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 currently 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.
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* (\( F_{sp^3} \)) 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 \( \alpha \)- or \( \beta \)-position of the nitrogen atom. Overwhelming examples in the literature are the \( \beta \)-fused azetidine spirocycles.

### 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.
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. The spiroazetidino 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.
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. 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. It has a spirocyclic azetidine–piperidine backbone. The third spiroazetidine-containing drug in clinical trials is Vitae’s VTP50469 (6) for treating leukemia. 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.

**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-diaza[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.
Inhibition of keto hexokinase (KHK, also known 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 conformationally 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
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 \textit{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.\(^8\)
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.\(^4\)
Similar 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 His57 and Asp81, the Ser139 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\(_{50} = 0.8 \, \mu M\)) similar to that of telaprevir (12, EC\(_{50} = 0.4 \, \mu M\)).\(^{10}\)
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.$^{11}$

![Structures](image)

**b. Utility of spiroazetidines in medicinal chemistry**

Spirocyclic 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 receptor (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 displayed 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.$^{13}$
Eventually, meticulous fine-tuning of the SAR culminated in PF-5190457 (5, $pK_i = 8.36$, i.e., $K_i = 4.4 \text{ nM}$), 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, FAAH inhibitors are explored as treatment of pain. A group of chemists at Janssen prepared heteroarylurea FAAH inhibitors with dozens of diamine linkers. One of them, 2,7-diazaspiro[3,5]nonane 19, was found to be an potent FAAH inhibitor ($h\text{FAAH} IC_{50} = 8 \text{ nM}$). 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.\(^{14}\)
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 \textit{in vitro} potency with an EC_{50} value of 0.8 nM and demonstrated 71% oral bioavailability in mice.\textsuperscript{15}
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$_N$2 alkylation of tetrazole 24 with t-buty1 bromoacetate gave rise to ester 25, which was followed by an S$_{\text{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.

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.
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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).
For spiroazetidine building blocks that are not commercially available, Carreira\textsuperscript{16,17} and Mykhailik\textsuperscript{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 currently 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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