Drug Design

DOI: 10.1002/anie.200602343

Oxetanes as Promising Modules in Drug Discovery**

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In drug discovery, it is common practice to block metabolically exposed sites in a biologically active molecule by the introduction of a *gem*-dimethyl unit (Figure 1). However, for a typical small molecule in medicinal chemistry, the replacement of hydrogen atoms by methyl groups leads to a significant increase in its lipophilicity, which in turn may adversely affect its metabolic and pharmacokinetic properties. Therefore, a stable, small, and less lipophilic molecular module with reduced susceptibility to metabolic attack would be a very desirable alternative. We have been interested in modules amenable to convenient synthesis and to facile incorporation into biologically active compounds, and have considered oxetanes to be of particular interest. Herein, we report the synthesis of discrete oxetane building blocks, which are readily prepared and attached onto molecular scaffolds. We show that incorporation of this motif results in remarkable improvements of key physicochemical characteristics and provides valuable opportunities for property-guided drug discovery.

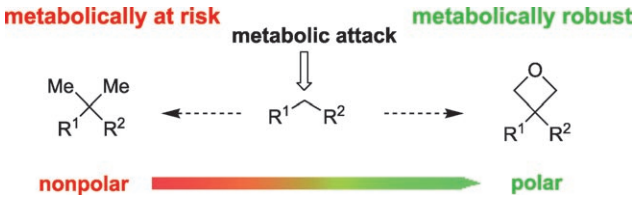
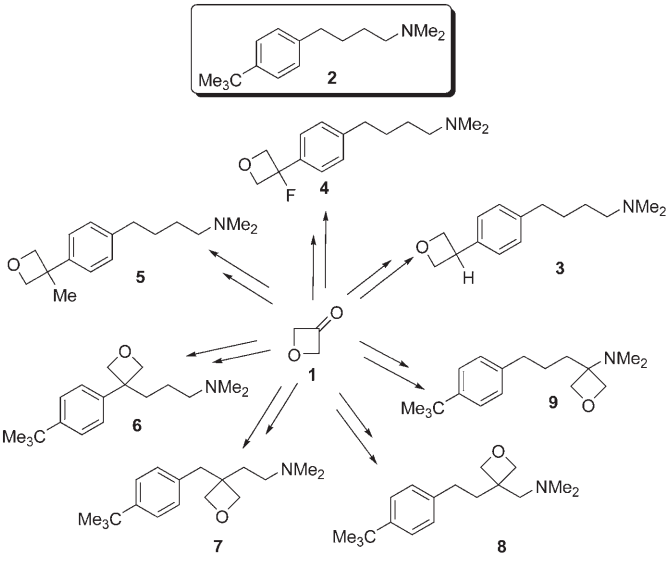


Figure 1. Comparison of gem-dimethyl and oxetane modules.

The analysis of the calculated van der Waals volumes reveals that an oxetanyl unit and a *gem*-dimethyl group occupy nearly the same volume. These calculations are in line with the experimental finding that the partial molar volumes of oxetane and propane in water are essentially the same.[1,2] We chose to incorporate oxetanes onto small molecules to make use of the polar nature of the oxetane to reduce the lipophilicity and metabolic liability. Little is known about the metabolic and chemical stability of oxetanes, and there are few synthetic methodologies of relevance to their incorporation and subsequent elaboration in compounds of pharmacological interest. Taxol,[3] oxetanocin,[4] and oxetin[5]—the best studied of oxetane containing biological actives—incorporate specifically 2,3-disubstituted oxetanes; however, the intrinsic chemical and pharmacological properties (or inherent advantages) of the oxetanes are far from clear.

