (Olumiant, **2**) was approved in 2017 for treating rheumatoid arthritis (RA). Also marketed as a treatment of RA since 2012, Pfizer′s tofacitinib (Xeljanz, **3**) is a JAK1/JAK3 inhibitor with moderate activity on JAK2.

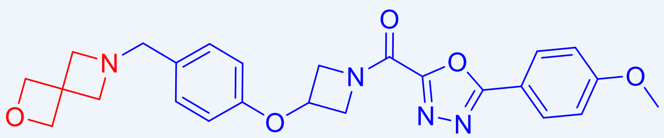
Drug discovery is extremely hard. Yet it can be comparatively easy if you know what you are doing, especially in the field of “me-too” drugs, also known as “patent busting” or euphorically “scaffold hopping” tactics. Pharmaceutical industry flourished on the tail of “me-too” beta blockers. Japanese Tobacco wisely infused features from both baricitinib (**2**) and tofacitinib (**3**) and arrived at delgocitinib (**1**) with a spirocyclic azetidine-pyrrolidine (1,6-diazaspiro[3.4]-octane) fragment.[3] The spiroazetidine moiety closely mimics the three-dimensional head pieces of its progenies: baricitinib (**2**) and tofacitinib (**3**). The discovery of delgocitinib (**1**) showcased the third merit for spirocycles: creating novel IPs.



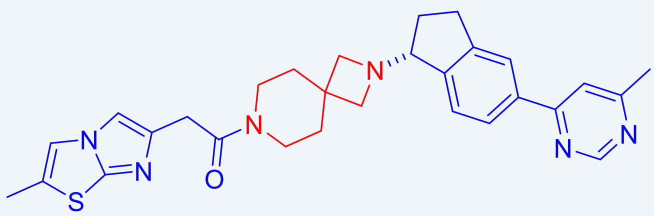
baricitinib (Olumiant, **2**)
Incyte/Lilly, 2017 (for RA)
JAK1/2 inhibitor

delgocitinib (Corectim, **1**)
Japanese Tobacco
2020 for atopic demotitis
pan-JAK inhibitor

tofacitinib (Xeljanz, **3**)
Pfizer, 2018 (for RA)
JAK1/3 inhibitor



AZD1979 (**4**)
AstraZeneca
MCHr1 antagonist



PF-5190457 (**5**)
inverse agonist of
ghrelin receptor

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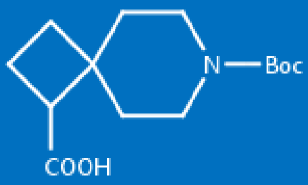
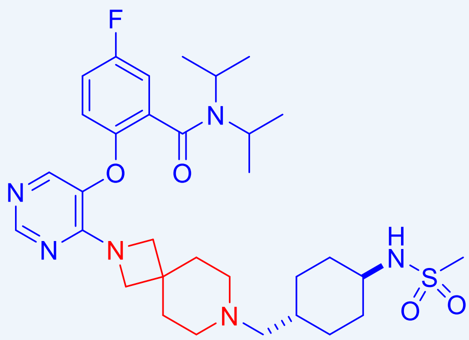


PBZ3122

At least three spiroazetidine-containing drugs have been advanced to clinical trials. One is AstraZeneca′s melanin concentrating hormone receptor 1 (MCHr1) antagonist AZD1979 (**4**) for the treatment of diabetes.[4] Its peripheral azetidine-oxetane (2-oxa-6-azaspiro[3.3]-heptane) spirocycle is an isostere for morpholine. Another spiroazetidine-containing drug in clinical trials is Pfizer′s inverse agonist of the ghrelin receptor (GR) PF-5190457 (**5**) for treating obesity.[5] It has a spirocyclic azetidine–piperidine backbone. The third spiroazetidine-containing drug in clinical trials is Vitae′s VTP50469 (**6**) for treating leukemia.[6] It is an inhibitor of the menin and MLL interaction. It also has a spirocyclic azetidine–piperidine framework. Its three-dimensional structure is a good solution for the challenge of tackling protein–protein interactions.



VTP50469 (**6**)
Inhibitor of
menin/ MLL
nteraction

Spiroazetidines in Drug Discovery

a. Spiroazetidines as bioisosteres

Spiroazetidines, like all spirocyclic scaffolds, are inherently three dimensional and offer structural novelty. In terms of isosterism, 2,6-diazaspiro[3.3]heptane can mimic piperazine and 2-oxa-6-azaspiro[3.3]heptane bears striking resemblance to morpholine. In the same vein, 2-azaspiro[3.3]heptane has served as an excellent isostere of piperidine and has found several important applications in drug discovery. Therefore, these spiroazetidines have found utility in drug discovery as isosteres for piperazine, morpholine, and piperidine, respectively.

