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The use of spirocyclic scaffolds in drug discovery

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ABSTRACT

Owing to their inherent three-dimensionality and structural novelty, spiro scaffolds have been increasingly utilized in drug discovery. In this brief review, we highlight selected examples from the primary medicinal chemistry literature during the last three years to demonstrate the versatility of spiro scaffolds. With recent progress in synthetic methods providing access to spiro building blocks, spiro scaffolds are likely to be used more frequently in drug discovery.

One widely used strategy in drug design is to rigidify the ligand conformation by introducing a ring.[1] The resulting cyclic analog will suffer a reduced conformational entropy penalty upon binding to a protein target. In addition to ring fusion, conformational restriction can also be imposed by introduction of a spiro-ring fusion. Spiro compounds are molecules containing two rings with just one shared atom (the spiroatom). Spiro rings such as spiroketals are present in numerous natural products;[2] simple spiroketals are known insect pheromones. A small number of spiro containing drugs has been investigated during the last several decades;[3] a few examples are shown in Figure 1. Recent progress on new synthetic routes to spiro building blocks will facilitate incorporation of spiro scaffolds into more pharmaceutically active molecules. Spiro containing systems not only have greater three-dimensionality than flat aromatic compounds, but also introduce structural novelty for patentability. Even though both spirocyclic and flat aromatic rings can impact ligand binding entropy, it has been suggested that compounds with too many flat rings have suboptimal physical properties and are less likely to be successfully developed as drugs.[4] As a result, spiro compounds have increasingly appeared in the recent literature.[5]

In this review, selected examples from medicinal chemistry literature published within the past three years (2011- March 2014) will be presented to illustrate the utility of spirocyclic scaffolds in drug discovery.

3-Membered spirocyclic systems: Cyclopropane and oxirane can be incorporated into spiro scaffolds, for example compounds **1**–**6** (Fig. 2). Even though the N–H and N–Me forms of aziridines are present in a number of bioactive natural products such as mitomycins, it is generally difficult to construct such aziridines from unfunctionalized olefins. A recent report of a facile synthetic route to N–H and N–Me aziridines will enable their incorporation into drug molecules.[6]

Free fatty acid receptor 1 (FFA1, or GPR40), is a GPCR highly expressed in pancreatic β-cells and is considered to be an attractive target for type 2 diabetes. Two spiro FFA1 agonists have been reported recently.[7,8] Compound **1a** (AM-5262) is a full agonist of FFA1 with an EC_{50} of 0.081 μM.[7] As compared to the unconstrained early lead AM-1638 (**1b**), the introduction of the spiro constraint improved the potency for FFA1 by twofold, and enhanced the off-target selectivity when tested at 10 μM against a panel of 101 GPCRs, ion channels, transporters, and enzymes. AM-5262 demonstrated >90% inhibition of only one member of this panel, while the non-spirocyclic AM-1638 inhibited four targets. AM-5262 has reasonable rat PK (half-life = 4.2 h; clearance = 0.25 L/h/kg; *F*% = 28) and showed enhanced glucose stimulated insulin secretion and improved glucose homeostasis in vivo. Astellas disclosed another FFA1 agonist, compound **2** (AS2575959), and described its effect on glucose metabolism and potential synergy with a dipeptidyl peptidase-IV (DPP-IV) inhibitor, sitagliptin.[8] The demonstrated synergistic effect on the glucose dependent insulin secretion and GLP-1 concentration increase appears to be the first between a FFA1 agonist and a DPP-IV inhibitor. The structural resemblance between the Amgen and Astellas compounds (**1a** and **2**, respectively) is quite apparent. However, the spiro ring fusion patterns are different.

Sudemycin D6 (**3**), was shown to be a modulator of pre-mRNA splicing with potent cytotoxic activity in various tumor cell lines (IC_{50} = 39 nM for SK-MEL-2, 22 nM for JeKo-1, 50 nM for HeLa, and 81 nM for SK-N-AS cell lines).[9] Compound **4** is a potent, once-daily oral NS5A inhibitor against HCV infection (EC_{50} = 31 pM

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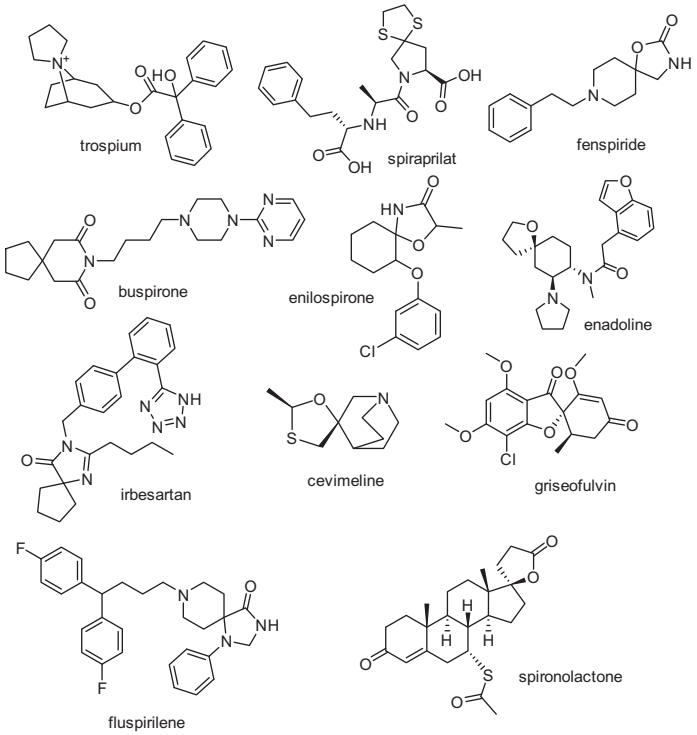

Figure 1. Some marketed drugs containing spirocyclic scaffolds.