To explore the utility of the oxetanyl moiety we selected *N*,*N*-dimethyl-4-(*p*-*tert*-butylphenyl)butylamine (**2**) as a model system. This compound contains a large hydrophobic unit, which renders it essentially insoluble in water in its neutral form, and also a polar head group, which makes it highly amphiphilic. Additionally, its readily accessible hydrophobic subunits may be perfect target sites for metabolic attack. For these reasons, **2** would most likely be designated an undesirable candidate in a medicinal chemistry program, thus making it a suitable starting point to probe the effect of introducing the oxetane unit at various positions. Several important synthetic aspects of the underlying oxetane chemistry targeted for development need to be considered for the assembly of oxetanyl analogues of **2**. This approach



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[**] We thank the Swiss National Science Foundation and F. Hoffmann–La Roche AG for support of this research. We are very indebted to I. Parrilla, F. Senner, and P. Warga for their great dedication in measuring important physicochemical data described in this work.

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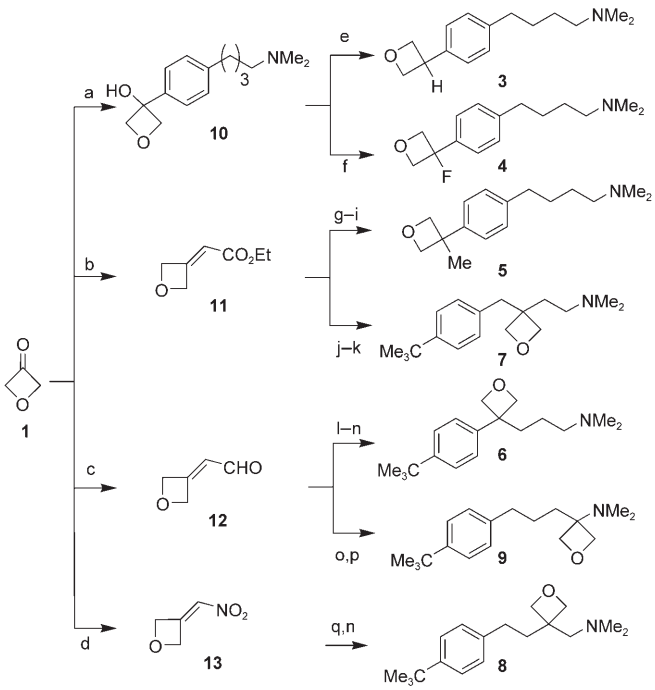
would involve the convenient preparation of oxetanes substituted with aliphatic, aromatic, and heterofunctional groups. Another structural feature of **2** is the *tert*-butyl group, which can be considered a special case of a *gem*-dimethyl group on a small aliphatic (ethyl) side chain. As the *tert*-butyl group is frequently encountered in medicinal chemistry,[6] its replacement by a 3-methyl-oxetan-3-yl unit and the study of concomitant property changes constitute a particularly interesting aspect of this work.

We chose structures **3**–**5** to examine the effect of replacing the *tert*-butyl group by an oxetane-containing unit. Structures **6**–**9** were prepared so as to carry out an "oxetane scan", in which the oxetanyl unit was placed at different topological distances from the basic amine. We focused on strategies in which an oxetane ring is conveniently introduced in a modular building block approach rather than built up de novo for each molecule.[7] The targeted oxetane derivatives were assembled using oxetan-3-one[8–10] (**1**) as the starting material (Scheme 1). The existing procedure for the preparation of oxetan-3-one[8,11] involves a five-step sequence, with preparative GC as a final purification step, and proceeds in 13% overall yield. Consequently, we developed an efficient and scalable route that could provide useful quantities of oxetan-3-one (**1**). The new route commences with the conversion of dihydroxyacetone into its dimethylacetal (MeOH, toluene *p*-sulfonic acid, trimethylorthoformate). Monotosylation of the isolated 1,3-dihydroxy-2,2-dimethoxypropane, followed by treatment with NaH, furnished 2,2-dimethoxyoxetane (37% overall yield).[12] Hydrolysis of the acetal proved difficult. However, heating a solution of 2,2-dimethoxypropane in the presence of Montmorillonite K10 at reflux provided the desired oxetan-3-one in 62% yield. This four-step sequence affords the target compound in an overall yield of 23% after purification by distillation.[13]

The addition of an aryl lithium compound to oxetan-3-one (**1**) furnished the 3-aryl-3-hydroxyoxetane **10**, which in turn was converted into the 3-aryl-3-fluorooxetane **4** by treatment with DAST (Scheme 1). Alternatively, the 3-hydroxy-oxetane **10** can be reduced to **3** in a one-pot procedure involving tosylation followed by reduction ($LiAlH_4$). These two protocols thus provide the simple aryl oxetanes.