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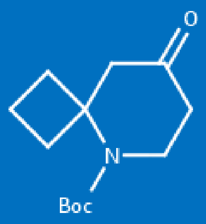

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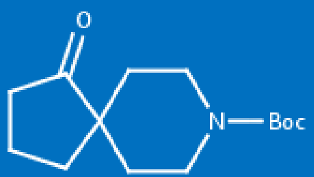

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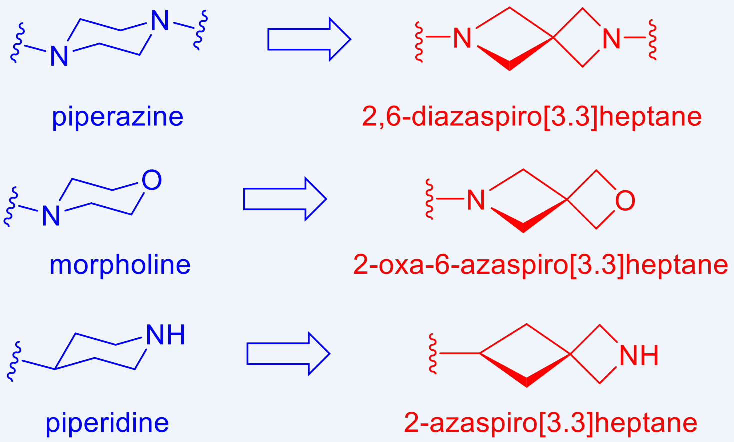

Inhibition of ketohexokinase (KHK, also know as fructokinase) promotes fructose metabolism thus KHK inhibitors have potential as diabetes treatment. From high-throughput screening (HTS) and further optimization guided by structure-based drug design (SBDD), Maryanoff and coworkers arrived at pyrimidinopyrimidine piperazine **7** as a potent, selective human hepatic KHK (KHK-C isoform) inhibitor. Since piperazine is considered a conformational constrained isostere for ethyldiamine, 2-oxa-6-azaspiro[3.3]heptane is even further constrained than piperazine. Along this line of speculation, spiroazetidine analog **8** was prepared and tested to have similar enzymatic potency in inhibiting recombinant human hepatic KHK-C as piperazine **7**, indicating that conformational rigidity is tolerated for the structure-activity relationship (SAR) for this pyrimidinopyrimidine series of KHK-C selective inhibitors. Furthermore, both piperazine **7** and spiroazetidine **8** exhibited reasonably potent cellular KHK inhibition (400 and 360 nM, respectively), which relates to their intrinsic potency versus KHK and their ability to enter cells.[7]

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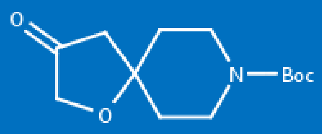

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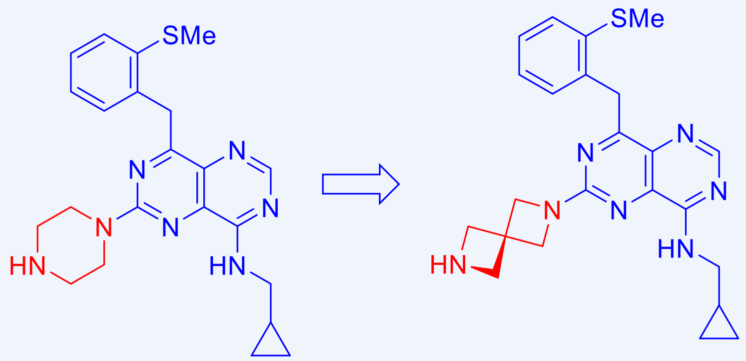

piperazine **7**
KHK-C, IC_{50} = 12 nM
cellular, IC_{50} = 400 nM

spiroazetidine **8**
KHK-C, IC_{50} = 8 nM
cellular, IC_{50} = 360 nM

Like KHK inhibitors, stearoyl-CoA desaturase (SCD) inhibitors are also potential treatments of metabolic disorders such as diabetes and obesity. To minimize adverse events associated with eyes and skin, liver-targeted SCD inhibitors are preferred. Merck Frost arrived at piperazine **9** as a liver-selective SCD inhibitor with an enzymatic potency of 28 nM against rat SCD. The strategic presence of polar acid moiety was important because carboxylic acids and tetrazoles are recognized organic anionic transporter proteins (OATPs), thus providing the desired *in vivo* properties, i.e., a high liver concentration (target organ for efficacy) and a low systemic concentration to minimize exposures in off-target tissues and cell-associated adverse events (eyes and skin).[8]

