Drug Design

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Synthesis of Azaspirocycles and their Evaluation in Drug Discovery**

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In modern drug discovery, high-throughput screening may generate promising lead compounds that are less than ideal with respect to important parameters such as absorption, distribution, metabolism, and excretion (ADME). In the process of lead optimization, various structural features are fine-tuned and physicochemical properties such as the basicity (pK_a[1]), lipophilicity ($\log D$), solubility (Sol_{int}), and clearance rates (CL_{int}) are improved.[2] Herein, we introduce heteroatom-substituted spiro[3.3]heptanes as viable and promising surrogates for piperazines, piperidines, morpholines, and thiomorpholines—all of which are common building blocks in medicinal chemistry (Figure 1).

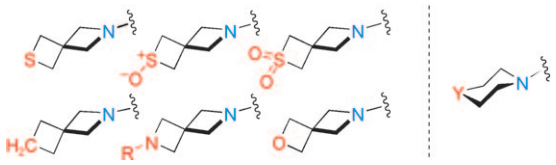
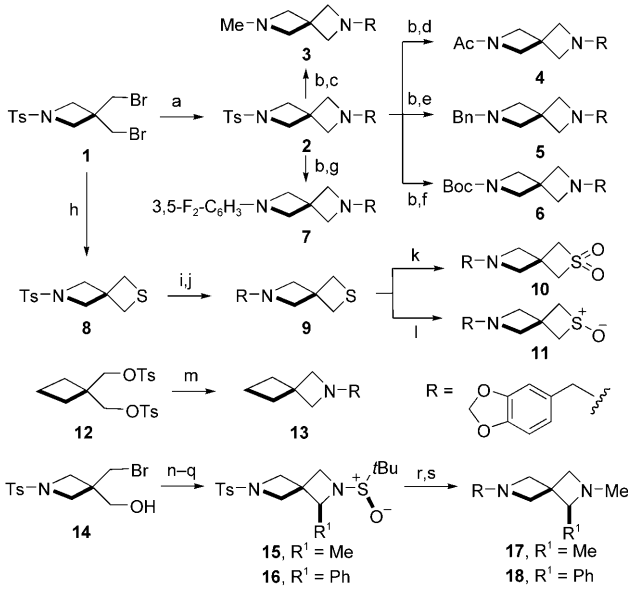


Figure 1. Heteroatom-substituted spiro[3.3]heptanes as novel building blocks in drug discovery.

The vast majority of compounds generated in pharmaceutical and chemical research contain saturated and unsaturated six- and five-membered rings that may be fused or linked.[3] In striking contrast, four-membered rings are found comparatively rarely. As previously described with oxetanes, the incorporation of four-membered heterocycles into drug-like scaffolds provides an opportunity to uniquely tune the physicochemical and biochemical properties of the parent compound.[4] Furthermore, these structures allow the exploration of novel chemical space in biology and medicine.[5]

We have suggested a number of useful ways of perceiving oxetanes as structural analogues. For example, oxetanes may be considered as structural mimics of carbonyl groups; consequently 2-oxa-6-azaspiro[3.3]heptane has a close structural resemblance to azetidin-3-one. Interestingly, this same oxetanyl spiroazetidine also exhibits similarities to morpholine.[6] In view of the use of saturated heterocycles in medicinal chemistry,[7] we turned our attention to other members of the spiro[3.3]heptane family. For a survey of fundamental physicochemical and biochemical properties, we chose molecules of topological C_2 symmetry with a common piperonyl-tagged[6] azetidine moiety as model compounds.

2,6-Diazaspiro[3.3]heptanes **2–7** and azetidine-thietane spirocycles **9–11** were prepared from dibromide **1**[8] (Scheme 1). After removal of the *N*-tosyl group of common