We next developed routes to oxetanes bearing a quaternary center at C-3. Oxetan-3-one (**1**) undergoes reaction with stabilized ylides to furnish the α,β-unsaturated ester **11** and aldehyde **12**. The analogous nitroalkene **13** was obtained in 81% yield from condensation of **1** with nitromethane. It is worth noting that all of these unsaturated compounds, to the best of our knowledge, have not been previously reported and yet are easily handled and stored. Given the ease with which **11**–**13** were prepared, the stage was set to examine these as Michael acceptors. We were pleased to find that all three compounds readily undergo 1,4-addition by various nucleophiles, including amines, aryl and vinyl boronic acids (catalyzed by Rh complexes), as well as organocuprates, thus making them useful building blocks.

With compounds **3**–**9** in hand, we examined the physical and pharmacological properties imparted by the oxetane ring. The thermodynamic solubilities measured in buffered solutions at pH 9.9 are shown in Table 1. We chose these



Scheme 1. a) $(Me_2N)(CH_2)_4(C_6H_4)$-*p*-Li, THF, −78 °C, 71%; b) Ph_3PCHCO_2Et, CH_2Cl_2, RT, 89%; c) $Ph_3PCHCHO$, CH_2Cl_2, RT, 81%; d) 1. NEt_3, $MeNO_2$, RT; 2. NEt_3, MsCl, CH_2Cl_2, −78 °C, 81%; e) 1. NaH, Et_2O, 0 °C; 2. TsCl, 0 °C; 3. $LiAlH_4$, −78 °C, 58%; f) DAST, CH_2Cl_2, −78 °C, 40%; g) $(Me_2N)(CH_2)_4(C_6H_4)$-*p*-$B(OH)_2$, cat. [{Rh(cod)Cl}$_2$], KOH, aq dioxane, RT, 83%; h) DIBAL-H, −78 °C; i) [$(Ph_3P)_3RhCl$], toluene, 105 °C, 33% (2 steps); j) 4-*t*BuBnMgBr, TMSCl, CuI, THF, −18 °C, 70%; k) Me_2NH, $NaCNBH_3$, MeOH, 28% (2 steps); l) 4-*t*BuPhB$(OH)_2$, cat. [{Rh(cod)Cl}$_2$], KOH, aq dioxane, RT, 78%; m) 1. $MeNO_2$, NEt_3, RT; 2. NEt_3, MsCl, CH_2Cl_2, −78 °C, 58%; n) 1. H_2, $Pd(OH)_2$/C, CH_2O, RT, MeOH; 2. $NaCNBH_3$, CH_2O, MeOH, 34% o) 1. $HNMe_2$, DBU, THF, −18 °C; 2. 4-*t*BuPhCHPPh_3; p) H_2, Pd/C, MeOH, 36% (3 steps); q) (*E*)-4-*t*BuC_6H_4CHCHB$(OH)_2$, cat. [{Rh(cod)Cl}$_2$], KOH, aq dioxane, RT; 34% (overall sequence). Ms = methanesulfonyl, Ts = toluene-*p*-sulfonyl, DAST = (diethylamino)sulfur trifluoride, cod = cycloocta-1,5-diene, DIBAL-H = diisobutylaluminum hydride, TMS = trimethylsilyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, Bn = benzyl.

conditions to better differentiate the solubilities of amines with slightly different basicities, and to avoid complications resulting from the tendency of charged lipophilic compounds to form micelles under neutral pH conditions (a tendency observable for some of these compounds). At basic pH, **2** is essentially insoluble, whereas the oxetane derivatives **3**–**5** remain highly soluble (Table 1). The solubility data closely parallel the lipophilicity data represented by log*D* and log*P* values. The latter constitutes the intrinsic lipophilicity of the neutral base derived from the experimental pK_a and log*D* values, which correspond to the octanol/water distribution coefficients measured at pH 7.4. The replacement of the remote *tert*-butyl group by the 3-methyl-3-oxetanyl group results in a lowering of the log*D* (and log*P*) value by one unit. Interestingly, the magnitude of this decrease in lipophilicity is comparable with the increase in lipophilicity when going from ethylbenzene (log$P \approx 3.2$) to *tert*-butylbenzene (log$P \approx 4.1$).[14] The introduction of an oxetanyl module into a methylene unit thus constitutes a "liponeutral" bulk increase.