Spiroazetidine isostere **10** has a similar enzymatic potency as its prototype **9**. Moreover, both of them are also virtually inactive in a whole cell assay in a human hepatocellular carcinoma line (HepG2), indicating that both spiroazetidine **10** and piperazine **9** do not enter off-target cells via passive diffusion since HepG2 cells are devoid of active OATPs. Gratifyingly, both spiroazetidine **10** and piperazine **9** do cross cell membranes through active transporters when assessed in a rat hepacyte (Rat Hep, which contains functional OATPs) assay.[8]

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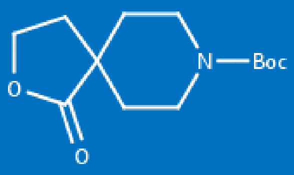

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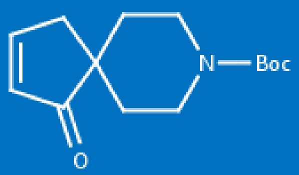

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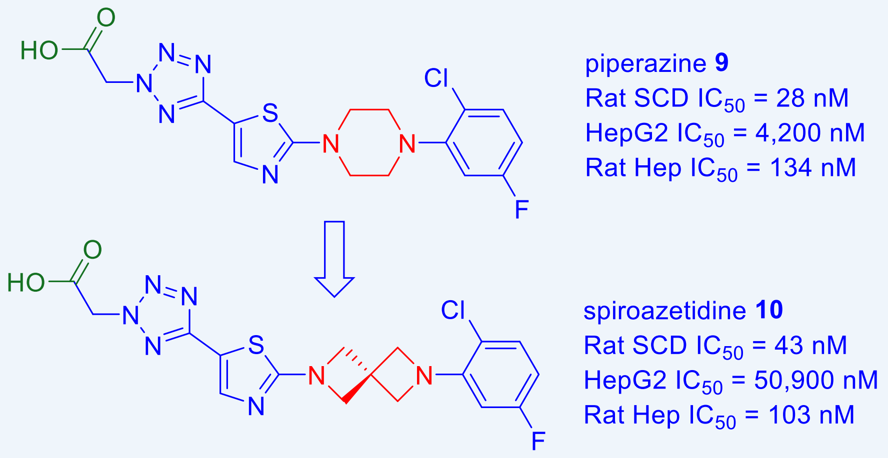
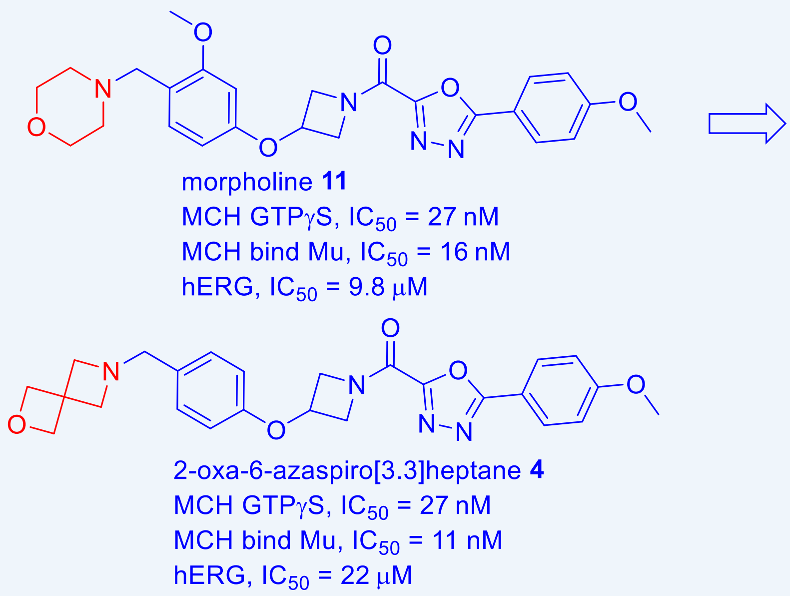


Continuing the theme of diabetes treatments, MCHr1 antagonists have been explored for weight control. AstraZeneca discovered a series of oxadiazole-containing compounds as represented by morpholine **11**. Their efforts culminated in clinical candidate AZD1979 (**4**) with a novel peripheral spiroazetidine moiety. Spiroazetidine **4** displayed appropriate lipophilicity for a CNS indication, showed excellent permeability with no efflux, and possessed good off-target selectivity, including hERG. Preclinical good-laboratory practice (GLP) toxicology and safety pharmacology studies were without findings and AZD1979 (**4**) was taken into clinical trials.[4]