Scheme 1. R = Piperonyl. Reagents and conditions: a) Piperonylamine, 90%; b) Mg, MeOH, ultrasound; c) CH_2O, $NaBH(OAc)_3$, 71% (2 steps); d) Ac_2O, Et_3N, 71% (2 steps); e) PhCHO, $NaBH(OAc)_3$, 62% (2 steps); f) Boc_2O, Et_3N, 66% (2 steps); g) 1-Br-3,5-F_2-C_6H_3, (±)-binap, [$Pd_2(dba)_3$], KO*t*Bu, 69% (2 steps); h) Na_2S, 86%; i) see (b), then $C_2H_2O_4$; j) piperonal, $NaBH(OAc)_3$, 70% (3 steps); k) $K_2OsO_4 \cdot 2H_2O$, NMO, 99%; l) H_2O_2, AcOH, 68%; m) see (a), 69%; n) Swern oxidation, 91%; o) (*R*)-*t*Bu-S(O)NH_2, $Ti(OEt)_4$, 79%; p) MeLi, 52:48 d.r.; PhLi, 73:27 d.r.; q) KO*t*Bu, 40% (2 steps; for **15**), 73% (2 steps; for **16**); r) see (b) and (j); s) HCl, then (c), 42% (4 steps; **15**→**17**), 46% (4 steps; **16**→**18**). Ts = *p*-toluenesulfonyl, Bn = benzyl, Boc = *tert*-butoxycarbonyl, binap = 2,2′-bis(diphenylphosphino)-1,1′-binaphthalene, dba = *trans*,*trans*-dibenzylideneacetone, NMO = *N*-methylmorpholine *N*-oxide.

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intermediate **2**, functionalization of the amine furnished the desired diazaspiro[3.3]heptanes **3–7** in good yields.[9] Treatment of **1** with Na_2S delivered thietane **8**, which was elaborated to **9–11**. Clean oxidation to sulfone **10** was observed using $K_2OsO_4 \cdot 2H_2O$/NMO. Homospiropiperidine **13** was readily synthesized from **12**.[10,11]

The general route described above was successfully adapted to introduce substituents α to the nitrogen atom in a stereoselective manner. Swern oxidation of bromoalcohol **14** and subsequent condensation with Ellman's auxiliary[12] afforded the sulfinylimine. Whereas reagents such as MeMgBr failed to add to the sterically hindered electrophile,[13] more reactive MeLi as well as PhLi added at −78 °C. Ring closure was then performed at 0 °C using KO*t*Bu,[14] and subsequent elaboration led to enantiomerically pure diazaspiro[3.3]heptanes **17** and **18**.

The spirocycles and their six-membered-ring counterparts were analyzed with respect to their lipophilicity, aqueous solubility, metabolic stability, and amine basicity (Table 1). A survey of the pK_a values reveals a general trend that the amine of the spirocycle is generally more basic. If piperidine **34** and homospiropiperidine **13**, which have identical basicity, are taken as a common reference, the decrease in the pK_a values in the homospiro series as a consequence of Y is typically a factor of 0.5–0.6 lower than that observed for the corresponding members in the six-membered monocyclic series (Figure 2). This holds true for the whole range of pK_a values, and is consistent with the fact that the Y functionality (see Table 1) exerts its influence in the six-membered monocyclic series through two paths of three σ bonds,[1] but through four σ bonds in the homospirocyclic series. In this correlation we exclude the dibasic compounds, as their pK_a values cannot be assigned unambiguously to specific protonation sites (for further discussions, see the Supporting Information). The pK_a data in Table 1 are consistent with previously discussed pK_a decrements,[1] and nicely complement hitherto unavailable data for basicity modulation by heterofunctionalities Y at the β or γ positions to an amine unit.

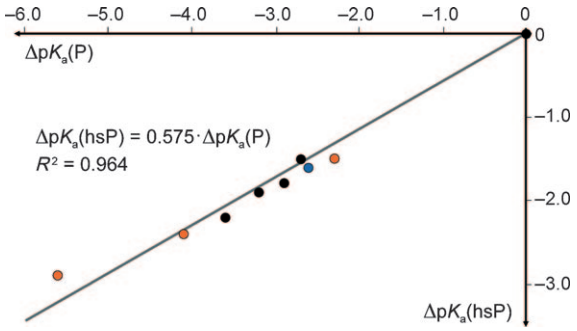
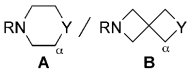


Figure 2. Correlation of pK_a decrements in the homospirocyclic series, ΔpK_a(hsP), against the six-membered monocyclic series, ΔpK_a(P), relative to the reference compounds **13** and **34**, respectively, containing no heterofunctionality (Y = CH_2, see Table 1). The black, orange, and blue dots refer to Y = NR, $S(O)_x$ (x = 0–2), and O functionalities, respectively.