Table 1: Physicochemical and biochemical properties.

Compound	Sol.[a]	hCL_{int}[b]	mCL_{int}[b]	$\log D$[c] ($\log P$[d])	pK_a[e]
2	<1	16	417	1.8 (4.3)	9.9
3	4000	2	27	−0.1 (2.4)	9.9
4	6100	6	50	−0.5 (2.0)	9.9[f]
5	4400	0	43	0.8 (3.3)	9.9
6	270	0	147	1.7 (3.9)	9.6
7	4100	6	13	1.7 (3.5)	9.2
8	25	42	383	3.3 (4.0)	8.0
9	57	13	580	3.3 (3.6)	7.2

[a] Thermodynamic solubility ($\mu g\,mL^{-1}$) in 50 mM phosphate buffer at pH 9.9 and 22.5 ± 1 °C. [b] Intrinsic clearance rates in $min^{-1}\,mg^{-1}\,\mu L$ measured in human (hCL_{int}) and mouse (hCL_{int}) liver microsomes (see text). [c] Logarithmic octanol/water distribution coefficients at pH 7.4. [d] Intrinsic lipophilicity of the neutral base, according to $\log P = \log D^{pH} + \log(1+10^{(pK_a-pH)})$. [e] Amine basicities in H_2O measured spectrophotometrically at 24 °C. [f] The pK_a value for **4** in H_2O is estimated on the basis that its pK_a value (9.2) measured in MeOH/H_2O (1:1) is identical to the pK_a values of amines **2**, **3**, and **5**, also measured under these conditions.

While it comes as no surprise that **3**, which lacks the 3-methyl group on the oxetane ring, is less lipophilic than **5**, the further decrease in lipophilicity upon H/F exchange at the 3-position (**4**) is noteworthy. Typically, the replacement of one hydrogen atom by fluorine in an otherwise identical molecule results in a slight increase in the lipophilicity ($\Delta\log P \approx 0.25$).[15]

The chemical stability of all compounds was tested by stirring solutions of these for 2 h at 37 °C in aqueous buffers at pH 1–10. Oxetane-containing compounds (**3**–**9**) proved stable over the range of acidic to alkaline conditions. Compound **3** is stable in the pH range 2–10, but shows partial decomposition at pH 1, although 84% of **3** is recoverable.

Reference compound **2** was designed to be amphiphilic, because it consists of a large apolar residue connected to a basic, polar amine. Amphiphilic behavior is known to correlate with the free energy of amphiphilicity $\Delta\Delta G_{amp}$, which is defined as the difference in the free energies in transferring a compound from the aqueous phase to the air/water interface and from the aqueous phase into a micelle.[16] Compounds displaying high absolute values for $\Delta\Delta G_{amp}$ are more likely to induce phospholipidosis in cells.[17] In our experience, a basic compound ($pK_a > 7$) with $\Delta\Delta G_{amp}$ values less than 7.0 $kJ\,mol^{-1}$ is likely to cause phospholipidosis in vivo. Model compound **2** exhibits a free energy of amphiphilicity of −8.3 $kJ\,mol^{-1}$, clearly showing its pronounced amphiphilic nature and thus raising concerns about potential phospholipidosis. By contrast, oxetane **5**, with an experimental value of −3.2 $kJ\,mol^{-1}$, is well below the critical threshold.