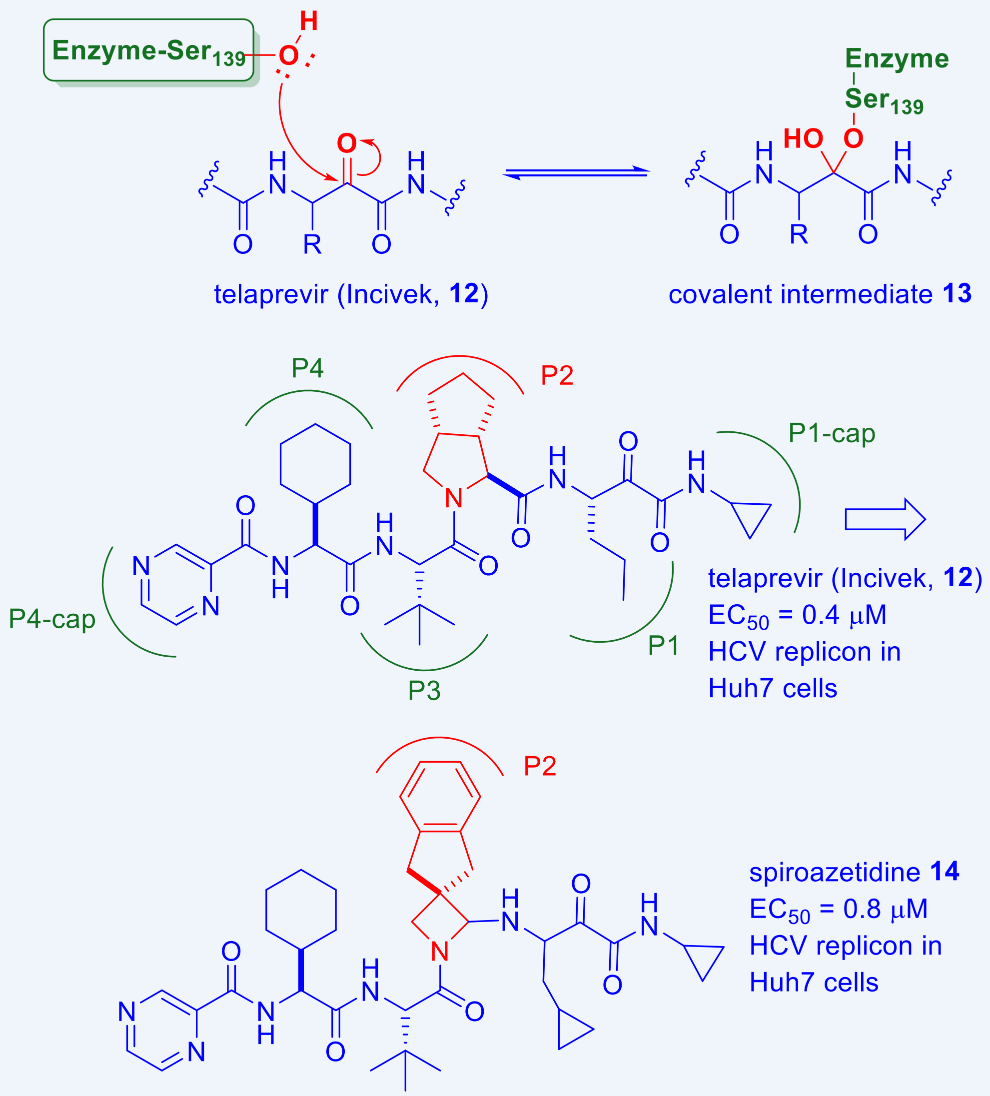


PharmaBlock Products

PBN20122077

PB08298

Simlar to Schering–Plough's boceprevir (Victrelis), Vertex's telaprevir (Incivek, **12**) is a hepatitis C virus (HCV) NS3/4A *serine* protease inhibitor. They are also competitive, covalent reversible inhibitors. Working in concert with His_{57} and Asp_{81}, the Ser_{139} on HCV NS3/4A serine protease enzyme adds to the ketone warhead on telaprevir (**12**) to form a covalent tetrahedral hemiacetal intermediate **13**, which closely mimics the transition state of the hydrolysis processes by the serine protease.[9] Introduction of spiroazetidine moieties at the P2 unit of telaprevir (**12**) resulted in inhibitors with good potency as measured using inhibition of HCV RNA replication in Huh7 cells in a subgenomic HCV replicon system. In particular, spiroazetidine **14** displayed a potency (EC_{50} = 0.8 μM) similar to that of telaprevir (**12**, EC_{50} = 0.4 μM).[10]



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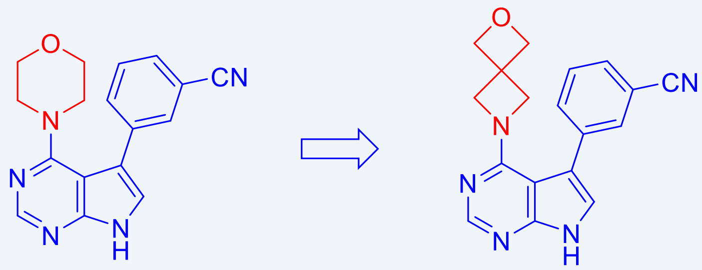
PB97358-1

Not surprisingly, not all spiroazetidine isosteres worked as a panacea.