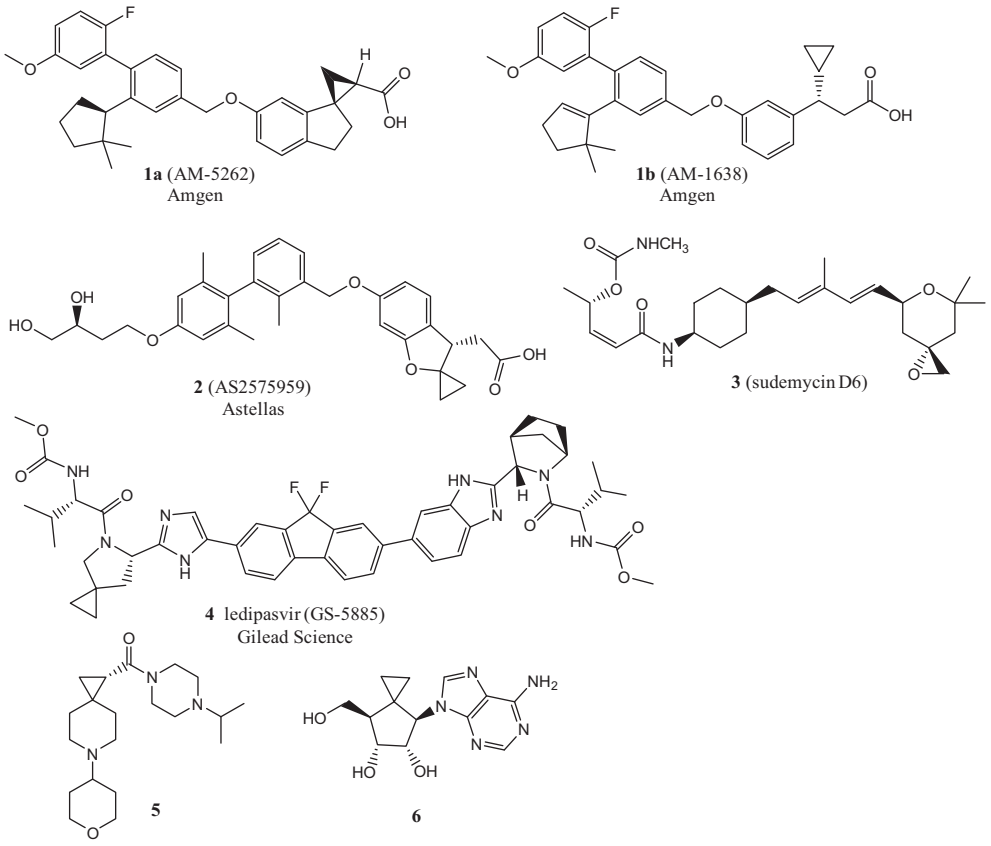

Figure 2. Spirocyclic compounds incorporating 3-membered rings.

against genotype 1a replicon) from Gilead.[10] It has a plasma half-life of 37–45 h in healthy volunteers, and phase 2 trials showed **4** to be very safe and well tolerated. Recently, a novel spiro antagonist (**5**) of histamine-3-receptor (H3R) was reported.[11] Compound **5** is potent against H3R (IC_{50} = 8.3 nM; hERG >33 μM) and selective over a panel of 144 secondary pharmacological receptors.

Spirocyclic nucleoside analog **6** was reported to demonstrate significant antiviral activity against HCV (EC_{50} = 0.273 μM, and 0.368 μM for genotypes 1A and 1B, respectively).[12]

4-Membered spirocyclic systems: Compounds **7**–**17** (Figs. 3 and 4) are recent examples of spirocyclic scaffolds incorporating 4-membered rings. Among nucleoside modifications that have been explored by Pharmasset and Janssen as anti-HCV compounds are 2′-spirocyclic ethers.[13,14] Spirocyclic 2′-oxetane guanosine triphosphate **7** inhibits both wild-type and S282T mutant RNA polymerase, NS5B, with an IC_{50} of 9.5 μM against wild-type NS5B polymerase and 10.4 μM for S282T mutant.[13] Phosphoramidate prodrugs of 2′-deoxy-2′-spirooxetane cytidine and uridine (e.g., **8**) exhibited EC_{50} values from 0.2 μM to >98 μM in the Huh7-replicon cell line without apparent cytotoxicity.[14]

In recent years, oxetanes have attracted attention in the drug discovery community.[15] HCV NS3 protease inhibitors represent another type of therapy for treatment of hepatitis C viral infection. Spiroazetidine **9** is a less potent analog of boceprevir with an IC_{50} = 0.8 μM against NS3 protease.[16]

Spirooxetane **10** is a potent inhibitor of RSV (respiratory syncytial virus) A polymerase (replicon EC_{50} = 10 nM).[17] Additional investigations indicated that **10** was rapidly absorbed into the systemic circulation following intratracheal dosing. Therefore insufficient compound was retained in the lung and bronchoalveolar lavage fluid, suggesting further improvement is still needed.

Compounds **11**–**13** (Fig. 4) are 2,7-diazaspiro[3,5]nonane inverse agonists of ghrelin receptor (GR), a GPCR target that plays a role in obesity and glucose homeostasis.[18] Compound **11** (hGR IC_{50} = 4.6 nM) has an undesired off-target effect; specifically it demonstrated muscarinic acetylcholine receptor (mAChR) M2 activity (pK_i = 6.57 (or K_i = 269 nM)).[18a] Cyclization near the azetidine nitrogen yielded **12** (hGR pK_i = 8.2 (or K_i = 6.3 nM)) with improved selectivity over mAChR M2 (pK_i = 4.85 (or K_i = 14125.4 - nM)) as well as high receptor occupancy.[18b] A further optimized compound (**13**, PF-5190457, pK_i = 8.36 (or K_i = 4.4 nM)) showed a better balance of receptor activity and off-target selectivity (M2 K_b/ghrelin receptor K_i ratio of 266).[18c]

Compound **14** is an inhibitor of fatty acid amide hydrolase (hFAAH apparent $IC_{50} \sim$ = 8 nM).[19] Spiro compound **15** is a very potent inhibitor (IC_{50} = 3.3 nM) of histone methyltransferase G9a with excellent selectivity of >1000-fold over 21 other methyltransferases.[20]

Structure-based design has been successfully applied to discover several types of potent inhibitors for BACE1 (β-site APP-cleaving enzyme 1), which has been a subject of investigations as a target for Alzheimer's disease.[21] The design of potent BACE1 inhibitors which are also brain penetrant has been a major challenge for many research teams. Amgen reported a series of hydoxyethylamine (HEA) based BACE1 inhibitors (e.g., **16** and **17**) that lower Aβ levels in rat brains after oral administration.[22,23] Compound **16** is a potent (IC_{50} = 5.5 nM in FRET assay, 9.6 nM in cell), permeable inhibitor with low rat P-glycoprotein (P-gp) efflux ratio of 1.7. These compounds are also potent CYP3A4 inhibitors (e.g., CYP3A4 IC_{50} for **17** is 50 nM);[22] potent CYP3A4 inhibition is generally undesirable for a drug molecule.