We next examined the susceptibility of the oxetane-containing compounds towards metabolic degradation in human and mouse microsomes by incubating the compounds at 2 μM for up to 30 minutes at 37 °C. The levels of non-metabolized compound were determined by calibrated HPLC/MS/MS at regular time intervals. The intrinsic clearance (CL_{int}, in $min^{-1}\,mg^{-1}\,\mu L$ microsomal protein) is the rate constant of the first-order decay of a given compound, normalized for the protein concentration in the incubations. Examination of the parent structure **2** reveals medium and high clearance rates from human and mouse microsomes, respectively (Table 1). By contrast, oxetane derivatives **3**–**5** show a significantly reduced susceptibility to microsomal attack. Compound **5** displays a marked increase in metabolic stability, with no detectable degradation in human microsomes, and a clearance rate an order of magnitude lower than that of **2** in mouse microsomes. Likewise, the replacement of the *tert*-butyl unit by an oxetane or fluorooxetane module, as in **3** and **4**, results in improved metabolic stabilities. It should be noted that **2**–**5** may suffer metabolic attack at various sites, and the substantial increase in metabolic stability found for the three oxetane-containing compounds **3**–**5** correlates with an overall reduction in lipophilicity. The enhanced stability of **5** is noteworthy given that **5** is more lipophilic than **3** or **4**.

We then decided to investigate the effect of oxetane incorporation on the hERG (*human ether-à-go-go*) potassium channel. Blockade of this channel may lead to a prolongation of the QT interval (electrophysiological heart parameter) and is therefore a fundamental test for in vitro safety.[18] It is known that lipophilic compounds containing a basic amine are often substrates of this channel, and that the introduction of polar functional groups into the extremity of the structure of such compounds can reduce their hERG liability. Indeed, this is confirmed by a comparison of the lipophilicity and hERG assay results for **2** and **5**. The marked reduction in lipophilicity by introduction of the oxetane unit resulted in a clear reduction of hERG liability for **5** (hERG $IC_{50} = 35$ μM) in comparison with **2** (hERG $IC_{50} = 7.5$ μM).

Finally, we prepared a series of compounds in which the distance between the amine and oxetane ring incrementally varied, thus giving rise to an "oxetane scan" (Scheme 1). The resulting series **6**–**9** allowed us to assess the influence of the oxetane unit on the basicity of the neighboring amine. The pK_a values for the various amines were determined spectrophotometrically by using a ProfilerSGA/Sirius Instrument. While an oxetane ring incorporated in a remote position from the amine (as in 3-5) did not affect the basicity of the compound, its incorporation α or β relative to the amine has a pronounced effect, lowering the basicity by nearly 3 and 2 pK_a units, respectively. The basicity-lowering effect of the compound may be diagnosed even when the oxetane is separated from the amine function by four single bonds (**6**). The remarkable power of an oxetane ring to reduce amine basicity renders it an interesting structural motif for the tempering of a nearby basic functional group.

The introduction of an oxetane moiety in the γ or δ position results in a marked increase in solubility. Its incorporation close to the amine group still results in reasonable solubility for **8** and **9**. Likewise, increased resistance towards metabolic attack, as assayed in human and mouse microsomes, can be diagnosed for **6** and **7**, in contrast to **8** and **9**, which display reduced metabolic stability probably because of their enhanced lipophilicity. Preliminary data (not shown) indicate that the primary metabolic attack occurs at the amine and *tert*-butyl groups, but not at the oxetane moiety.

In conclusion, we have demonstrated that, starting from the readily available building block oxetan-3-one, a prototypical set of compounds can be made, which contain the oxetane motif at different positions. These compounds

improve remarkably the physico- and biochemical properties of the underlying scaffold. Enhanced solubility, reduced lipophilicity, amphiphilicity, and hERG liability, as well as pronounced modulation of the basicity of a nearby amine group are documented. The various oxetane-containing compounds display robust chemical stability under various basic and acidic conditions, as well as an improved resistance towards metabolic degradation in most cases. The synthetic routes outlined in this study illustrate the relative ease with which the key oxetane building blocks can be incorporated at various sites of a given lead structure. Taken together, our data make a case for a more general use of this under-represented structural motif in drug discovery and beyond.

Received: June 12, 2006
Published online: October 2, 2006

Keywords: drug design · metabolism · oxetanes · small ring systems · synthesis design

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