Leucine rich repeat kinase 2 (LRRK2) inhibitors are potentially useful in the treatment of Parkinson′s disease (PD). From HTS and lead optimization, Pfizer arrived at morpholine **15** (PF-06447475) as a highly potent (LRRRK2 enzymatic assay: IC_{50} = 3 nM), selective, brain penetrant, and *in vivo* active LRRK2 inhibitor. Attempt to replace the morpholino group with the 2-oxa-6-azaspiro[3.3]heptane isostere led to spiroazetidine **16**, which was virtually inactive toward LRRK2, regrettably.[11]



morpholine **15**
LRRK2 IC_{50} = 3 nM

2-oxa-6-azaspiro[3.3]heptane **16**
LRRK2 IC_{50} >2,300 nM

b. Utility of spiroazetidines in medicinal chemistry

Spirocycic azetidine–piperidine (2,7-diazaspiro[3,5]nonane) fragment served as the backbone of a series of inverse agonists of the ghrelin receptor (GR) discovered by Pfizer.[12,13,5]

GR is a G-protein coupled rececptor (GPCR) that plays a role in obesity and glucose homeostasis. Starting from an HTS hit, azetidine–piperidine **17** was discovered as a potent GR *inverse agonist* (hGR IC_{50} = 4.6 nM, K_i = 7.0 nM, an *inverse agonist* is a drug that binds to the same receptor as an agonist but induces a pharmacological response opposite to that of the agonist) with 43% bioavailability in Sprague–Dawley rat. But it suffered from an undesired off-target effect; namely it ddisplayed muscarinic acetylcholine receptor (mAChR) M2 activity (K_i = 269 nM).[12]

Conformational restriction of the right-hand portion of compound **17** led to chiral indane **18**, which maintained its potency (hGR pK_i= 8.2, i.e., K_i = 6.3 nM) with improved selectivity over mAChR M2 (pK_i = 4.85, i.e., K_i = 14.1 μM) as well as high receptor occupancy.[13]

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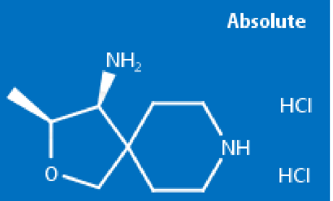

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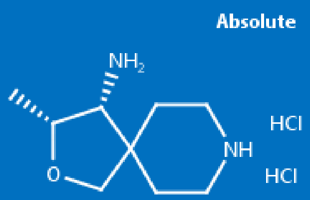

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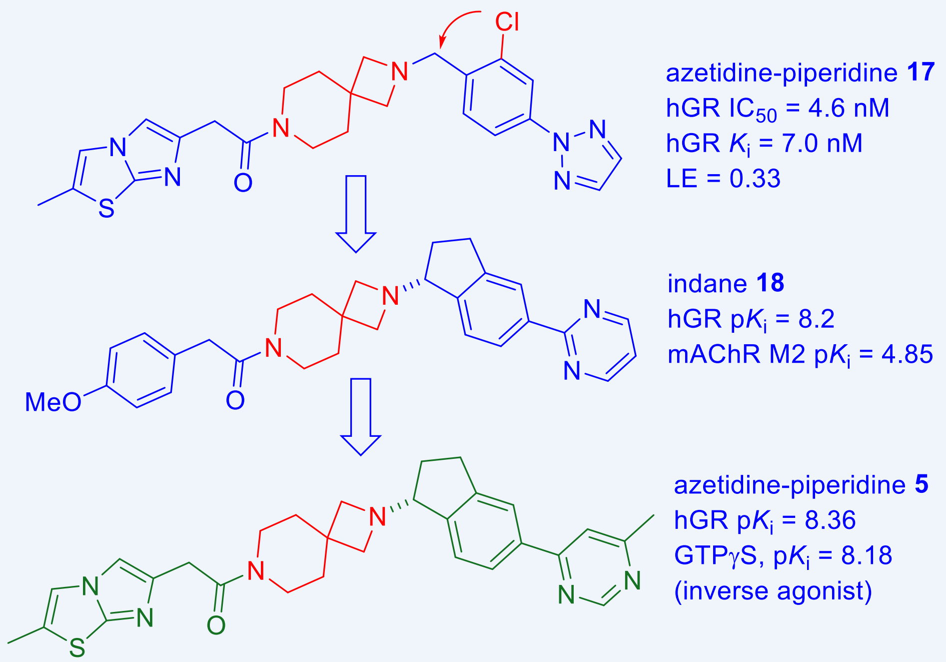
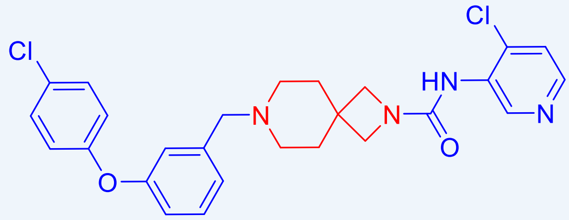
Eventually, meticulous fine-tuning of the SAR culminated in PF-5190457 (**5**, pK_i = 8.36, i.e., K_i = 4.4 nM), which showed a better balance of receptor activity and off-target selectivity (M2 K_b/GR K_i ratio = 266). As a potent, selective, and orally bioavailable GR inverse agonist, azetidine–piperidine **5** was advanced to clinical trials for the treatment of diabetes on the basis of its promising pharmacological and safety profile.[5]