5/5 and 5/6 spiro-ring fused systems: The majority of the spirocyclic scaffolds described in the literature incorporate 5- and 6-membered ring systems. Using an X-ray based fragment screen, scientists at Pfizer identified BACE1 inhibitors with a spiropyrrolidine scaffold; further structure-based optimization led to a 1 μM inhibitor (**18**, Fig. 5).[24] Compound **19** was reported to be a potent antagonist (IC_{50} = 8.6 nM) of the protease activated receptor 1 (PAR1), which plays an important role in thrombin mediated platelet aggregation.[25] However, the current structures (**19** and analogs) were metabolized quickly and further improvements are required.

The racemic 1-thia-4,7-diaza-spiro[4.4]nonane-3,6-dione containing compound **20** is a potent antagonist of 5-hydroxytrptamine 6 (5-HT_6) with K_i of 26 nM. The more potent enantiomer of **20** had a K_i of 15 nM against 5-HT_6 with good selectivity over other members of the serotonin family.[26]

Small molecule protein/protein interaction inhibitors which disrupt the MDM2/p53 interaction have been a subject of clinical investigations.[27] Wang's group at the University of Michigan has discovered a class of spirooxindole-based inhibitors.[28] Their optimization efforts led to the discovery of compound **21** (MI-888), which showed remarkable activity in xenograft models of human cancer after oral administration and appears to be the most efficacious MDM2 inhibitor (K_i = 0.44 nM) reported so far. Spirooxindole-3,3′-thiazolidine containing compound **22** (IC_{50} = 40 nM) also showed interesting activity in human tumor cell lines.[29] Compound **23** represents another class of inhibitors based on the spiroisoxazoline oxindole scaffold; the disruption of the p53/MDM2 complex was demonstrated in a live-cell biomolecular fluorescence complementation assay.[30] Compounds **24** (RO2468) and **25** (RO5353) are potent (IC_{50} = 6 nM and 7 nM, respectively), orally active MDM2/P53 antagonists from Roche that are clearly structurally related to **21** by replacement of a benzene ring of the oxindole

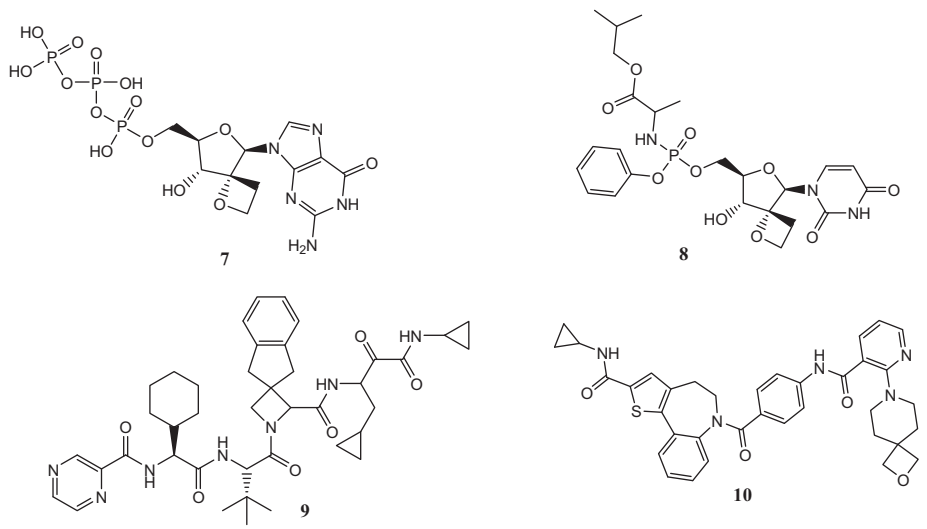

Figure 3. Spirocyclic compounds containing 4-membered rings.

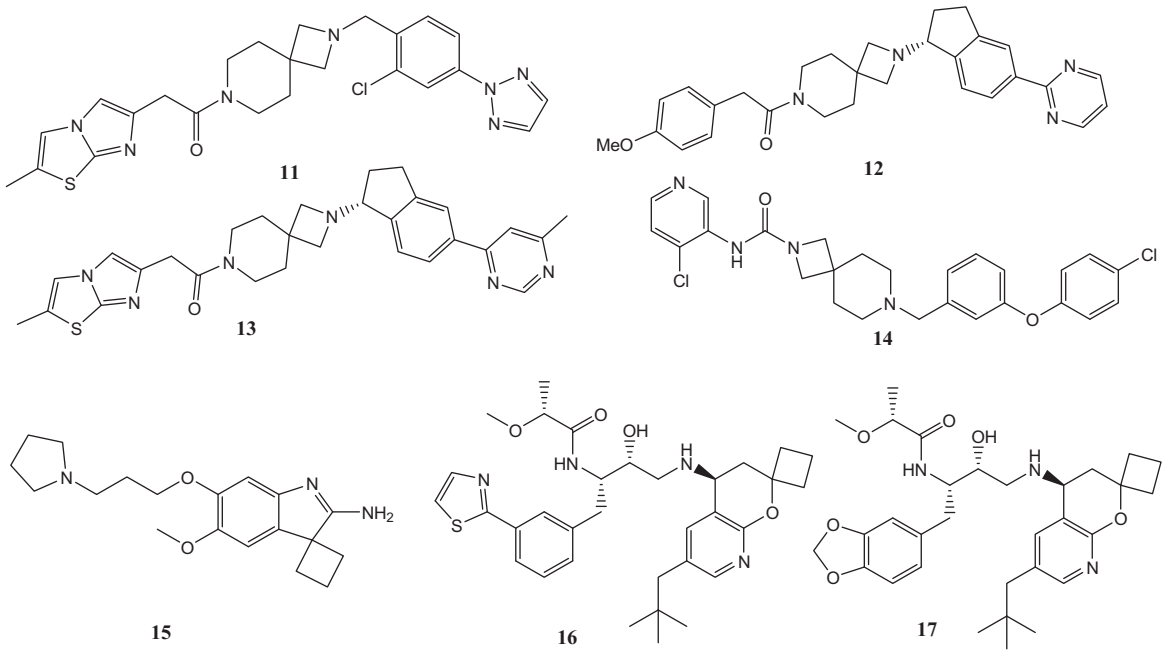

Figure 4. Further examples of spirocyclic scaffolds containing 4-membered rings.