Table 1: Physicochemical and biochemical properties.

Compound[a]		log*D*[b] (log*P*)[c]	Sol_{int}[d]	CL_{int} (h/m)[e]	pK_a[f]
 A B					
A, Y = NTs	**25**	3.4 (3.4)	13	310/586	6.0
B, Y = NTs	**2**	2.5 (2.8)	23	25/114	7.4
A, Y = NMe	**26**	0.5 (1.1)	> 59800	18/21	7.9
B, Y = NMe	**3**	−0.5 (1.6)	26800	12/4	9.5
A, Y = NAc	**27**	0.9 (1.0)	> 14900	13/39	6.4
B, Y = NAc	**4**	0.0 (0.5)	> 10600	6/10	7.7
A, Y = NBn	**28**	2.8 (3.2)	n.d.[g]	26/156	7.6
B, Y = NBn	**5**	1.6 (2.7)	n.d.[g]	6/41	8.4
A, Y = NBoc	**29**	3.1 (3.1)	287	32/1150	6.7
B, Y = NBoc	**6**	2.2 (2.8)	2620	2/35	7.8
A, Y = NAr[h]	**30**	> 3.7 (> 3.8)	12	184/495	6.9
B, Y = NAr[h]	**7**	> 3.0 (> 3.8)	12	29/241	8.1
A, Y = S	**31**	2.2 (2.4)	1740	28/2300	7.3
B, Y = S	**8**	1.6 (2.3)	4560	18/330	8.1
A, Y = SO_2	**32**	0.1 (0.1)	1440	7/100	4.0
B, Y = SO_2	**9**	0.5 (0.6)	4930	21/30	6.7
A, Y = SO	**33**	0.5 (0.5)	> 33000	0/6	5.5
B, Y = SO	**10**	0.1 (0.3)	> 32000	9/0	7.2
A, Y = CH_2	**34**	0.9 (3.1)	2060	8/18	9.6
B, Y = CH_2	**13**	1.0 (3.2)	3280	9/26	9.6
A, Y = O	**35**	1.5 (1.6)	36300	9/8	7.0
B, Y = O[6]	**36**	0.5 (1.2)	100000	3/7	8.0
A, Y = NMe;α-Me	**37**	n.d.[g]	n.d.[g]	0/23	7.9
B, Y = NMe;α-Me	**17**	n.d.[g]	n.d.[g]	9/39	9.3
A, Y = NMe;α-Ph	**38**	2.9 (3.1)	796	16/91	7.1
B, Y = NMe;α-Ph	**18**	2.0 (3.1)	2490	16/67	8.4

[a] R = piperonyl. [b] Log *n*-octanol/water distribution coefficient at pH 7.4. [c] Intrinsic lipophilicity of neutral base according to $\log P = \log D + \log_{10}(1 + 10^{(pK_a - pH)})$. [d] Intrinsic molar solubility [μmol L^{-1}] of the neutral base obtained from the experimental thermodynamic solubility in phosphate buffer (50 mM) at pH 9.9 and (22.5 ± 1) °C, and corrected for pK_a. [e] Intrinsic clearance rates in min^{-1}/(mg(protein)/μL) measured in human (h) and mouse (m) liver microsomes. [f] Amine basicity in H_2O measured spectrophotometrically at 24 °C; for details, see the Supporting Information. [g] n.d. = not determined. [h] Ar = 3,5-difluorophenyl.

The spiro[3.3]heptanes generally exhibit lower lipophilicities than their monocyclic counterparts (average Δlog*D* = −0.75). Interestingly, their neutral bases are also more polar, as can be seen from their log*P* values (average Δlog*P* = −0.21). One notable exception is sulfone **9**, which has higher lipophilicity than its thiomorpholine counterpart **32**, but it is still more soluble in aqueous phosphate buffer. Most of the spirocyclic compounds have a higher intrinsic solubility, even for substances that have very similar log*P* values (for example, **8** and **31**, or **13** and **34**), or when Δlog*P* > 0 (**9** and **32**). The differences are in some cases quite remarkable; for example, *tert*-butyl carbamates **29** and **6** show a solubility difference of roughly an order of magnitude.