The azetidine–piperidine (2,7-diazaspiro[3,5]nonane) fragment also found success in the fatty acid amide hydrolase (FAAH) inhibitors program as well. FAAH is an integral membrane serine hydrolase responsible for the degradation of fatty acid amide signaling molecules such as endocannabinoid anandamide (AEA), which has been shown to possess cannabinoid-like analgesic properties. Therefore, FAHH inhibitors are explored as treatment of pain. A group of chemists at Janssen prepared heteroarylurea FAHH inhibitors with dozens of diamine linkers. One of them, 2,7-diazaspiro[3,5]nonane **19**, was found to be an potent FAHH inhibitor (hFAHH IC_{50} = 8 nM). In addition, it was found to inhibit FAAH centrally, elevate the brain levels of three fatty acid ethanolamides [FAAs: AEA, oleoyl ethanolamide (OEA) and palmitoyl ethanolamide (PEA)], and was moderately efficacious in a rat model of neuropathic pain.[14]



2,7-diazaspiro[3,5]nonane **19**
hFAHH IC_{50} = 8 nM

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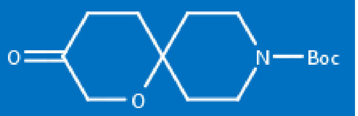

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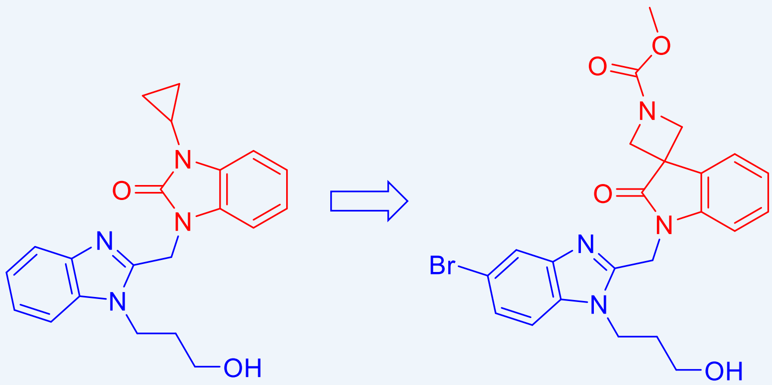
Respiratory syncytial virus (RSV) is a major cause of pneumonia and bronchiolitis in young children, immunocompromised adults, and elderly. Unfortunately, there is no effective treatment except an expensive monoclonal antibody (palivizumab) with a dubious safety profile. BMS's pyridinoimidazolone **20** is an efficacious RSV F fusion glycoprotein inhibitor. Using **20** as a starting point, a “patent-busting” or “scaffold-hopping” exercise led to the discovery of a series of novel spiroazetidine 2-oxo-indoline derivatives. The lead compound **21** exhibited excellent *in vitro* potency with an EC_{50} value of 0.8 nM and demonstrated 71% oral bioavailability in mice.[15]



pyridinoimidazolone **20**
RSV EC_{50} = 22 nM
CC_{50} >100 μM

spiroazetidine **21**
RSV EC_{50} = 0.8 nM
CC_{50} >100 μM
%*F* = 71%

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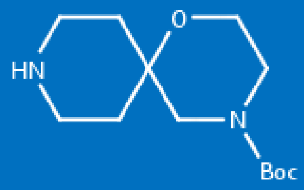

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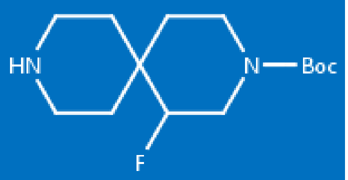

