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3,4-Dihydro-2*H*-benzo[1,4]oxazine derivatives as 5-HT$_6$ receptor antagonists

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Abstract—A series of novel 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives has been designed and synthesized as 5-HT$_6$ receptor antagonists. Many of the compounds displayed subnanomolar affinities for the 5-HT$_6$ receptor and good brain penetration in rats. The relationship of structure and lipophilicity to hERG inhibition of this series of compounds is discussed.

The human 5-HT$_6$ receptor is one of the more recently identified members of the serotonergic receptor family. The density of 5-HT$_6$ receptors in rat brain is highest in the olfactory tubercles, striatum, nucleus accumbens, and hippocampus. Its distribution, together with its high affinity for several tricyclic antipsychotics, suggests the 5-HT$_6$ receptor may be involved in memory disorders, psychosis, depression, and appetite control.[1–3] Moreover, 5-HT$_6$ receptor antagonists have been shown to increase extracellular concentrations of neurotransmitters that are important for cognition. Therefore, there has been increasing interest in the 5-HT$_6$ receptor as a therapeutic target over the past few years, as evidenced by numerous publications appearing in the literature.[1]

Our initial lead was RO-65-7674 (**2**),[4] a potent and selective 5-HT$_6$ antagonist with good DMPK properties in preclinical studies. However, the whole brain homogenate to plasma ratio of **2** in rats was found to be low (B/P = 0.1). It is also a potent hERG channel inhibitor with an IC$_{20}$ of 0.2 μM. Hence, our goal was to discover a 5-HT$_6$ antagonist with an improved brain to plasma ratio and lower hERG inhibitory activity compared to **2**. Herein, we report the design, synthesis, and biological activity of a series of novel 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives **1** as 5-HT$_6$ receptor antagonists.

In addition to **2**, arylsulfonamides **3** and **4** were also reported to be 5-HT$_6$ receptor antagonists,[5,6] albeit with low brain penetration in the case of **3**. In comparing **2**, **3**, and **4**, common features are evident, including a central aromatic scaffold, a basic side chain, and an aryl sulfonyl or arylsulfonamide group. These groups are arranged in a spatially similar fashion (Scheme 1). Therefore, we envisioned that molecules of template **1**, which possess all these features, would also be high-affinity 5-HT$_6$ receptor antagonists. Moreover, template **1** lacks the sulfonamide NH hydrogen bond donor in **3**, which should help improve its brain penetration.

The synthesis of compounds **1** is exemplified by the reaction sequences shown in Schemes 2 and 3. The latter is more divergent, allowing more rapid synthesis of compounds with different substituted arylsulfonyl groups.

Substituted 6-bromo-2-nitrophenols were either commercially available or synthesized by bromination of the corresponding 2-nitrophenols in acetic acid in good

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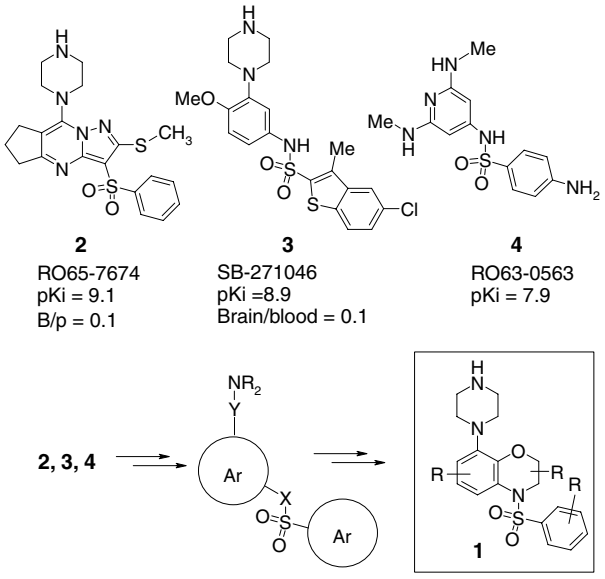
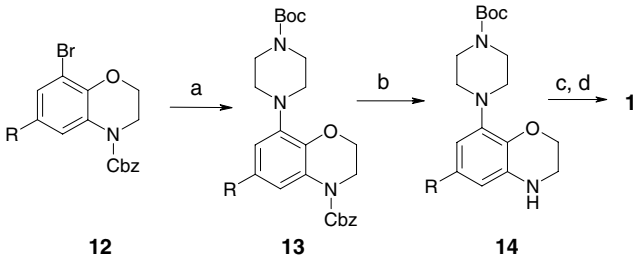