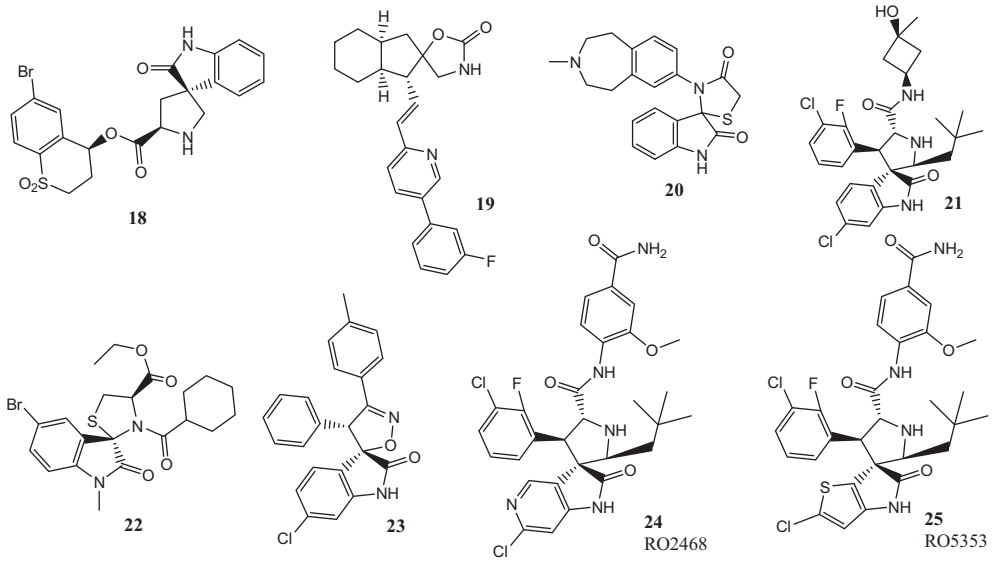

Figure 5. Compounds containing 5/5 spiro-ring fusions.

with a heterocycle (chlororopyridine in **24** and chlorothiophene in **25**, respectively).[31]

Spirohydantoin BMS-688521 (**26**, Fig. 6) is an antagonist (IC_{50} = 2.5 nM) of the interaction between integrin LFA-1 (leukocyte function associated antigen-1) and ICAMs (intercellular adhesion molecules) and has been advanced into clinical trials.[32]

XEN402 (**27**) is a spirooxindole containing inhibitor of the $Na_v1.7$ ion channel which is in clinical trials for treatment of pain in patients with congenital erythromelalgia.[33] Spiroindoline **28** is a SYK kinase inhibitor with an IC_{50} of 0.189 μM and good selectivity over a panel of kinases.[34] A structurally related compound (**29**) was reported to be a probe for the human (EC_{50} of 3.2 μM) and rat muscarinic M1 receptor subtype.[35] Compound **30** is a new antagonist of the CGRP receptor (K_i = 0.04 nM, P-gp efflux ratio of 1.4), a GPCR target for migraine.[36] Compound **31** is a potent p38α kinase inhibitor (IC_{50} = 1.8 nM) with good bioavailability in rat (65%).[37]

Compound **32** from GlaxoSmithKline is an analog of **4** with a different spiro ring fusion. Compound **32** is a potent HCV NS5A inhibitor (pEC_{50} = 10.4 (EC_{50} = 39.8 pM) for NS5A gt1A).[38] Compound **33** (SMU-B, Fig. 7), structurally related to **31**, has been reported to be a potent, orally available dual c-Met (IC_{50} = 1.87 nM) and ALK (IC_{50} <0.5 nM) inhibitor.[39] SMU-B demonstrated significant tumor growth inhibition in GTL-16 human gastric carcinoma xenograft models.

The discovery of sodium glucose cotransporter 2 (SGLT2) inhibitor, tofogliflozin, for treatment of type 2 diabetes was reported by Chugai.[40] Compound **34** is highly selective for SGLT2 (IC_{50} of 2.9 nM for hSGLT2 vs 8444 nM for hSGLT1). Tofogliflozin is currently in phase 3 clinical trials. Compound **35** is a potent inhibitor of 11-β HSD1 (IC_{50} = 1.1 nM).[41] A spiro scaffold has been used to mimic Pro-Leu-Gly-NH_2 as shown in compound **36**, which behaves as a negative allosteric modulator of dopamine D2 receptor.[42]