Although metabolic susceptibility is dependent on the structural context, and thus cannot generally be attributed on a given structural subunit of interest, we found that most spirocyclic compounds were oxidatively degraded at lower rates than the six-membered monocyclic analogues in both human (h) and mouse (m) liver microsomes. Striking differences are observed for the more lipophilic pairs; in particular,

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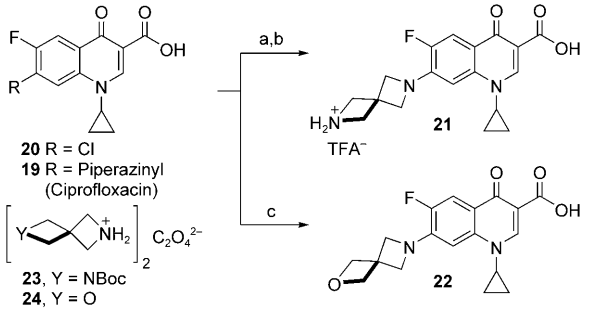
carbamate **6**, sulfonamide **2**, and aniline **7** display pronounced greater metabolic stability than their monocyclic analogues. This finding suggests that the higher aqueous solubility may result in the more polar compounds having fewer interactions with cytochromes P450 and other membrane-bound oxidizing enzymes, thereby avoiding metabolic degradation. Only in a few cases, particularly where the six-membered ring derivatives exhibit low intrinsic clearance rates, the spirocyclic analogues show similar or even slightly higher rates. In general, the data in Table 1 suggest that the concept of replacing a six-membered monocyclic unit in a drug candidate by a corresponding spiro[3.3]heptane analogue is worth implementing, as it may significantly improve relevant aspects of the pharmacokinetic profile by increasing the aqueous solubility and reducing both lipophilicity and metabolic degradation.

To demonstrate the usefulness of spirocyclic building blocks **23**[8] and **24**[6] in applied medicinal chemistry, the synthesis of analogues of the antibacterial compound ciprofloxacin (**19**) was undertaken (Table 2).[15] The commercially available aryl chloride **20** was treated with **23** or **24** (KO*t*Bu/DMSO) at 130 °C to give **21** or **22**, respectively, in good yields. These were then tested against clinical isolates of *S. aureus*. Ciprofloxacin trifluoroacetate was measured for the purpose of comparison. Azetidine analogue **21** displayed 4–8-fold weaker inhibition than ciprofloxacin. By contrast, **22** displayed comparable activity. The fact that the analogues **21** and **22** show comparable activities in an otherwise non-optimized scaffold suggests that spirocyclic amines can be considered in lead optimization. Furthermore, inhibitory properties may be retained with refinement of pharmacokinetic and -dynamic properties and the overall efficacy may even be improved. For example, metabolic studies reveal azetidine **21** and oxetane **22** to have high stabilities, with no observable metabolism in human microsomal assays (Table 2), whereas ciprofloxacin trifluoroacetate shows slight metabolic degradation. Likewise, **21** was as stable as ciprofloxacin in a human hepatocyte assay, whereas the homospiromorpholine analogue **22** remained essentially unaffected under the same whole-cell assay conditions.

Table 2: Synthesis, activity (MIC) against *S. aureus*, and metabolic stability of ciprofloxacin (**19**) and analogues **21** and **22**.[a]



	MIC	CL_{int} (human)	
	S. aureus [$\mu g\,mL^{-1}$]	microsomal [min^{-1} ($mg_{protein}/\mu L$)$^{-1}$]	hepatocytes [min^{-1} (10^6 cells/μL)$^{-1}$]
19	0.125	5	7
21	0.5–1.0	0	5
22	0.125	0	1

[a] Reagents and conditions: a) **23**, KO*t*Bu, DMSO, 62%; b) TFA, 99%; c) **24**, see (a), 68%. TFA = trifluoroacetic acid.

In summary, we have developed an efficient and scalable synthesis of various heteroatom-substituted spiro[3.3]heptanes with topological C_2 symmetry, and have shown that important pharmacokinetic properties such as lipophilicity and metabolic stability may be advantageously altered when compared with their traditional piperidine, piperazine, or thiomorpholine counterparts. Moreover, the spirocyclic building blocks can be easily grafted onto frameworks of druglike structures, such as the fluoroquinolones, to give compounds that retain notable activity and populate chemical space otherwise not previously accessed. In this regard, we expect the spirocyclic systems to find wide applications in medicinal chemistry and beyond.

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