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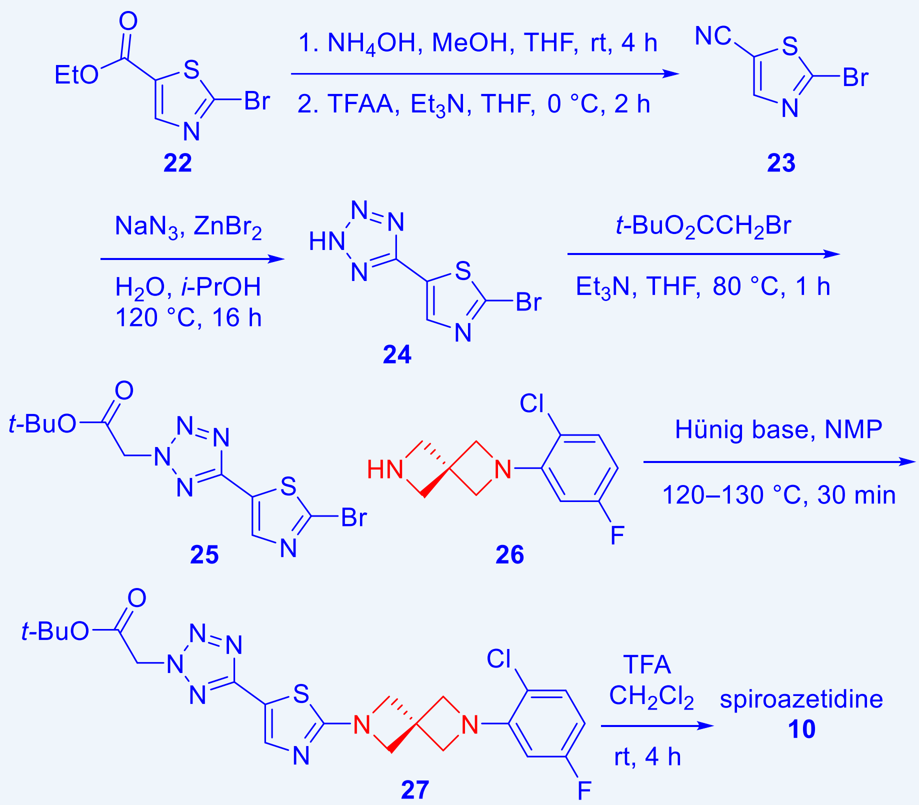
Synthesis of Some Spiroazetidines

Synthesis of Merck Frost's liver-selective SCD inhibitor spiroazetidine **10** commenced with preparation of bromothiazole-nitrile **23** from bromothiazole-ester **22** in two steps. Tetrazole **24** was then elaborated by condensation of bromothiazole-nitrile **23** with sodium azide. S_N2 alkylation of tetrazole **24** with *t*-butyl bromoacetate gave rise to ester **25**, which was followed by an S_NAr reaction with aryl-2,6-diazaspiro[3.3]heptane **26** to assemble the adduct **27**. TFA-promoted deprotection of the ester then delivered spiroazetidine **10**.[8]



AstraZeneca began synthesis of their MCHr1 antagonist clinical candidate AZD1979 (**4**) with a reductive amination of *p*-anisaldehyde with 2-oxa-6-azaspiro[3.3]heptane to prepare benzylamine **28**. Subsequently, a Mitsunobu reaction between benzylamine **28** and Cbz-protected azetidine **29** provided azetidine ether **30**. After deprotection, the resulting "naked" azetidine **31** was coupled with oxadiazole **32** with the aid of catalytic amount of sodium cyanide to deliver the desired 2-oxa-6-azaspiro[3.3]heptane **4**.[4]

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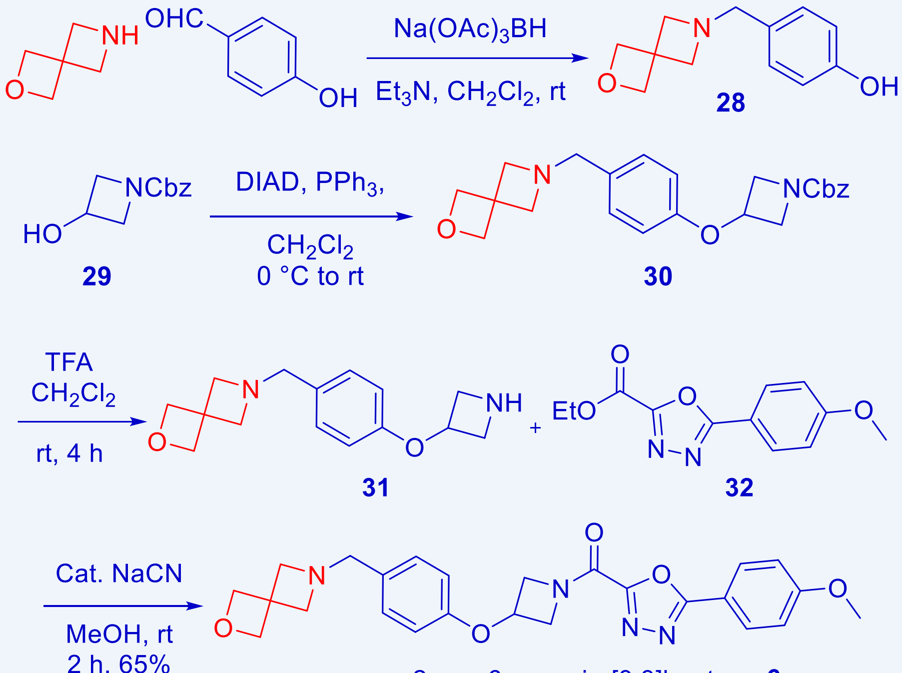