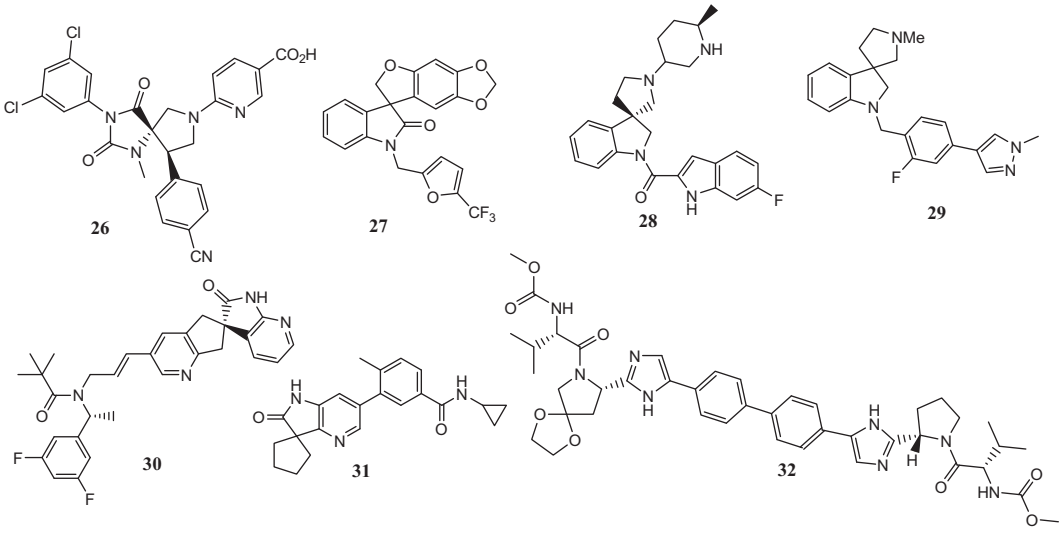

Figure 6. Further examples with 5/5 spiro-ring fusions.

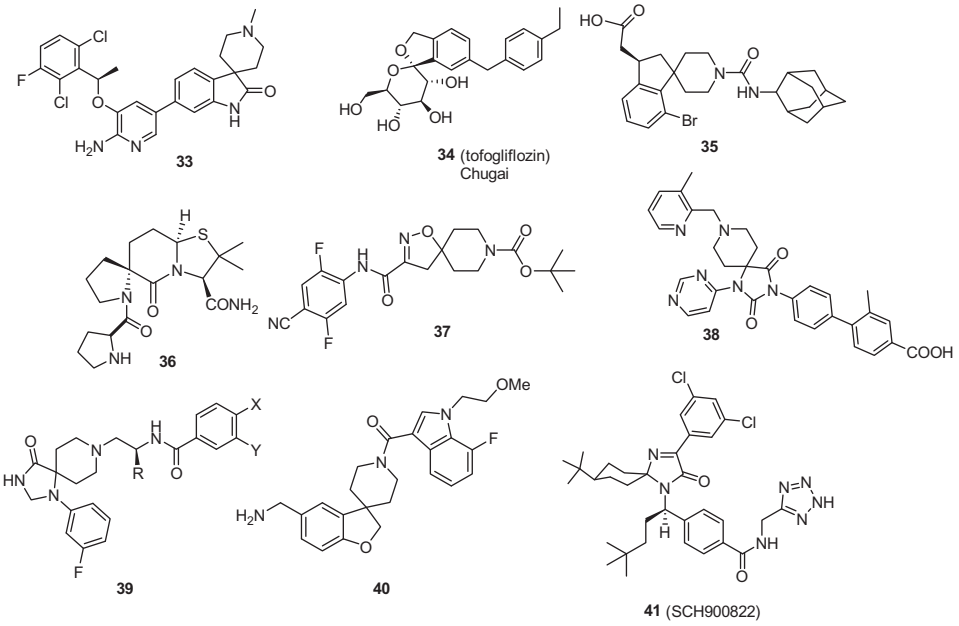

Figure 7. Compounds with 5/6 spiro-ring fusions.

GPR119 agonists have provided another mechanism to regulate glucose homeostasis and treat type 2 diabetes. Based on virtual screening and combinatorial chemistry follow-up, scientists at Boehringer Ingelheim discovered a novel class of GPR119 agonists, exemplified by **37** (EC_{50} = 93 nM).[43] Affinity selection mass spectrometry (AS-MS) was used by scientists at Merck to discover some spirooxindoles as inhibitors of hypoxia-inducible factor prolyl hydroxylase 1–3 (HIF PHD1-3).[44] Subsequent optimization yielded spirohydantion **38** (PHD1 IC_{50} = 0.2 nM; PHD2 IC_{50} = 0.2 nM; PHD3 IC_{50} = 1.6 nM; hERG binding of 22 μM; and CYPs >50 μM) as a preclinical candidate for treatment of anemia.

Phospholipase D (PLD) is an antipsychotic target. ML298 (**39** where R = H, X = Y = F) is a selective PLD2 inhibitor (PLD1 IC_{50} >20,000 nM, PLD2 IC_{50} = 355 nM) while a close analog of ML298 (ML299, **39** where R = CH_3, X = Br, Y = H) is dual PLD1/PLD2 inhibitor (PLD1 IC_{50} = 6 nM, PLD2 IC_{50} = 20 nM).[45] A spiropiperidineamide-based human β-tryptase inhibitor was reported by scientists from Sanofi-Aventis (**40**). This potent inhibitor **40** (K_i = 15 nM) also exhibited excellent metabolic stability without significant hERG channel inhibition.[46] Spiroimidazolone containing compound **41** was reported to be a potent and selective glucagon receptor antagonist (hGCGR cAMP IC_{50} = 46 ± 12 nM; hGLP-1R cAMP IC_{50} >10,000 nM).[47] At 30 mg/kg, **41** showed a significant glucose-lowering effect in a mouse model of type 2 diabetes.

CCR1 is a chemokine receptor that is believed to play an important role in chronic inflammatory diseases. Astra-Zeneca described compound such as **42** (Fig. 8) that are potent antagonists of human (IC_{50} = 1.8 nM) as well as rat CCR1 (IC_{50} = 16 nM).[48]

A number of 5/6 spirofused BACE1 inhibitors have been reported over the last three years. Aminooxazoline-based BACE1 inhibitor **43** from Amgen displayed good potency against BACE1 in both enzyme (IC_{50} = 8 nM) and cellular (IC_{50} = 36 nM) assays; it also showed good selectivity over cathepsin D (IC_{50} = 4470 nM).[49] Compound **43** is brain penetrant, and a single oral 30 mg/kg dose resulted in significant CNS Aβ40 reduction in naïve rats. The right hand benzene ring of the tricyclic system in