Scheme 1. Design of novel benzoxazine derivatives as 5-HT_6 ligands.

yields. The nitro compound **6** was reduced to aniline **7** by sodium dithionite in a mixture of ethanol and water at ~70 °C in 68% yield. Compound **7** was then reacted with chloroacetyl chloride in a mixture of acetonitrile and water in the presence of sodium bicarbonate at room temperature for 30 min. followed by heating at 60 °C for 10 h to afford benzoxazinone **8.**[7] Reduction by BH_3 in THF followed by sulfonylation in dichloromethane in the presence of pyridine gave aryl bromide **10**. The piperazine moiety was installed by a palladium/Binap-catalyzed aryl amination reaction using 1-Boc-piperazine.[8] The final piperazinyl benzoxazine compound **1m** was obtained after deprotection in ethanolic HCl and isolated as the HCl salt.

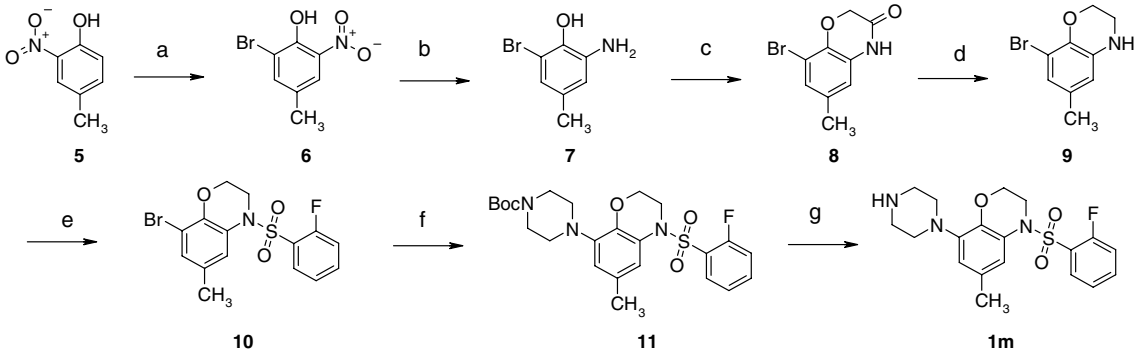
Alternatively, bromodihydrobenzoxazine **9** was protected with Cbz to give **12**. Subsequent amination with 1-Boc-piperazine followed by hydrogenolysis to remove Cbz gave intermediate **14**, which was then reacted with different arylsulfonyl chlorides to afford the desired compounds**1** after removal of the Boc group under acidic conditions (Scheme 3).



Scheme 3. Synthesis of 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives. Reagents and conditions: (a) 1-Boc-piperazine, $Pd_2(dba)_3$, BINAP, toluene, NaO-*t*-Bu, 100 °C; (b) H_2, Pd/C, EtOH; (c) $ArSO_2Cl$, DCM, pyridine; (d) HCl, EtOH, reflux.

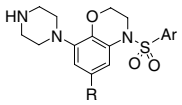
The in vitro binding activity of these compounds was determined by displacement of [^{3}H]LSD in HEK293 cells expressing the recombinant human 5-HT_6 receptor at 37 °C and pK_i values are presented in Table 1.

Halogen substitution on the 2 and/or 3 positions of the phenylsulfonyl group seems beneficial for 5-HT_6 affinity. For example, 2,3-dichlorophenylsulfonyl (**1a**) gave the highest affinity in this series with a pK_i of 10.2, whereas the parent unsubstituted compound **1b** had relatively modest affinity (pK_i = 7.9). A simple 2-fluoro substitution on the phenylsulfonyl group (**1c**) increased the pK_i from 7.9 to 8.9. Polar substituents on the phenylsulfonyl group such as methylsulfonyl or methylsulfonamide led to markedly lower activity (**1g** and **1h**). Naphthyl and some heteroaryl sulfonyl groups were well tolerated (**1i**–**k**). The effect of substitution at positions 2 and 6 of the 3,4-dihydro-2*H*-benzo[1,4]-oxazine core scaffold was also investigated. These substitution positions were chosen for synthetic considerations. Small lipophilic substituents such as Me, F, Cl, and methoxy on position 6 increased 5-HT_6 affinity (**1l**–**q**). The bulky 6-*tert*-butyl group lowered affinity (**1s**). Analogs with substitution on position 2 of the 3,4-dihydro-2*H*-benzo[1,4]oxazine core were synthesized according to Scheme 2 using corresponding substituted chloroacetyl chlorides. The 2,2-dimethyl and 2,2-spirocyclobutyl substituted compounds (Table 2, **15** and **16**) had slightly better affinity than the nonsubstituted analog (**1c**).