To produce GR inverse agonist PF-5190457 (**5**), Pfizer first carried out a reductive amination of chloroaldehyde **33** with indane-amine **34** to assemble azetidine–piperidine **35**. Scaffold **35** served as a linchpin-like modular framework for further functionalizations. Palladium-catalyzed Miyaura reaction between bromide **35** and bis(pinacolato)diboron produced boronate intermediate **36**, which underwent a Suzuki coupling with 4-bromo-6-methylpyrimidine to provide adduct **37**. After removal of the Boc protective group on **37**, the revealed azetidine–piperidine was coupled with the requisite acid to deliver PF-5190457 (**5**).[5]

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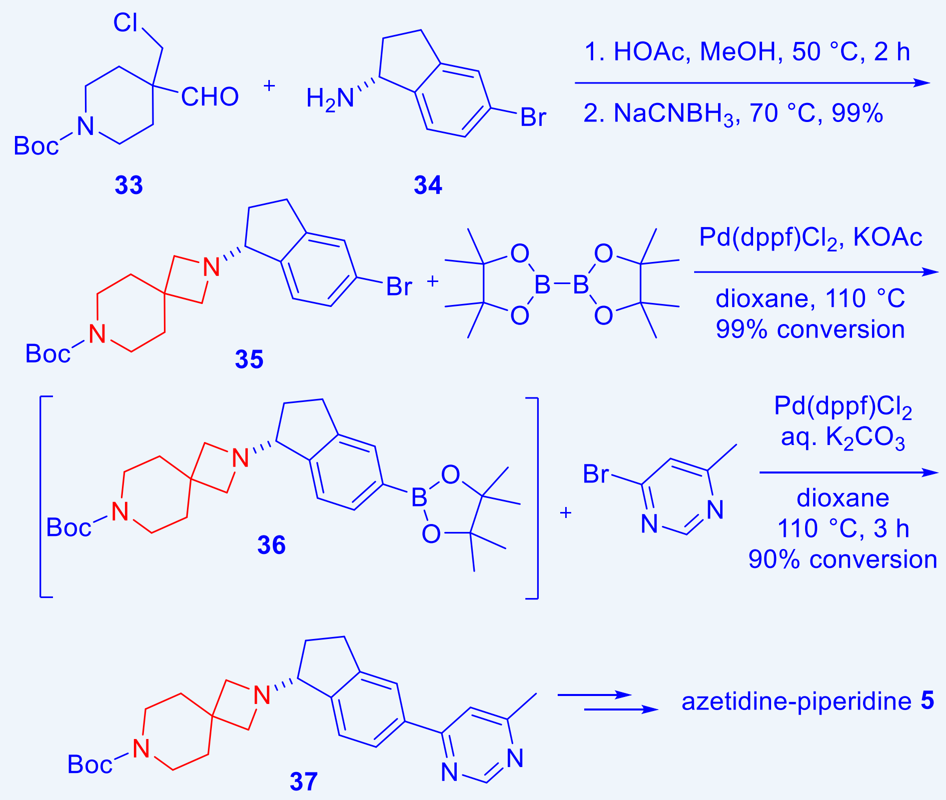

For spiroazetidine building blocks that are not commercially available, Carreira[16,17] and Mykhailik[18,19] have published excellent works on their preparations.

To conclude, spiroazetidines, like all spirocyclic scaffolds, are bestowed with three advantages over their flat counterparts: inherent three-dimensional structures may offer more interactions with target proteins; spiroazetidines may provide superior physiochemical properties thus more drug-like; and novel structures may offer fresh intellectual properties. Meanwhile, although no spiroazetidine-containing drugs are current approved for marketing, at least two of them have been advanced to clinical trials. Their applications in medicinal chemistry are destined to grow, especially since many of them are now commercially available.

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