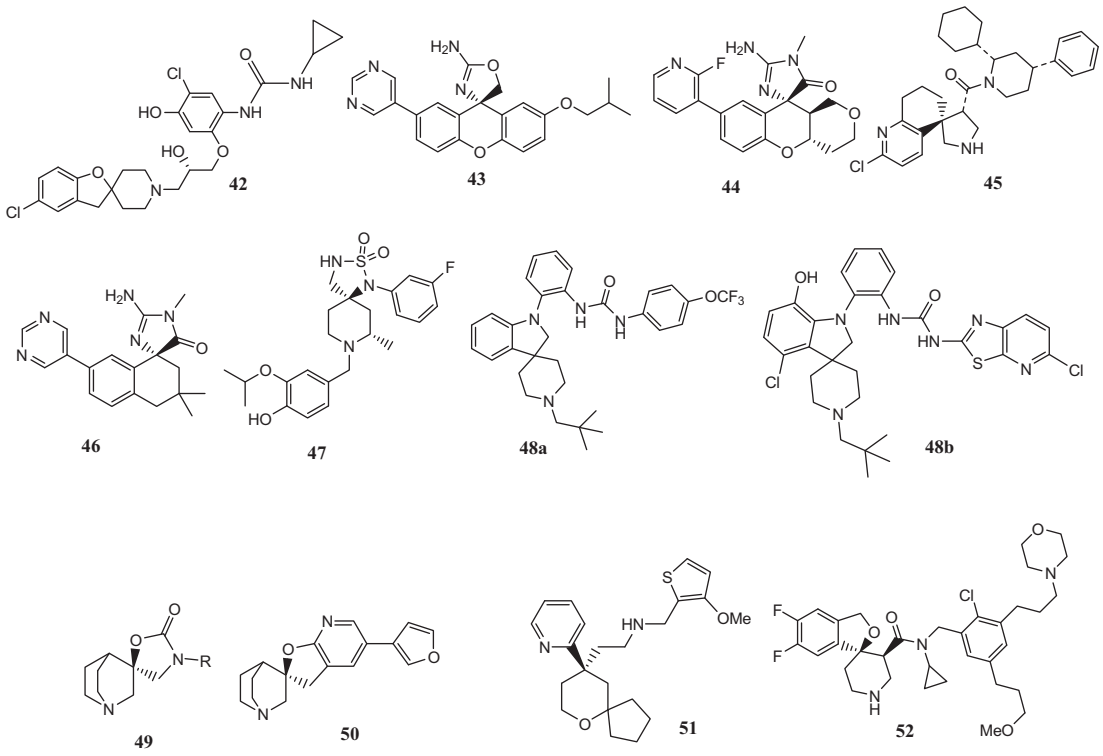

Figure 8. Further examples with 5/6 spiro-ring fusions.

43 can be replaced by a saturated ring as shown in **44**.[50] Spiropyrrolidine **45** exhibited good BACE1 inhibition (IC_{50} = 29 nM).[51] The aminohydantoin **46** is also a potent BACE1 inhibitor (IC_{50} = 48 nM) and reduced CSF Aβ in rodents and monkey.[52] Unlike the above mentioned HEA based BACE1 inhibitors (**16** and **17**, Fig. 4) which bind to the flap closed conformation of BACE1, both the aminooxazoline and aminohydantoin based inhibitors bind to the flap open BACE1 conformation. Compound **47** (Fig. 8) is spirocyclic sulfamide BACE1 inhibitor from Pfizer (IC_{50} = 0.10 μM).[53]

Spiropiperidine based compound **48a** from BMS was reported to be a potent, orally available P2Y1 antagonist (FLIPR IC_{50} = 2.6 nM; solubility of 4 μg/mL) and demonstrated a robust antithrombotic effect in the rat thrombosis and hemostasis models.[54a] Further optimization led to **48b** (FLIPR IC_{50} = 0.12 nM; solubility of 680 μg/ml),[54b] which is more efficacious than **48a**. Spiroquinuclidines and derived analogs (e.g., **49** and **50**) are known α7 nicotinic acetylcholine receptor modulators, which have been reviewed recently.[55] TRV130 (**51**) is a novel agonist of G protein μ opioid receptor (pEC_{50} = 8.1 (or EC_{50} = 7.9 nM)) discovered by Trevena;[56] it is now in clinical trials for treatment of acute severe pain. Compound **52** exhibited potent inhibition of renin (IC_{50} = 0.5 nM), a target for hypertension (Fig. 8).[57]

The two orexin receptor subtypes (OX_1R and OX_2R) are targets for treatment of insomnia. Spiropiperidine **53** is a potent and selective orexin-2 receptor (OX_2R) antagonist (IC_{50} = 3.3 nM; 450-fold selective against OX_1R).[58] Compound **54** is a potent neuropeptide Y5 receptor antagonist (K_i = 1 nM) with good metabolic stability in human (Cl_{int} <6 ml/mg/kg) and rat (Cl_{int} <25 ml/mg/kg) microsomes.[59] However, it failed to show efficacy in a diet-induced obese rat model. Compound **55** represents a novel class of mGluR5 allosteric modulators.[60] Compound **55** is potent (IC_{50} = 23 nM), and brain penetrant, and has shown efficacy in the mouse marble burying test, which is generally used as an indicator of potential anxiolytic activity.[60] Spiropiperidine compound **56** has been developed as positron emission tomography imaging agent for the σ1 receptor (K_i = 0.79 nM).[61] Interestingly, a spiro influenza virus A neuraminidase inhibitor (**57**) was discovered by Pinto and coworkers during synthetic follow-up of a carbocyclic analogues of zanamivir (Fig. 9).[62]

Lipid synthesis plays an important role in obesity, type 2 diabetes, and cancer. As the first committed step in the pathway to fatty acids, acetyl CoA carboxylase (ACC) has been intensively investigated.[63] Compound **58** is a potent acetyl-CoA carboxylase (ACC) inhibitor discovered by Takeda (IC_{50} = 32 nM for ACC1 and 5.4 nM for ACC2).[64a] Further optimization generated compound **59** (ACC1 IC_{50} = 21 nM; ACC2 IC_{50} = 4.9 nM), which is orally bioavailable and significantly decreased the values of the respiratory quotient in rats.[64b]

6/6 Spiro-ring systems: Based on screening of an in-house compound collection and subsequent optimization, Pfizer scientists identified some spiroketone-derived ACC inhibitors.[65] Further optimization led to spirolactam **60** (Fig. 10), a pre-clinical candidate for type 2 diabetes.[65] Compound **60** is potent for both ACC1 (IC_{50} = 10 nM) and ACC2 (IC_{50} = 4 nM) and has good in vivo metabolic stability (HLM <8 μL/min/mg).