Scheme 2. Synthesis of 3,4-dihydro-2*H*-benzo[1,4]oxazine derivative **1m**. Reagents and conditions: (a) Br_2, acetic acid, 95%; (b) $Na_2S_2O_4$, EtOH/water, heat, 68%; (c) $ClCH_2COCl$, 95%; (d) BH_3–DMS, THF, reflux, 89%; (e) 2-F-$PhSO_2Cl$, pyridine, CH_2Cl_2, 75%; (f) 1-Boc-piperazine, Binap, $Pd_2(dba)_3$, NaO-*t*-Bu, toluene, 52%; (g) HCl, EtOH, reflux, 90%.

Table 1. Biological activities of novel 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives: 5-HT_6 affinity, hERG inhibition and brain to plasma ratio in rats



Compound	R	Ar	5-HT_6 pK_i[a]	hERG at 1 μM[b]	clog *P*[c]	B/P[d]
1a	H	2,3-Dichloro-Ph	10.2			
1b	H	Ph	7.9			
1c (R1485)	H	2-F-Ph	8.9	9.9 ± 3.3%	2.90	1.8
1d	H	2-Cl-Ph	8.5	28.2 ± 5.4%	3.34	1.6
1e	H	3-Cl-Ph	8.7			1.4
1f	H	3-F-Ph	8.0			
1g	H	3-$MeSO_2NH$-Ph	6.7			
1h	H	2-$MeSO_2$-Ph	6.5			
1i	Cl	Naphthalen-1-yl	9.1			
1j	Cl	Benzo[1,2,5]oxadiazol-4-yl	9.3			
1k	Cl	Quinolin-8-yl	9.2			
1l	Me	Ph	8.6	23.2 ± 9.9%	3.25	1.0
1m	Me	2-F-Ph	9.4	26.5 ± 1.8%	3.45	
1n	Me	2-CN-Ph	8.7	14.2 ± 0.8%	2.79	
1o	F	2-F-Ph	9.2	26.0 ± 3.5%	3.11	
1p	Cl	2-F-Ph	9.4	45.2 ± 12%	3.54	2.7
1q	MeO	2-F-Ph	9.8			
1r	MeO	Ph	9.4	34.0 ± 3.8%	2.78	0.8
1s	*t*-Bu	2-F-Ph	8.1			
1t	F	4-Amino-Ph	8.9			

[a] Standard deviation of the pK_i estimates was 0.05–0.3.
[b] Inhibition of hERG outward current at 1 μM concentration; mean ± SEM; baseline = 0%, $n = 2$ cells.[11]
[c] $K_{o/w}$–clog *P* values (calculated values of octanol–water partition coefficient) calculated using KowWin (V1.57, Syracuse Research, North Syracuse, NY, USA).
[d] Whole brain homogenate to plasma ratio at 1 h post 2 mg/kg iv dosing in rats, drug concentrations were determined by HPLC–MS/MS.