Compound **61** from Novartis is a selective antagonist of hOX_2R (pK_i = 7.85 (K_i = 14.1 nM) vs 6.29 (K_i = 512.9 nM)) for hOX_1R), and induced sleep in mice.[66]

Compound **62** is a σ receptor agonist with modest selectivity over σ2 (σ1 K_i = 0.55 nM, σ2 K_i = 109 nM).[67] Further optimization of the fused ring and the N-substituent led to a more selective compound (**63**) (σ1 K_i = 1.1 nM with a σ1/σ2 selectivity ratio of >900).[68] Based on the previously reported anti-trypanosome activity of amantadine and rimantadine (anti-influenza A drugs) and the presence of essential metalloenzymes in trypanosomatid, Fytas et al. designed some novel N-hydroxyamides that showed excellent trypanocidal activity as exemplified by compound **64** (*Trypanosoma brucei* IC_{50} = 6. 8 nM, *Trypanosoma cruzi* IC_{50} = 0.21 μM).[69]

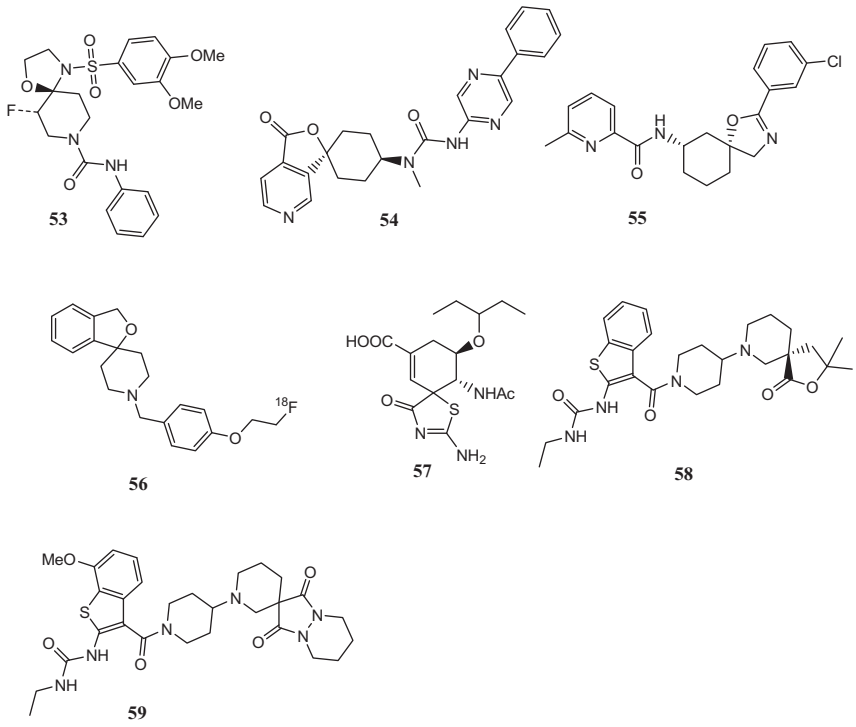

Figure 9. Additional examples with 5/6 spiro-ring fusions.

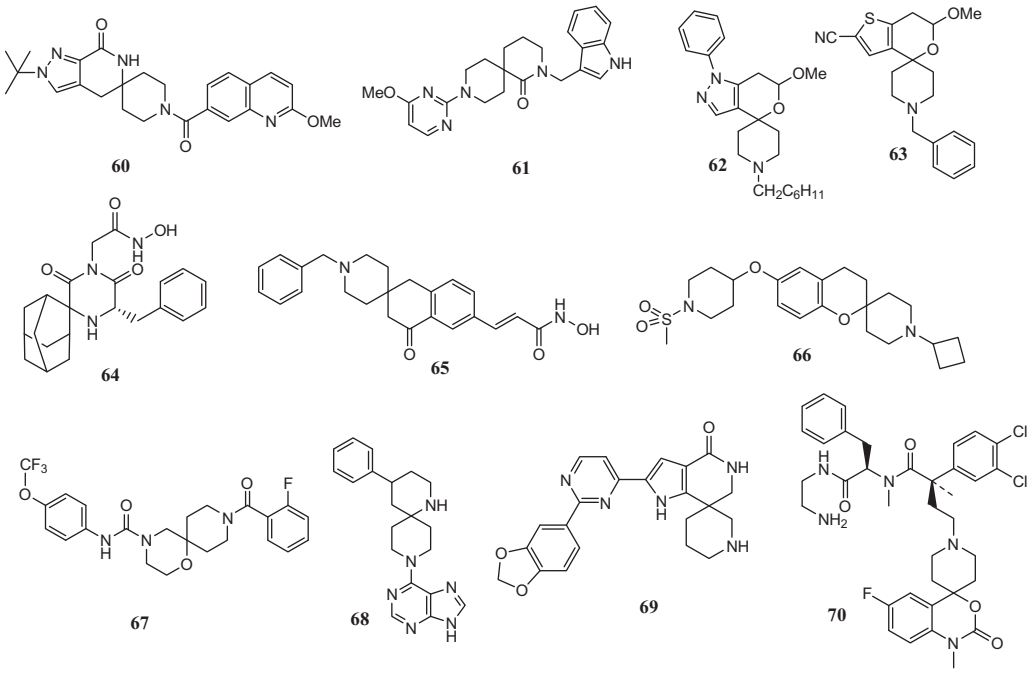

Figure 10. Compounds containing 6/6 spiro-ring fusions.