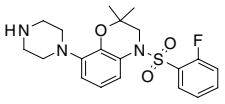
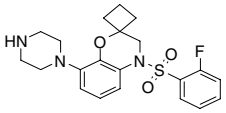
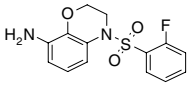
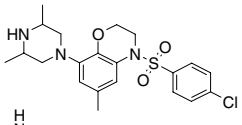
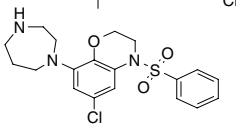
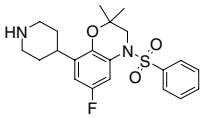
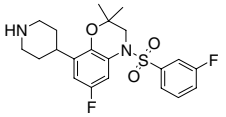
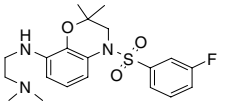
Several benzoxazine derivatives with basic side chains other than piperazine were synthesized (Table 2). Similarly to the synthesis of the piperazine derivatives, the homopiperazine, 2,6-dimethylpiperazine, and dimethylaminoethylamine derivatives (**18**, **19** and **22**) were synthesized by the palladium-catalyzed amination reaction of the corresponding aryl bromide with the required amine. The aniline compound (**17**) was synthesized using benzophenone imine as an ammonia surrogate.[9] The piperidine derivatives (**20**, **21**) were synthesized via the Suzuki coupling reaction of the corresponding aryl bromide with 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-3,6-dihydro-2*H*-pyridine- 1-carboxylic acid tert-butyl ester,[10] followed by hydrogenation and removal of the Boc group. The homopiperazine and the piperidine derivatives (**19**, **20** and **21**) had 5-HT_6 affinity comparable to the corresponding piperazine analog. Other side chains led to lower 5-HT_6 affinity. Among the basic side chains investigated, piperazine derivatives offered the combination of good 5-HT_6 affinity and ease of chemical synthesis.

Compounds with high 5-HT_6 affinity (p$K_i > 8.5$) and acceptable initial DMPK properties, such as good stability in microsomes and low cytochrome P450 inhibition, were selected for hERG inhibition measurement by conventional patch clamp electrophysiology assays.[11] Inhibition of hERG current by the tested compounds at 1 μM concentration ranged from 10% to 45% depending on the substitution (Table 1). Lipophilic substituents such as Me, F, and Cl on the aromatic rings seem to increase hERG blockade, with Cl substitution having a stronger effect (**1d**, **1m**, **1o** and **1p**). The more polar cyano substituted analog (**1n**) had weaker hERG inhibition than the fluoro analog (**1m**). In fact, a correlation seems to exist between calculated log *P* values and the measured hERG inhibition, with the methoxy compound **1r** being the outlier. Within this chemical series, the general trend is that the more lipophilic the molecule, the stronger its hERG inhibitory activity.

Several compounds were profiled in rat in vivo following 2 mg/kg single intravenous dose (iv). The brain to plasma ratios at 1 h post iv dose were calculated from drug levels in whole brain homogenate versus plasma. The drug levels were determined by using HPLC–MS/MS. The B/P ratios for the compounds tested ranged from 0.8 to 2.7, a significant improvement over our initial lead RO65-7674 (**2**) or SB-271046 (**3**).[5] This improvement is probably due to the fact that template **1** has fewer heteroatoms than **2**, and is also devoid of the sulfonamide NH hydrogen bond donor in **3**.

Compound **1c** (R1485) showed good B/P ratio (1.8) and low hERG inhibition (10% at 1 μM) while maintaining high affinity for the 5HT6 receptor (p$K_i = 8.9$). Its hERG IC_{20}value at 37 °C was determined using four concentrations in a patch clamp physiology assay and shown to be 2.2 μM, representing a significant improvement over our initial lead molecule **2** ($IC_{20} = 0.2$ μM). In

Table 2. 5-HT_6 affinity of 3,4-dihydro-2*H*- benzo[1,4]oxazines

Compound	Structure	5HT6 pK_i
15		9.5
16		9.2
17		6.2
18		7.4
19		8.4
20		8.4
21		8.3
22		6.9

the functional assay, **1c** was devoid of agonist activity and behaved as an antagonist at 5-HT_6 receptor. Profiling against standard receptor, ion channel, and uptake system panels including other serotonergic receptor subtypes indicated that R1485 was >100-fold selective over >50 other targets.

In summary, a series of novel 3,4-dihydro-2*H*-benzo[1,4]oxazine derivatives was designed and synthesized, and found to comprise potent 5-HT_6 receptor antagonists. The 5-HT_6 affinity of these compounds is dependent on the substitution on the aromatic rings. A general correlation between lipophilicity and hERG inhibition was observed in this chemical series. R1485 was selected for further development because of its overall superiority, and was eventually advanced to human clinical trials.

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