Compound **65** is a potent, orally bioavailable histone deacetylase (HDAC) inhibitor (IC_{50} = 0.121 μM), and showed tumor growth inhibition in an HCT-116 murine xenograft model.[70]

Spirobenzopyranpiperidine **66** is a potent and selective histamine-3 receptor (H3R) antagonist (hH3 K_i = 7 nM, rH3 K_i = 17 nM).[71] The bioavailability of **66** and its analogs is rather low; further optimization is required. Compound **67** was reported to be a potent inhibitor of soluble epoxide hydrolase (IC_{50} = 1.1 nM) with good bioavailability as well as some renal protective effect in a rat model.[72] Compound **68** inhibits a number of protein kinases[73] and **69** is an orally available inhibitor of MK2 (EC_{50} = 7.4 nM).[74]

Peptidomimetic **70** is a dual neurokinin 1 (NK1, pK_i = 8.6 (K_i = 2.5 nM)) and neurokinin 3 (NK3, pK_i = 8.1 (K_i = 7.9 nM)) receptor antagonist.[75]

7-Membered spirocyclic systems: Four spiro compounds which incorporate a seven membered ring have been reported (Fig. 11). Compounds **71**[76] and **72**[77] are inhibitors of stearoyl-CoA

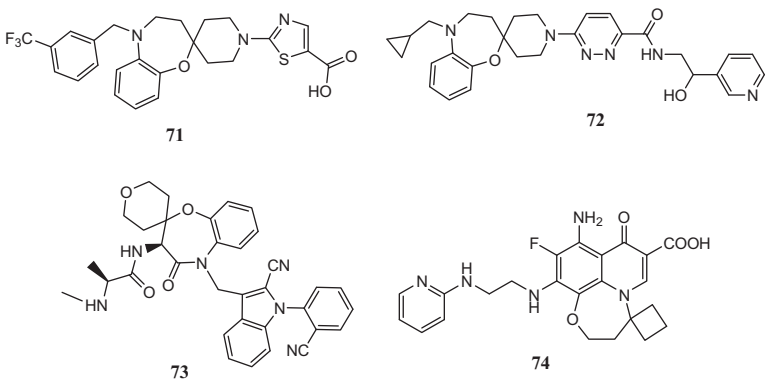

Figure 11. Compounds containing a 7-membered spirocyclic scaffolds.

desaturase-1 (SCD1), a therapeutic target for metabolic disorders and other indications.[78] Compound **71** is potent against mSCD1 (IC_{50} = 8 nM) with a reasonable PK profile and also showed a moderate reduction of the plasma desaturation index 4 h after oral dosing at 30 mg/kg. However, at higher doses over an extended period, some target-related side effects were observed. Compound **72** inhibited human SCD1 with an IC_{50} of 10 nM.[77]

Compound **73** inhibited the BIR2 (second baculovirus IAP repeat) domain (BIR2 IC_{50} = 0.039 μM, BIR3 IC_{50} = 3.06 μM) potently with adequate PK profile for further in vivo studies.[79] Spirocycle **74** is an orally available inhibitor of glycogen synthase kinase 3β (GSK-3β) (IC_{50} = 36 nM) with a significant dose-dependent plasma glucose lowering effect after the oral glucose tolerance test.[80]

Summary and perspective: The most utilized spiro ring systems reported over the last three years involve five and six membered rings (Figs. 5–10). Spiro ring systems have been successfully incorporated into ligands of GPCRs (e.g., **1**, **2**, **11**–**13**, **30**, **41**, **42**) and other receptors, enzyme inhibitors (kinases (e.g., **28**, **31**, **33**), aspartyl proteases renin (**52**) and BACE1 (**16**, **17**, **18**, **43**–**47**), epoxide hydrolase (**67**) etc.) and protein–protein interaction inhibitors (**21**–**26**). The presence of a spiro ring fusion tends to impose conformational restriction on a molecule. If the spiro ring lies in the periphery of a molecule, the conformational impact is rather limited. However, a centrally situated spiro ring significantly rigidifies the compound. In a number of cases, spiro rings were probably introduced mainly to provide novelty. In such cases, the spiro rings tend to be in the periphery of the molecules such as in **4**, **15**, and **32** and the small spiro ring can often be replaced by a geminal dialkyl group (e.g., geminal dimethyl) without causing significant conformational perturbation. For instance, in **15**, the cyclobutane ring can be replaced by geminal dimethyl with a resulting IC_{50} of 0.9 nM.[20] For BACE1 inhibitors **43**, **44**, **45**, **46**, and **47**, the spiro ring is crucial for presenting the basic moiety of each inhibitor to the active site catalytic Asp dyad.[21]

When a ligand binds to a protein, the bound conformation is usually not identical to the low energy conformation of the free ligand in solution; the energy difference between those two conformations is referred to as strain energy.[81] If the dominant conformation of the free ligand is the same as the bound conformation, the entropic penalty is alleviated upon protein–ligand complex formation and the ligand binding affinity is enhanced due to reduced strain energy.[82] Constraining the free ligand conformation to the bound conformation by cyclization has been widely applied, and in one extreme case, a free energy difference of 3.6–5.6 kcal/mol was reported.[82] When used appropriately, spiro cyclization provides an excellent way to enforce the desired conformation for ligand–protein binding; we may expect to see more examples where this tactic has been employed in the future. Introduction of conformational restriction by ring formation, including spiro ring formation, can not only modulate binding potency and specificity, but also potentially improve bioavailability and metabolic stability.[83] Furthermore, conformational restriction may reduce off-target activities. The fraction of sp^3 carbons (Fsp^3, ratio of number of sp^3 hybridized carbons and total carbon count in a molecule) and chiral carbon count have been used as descriptors of molecular complexity.[4c,d] Interestingly, the Fsp^3 descriptor not only appears to correlate with the probability of a compound progressing successfully from discovery, through clinical testing, to becoming a drug, but also correlates with compound solubility.[4c] Furthermore, increasing complexity also reduces promiscuity and P450 inhibition.[4d] Spiro-ring fusion provides a useful method of increasing molecular complexity and may offer greater benefit than introduction of flat rings.

With the recent advances in synthetic routes to spiro building blocks[14,84–86] and the interest in fragments with greater three-dimensionality,[87] more bioactive compounds containing spiro rings will be exploited in drug discovery. Even though all of the compounds discussed so far contain only one spiro element, in principle more than one spiro ring system can be incorporated into a drug molecule.[88]

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