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Cyclic guanidines as dual 5-HT$_{5A}$/5-HT$_7$ receptor ligands: Optimising brain penetration

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Abstract—The optimisation of molecular properties within a series of 2-amino dihydroquinazoline 5-HT$_{5A}$/5-HT$_7$ receptor ligands resulted in a significantly improved brain-to-plasma ratio, enhancing the pharmacological utility of these compounds. By modulating the lipophilicity and pK_a, a 20-fold increase in brain-to-plasma ratio could be achieved, leading to micromolar brain concentrations after oral administration. The enantiomers of one representative of this series of improved compounds were separated, and the configuration of the eutomer was determined by X-ray crystallography.
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5-Hydroxytryptamine (5-HT) receptor (R) subtypes have proven to be tractable targets for drug discovery, leading to well-established medicines for the treatment, for example, of anxiety (buspirone, 5-HT$_{1A}$R partial agonist),[1a] migraine ('triptans', 5-HT$_{1D}$R agonists),[1a] emesis (ondansetron, 5-HT$_3$R antagonist),[1b] or GI tract motility disorders (cisapride, 5-HT$_4$R agonist).[1c] The more recently discovered 5-HT$_6$ and 5-HT$_7$Rs have been the subject of extensive preclinical research.[1d,e] In contrast, the 5-HT$_{5A}$ subtype has been less well studied, despite its expression in the cerebral cortex, hippocampus, amygdale, thalamus, hypothalamus, basal ganglia, brain stem, and cerebellum,[2] and an association of the 5-HT$_{5A}$ receptor polymorphism with schizophrenia,[3] pointing to a potential role in the modulation of mood disorders.[4a,b] Recently disclosed, selective 5-HT$_{5A}$ receptor antagonists demonstrate antipsychotic-like potential in animal models.[5] These findings have generated an increased interest in the 5-HT$_{5A}$R as a potential drug target.

In a search for novel 5-HT$_{5A}$R ligands, we identified **1a** as the best representative of a novel class of guanidine-type 5-HT$_{5A}$R antagonists with high affinity and high functional activity, however, with no selectivity over the 5-HT$_7$R (see preceding publication). Compound **1a** penetrates into the brain after oral administration, with a brain-to-plasma ratio of ~0.2, which is modest for a compound targeting the CNS. Selected molecular properties of **1a** are summarised in Table 1, further pharmacokinetic properties are given in Table 5.

With the goal of developing a pharmacological tool for behavioural studies, we aimed to further improve the brain penetration. We speculated that this might be possible by adjusting the physicochemical properties (e.g., pK_a, log D) of these compounds more towards the optimal range for CNS drugs (pK_a < 10,[6] log D ~ 2[7]). Especially lipophilicity is known to be an important parameter governing brain penetration,[8] so we first attempted to improve the brain-to-plasma ratio of our compounds by increasing lipophilicity. We decided to explore the modulation of lipophilicity by attaching a variety of substituents onto the exocyclic nitrogen. Compound **2** (Table 2) was chosen as a model compound due to its straightforward synthetic access. Compounds **2b**–**2i** were prepared to explore the tolerance of substituents. A significant loss of 5-HT$_{5A}$R affinity was observed for all compounds except **2d**. Also, the stabilities in rat liver microsome incubations were very low (except **2i**), in sharp contrast to the unsubstituted

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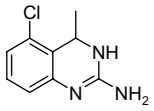
Table 1. Properties of the dual 5-HT_{5A}/5-HT_7R ligand, **1a**

 **1a**	h5-HT_{5A} pK_i = 8.3 [^{35}S]GTPγS h5-HT_{5A} pA_2 = 8.5 h5-HT_7 pK_i = 7.8 log $D_{7.4}$ = 0 pK_a = 9.9 MAB (rat)[a] = 65% MAB (human)[b] = 100%

[a] Maximal achievable bioavailability estimated from incubations with rat liver microsomes.
[b] Human liver microsomes.

compounds **1a** and **2a**. Additionally, the measured lipophilicity did not exceed log D = 1.3, despite the lipophilic nature of substituents. The latter fact might be explained by the high basicity of the compounds (pK_a = 9.9–10.7), leading to a very low fraction of unionised compound under the near-neutral conditions (pH = 7.4) of the log D measurement.[9]

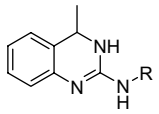
In a more systematic investigation, an attachment of lipophilic alkyl chains to the 2-amino function did not lead to higher log D values, as would be expected from Clog P calculations[10] predicting a lipophilicity increase of ~0.5log units per methylene unit (**2j**–**m**, Table 3). Moreover, the stabilities of the compounds in rat liver microsomes deteriorated with increasing chain lengths; this trend was less pronounced with human microsomes. We found that the attachment of small, lipophilic and electron-withdrawing fluoro-ethyl substituents was a superior way to improve log D, by increasing not only the intrinsic lipophilicity, log P, but also by lowering the high basicity (pK_a) of the guanidine core (**2o**, **2p**). The reduction of pK_a leads to a smaller pH-dependent term in Eq. 1[11] and thus contributes to the increase of the measured lipophilicity, log D.

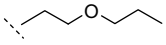
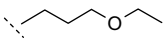
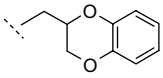
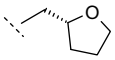
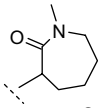
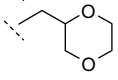
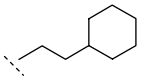
$$\log D = \log P - \log(1 + 10^{(\mathrm{pH}-\mathrm{p}K_a)}) \quad (1)$$

Whereas the monofluoro-ethyl (**2n**), and the trifluoro ethyl, substituent (**2p**) led to a ~10-fold drop in 5-HT_{5A}R affinity, the bis-fluoro-ethyl substituent (**2o**) gave an only slight decrease in affinity, a retained microsomal stability, a reduced basicity, and an improved lipophilicity.

The introduction of the difluoroethyl side chain into compounds with optimised aromatic substituents (see preceding publication) led to highly active compounds **1b**, **3**–**7** (Table 4) with improved physicochemical properties. The lipophilicity was especially increased in compounds carrying a lipophilic, electron-withdrawing aromatic chloro substituent, which further lowers the basicity of the guanidine core (**1b**, **3**, **6**, **7**), whereas compounds with 8-alkoxy substituents retain high basicities

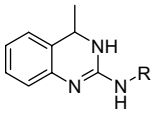
Table 2. Exploration of substituents R


2

Compound	R=	log $D_{7.4}$	MAB[a] (rat, %)	MAB[b] (human, %)	5-HT_{5A} K_i (nM)
2a[c]	H	−1.4	75	87	38
2b		0.1	1.5	55	241
2c		−0.3	2.1	41	176
2d		1.0	1.4	23	37
2e		−0.6	3.7	100	163
2f		−0.1	5.3	71	279
2g		−0.8	5.4	66	96
2h		1.3	1.3	5.1	124
2i		−0.6	46	83	122

[a] Maximal achievable bioavailability estimated from incubations with rat liver microsomes.
[b] Human liver microsomes.
[c] As HI salt.

Table 3. The low lipophilicity can be improved by substituents which lower the guanidine basicity

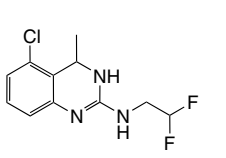


2

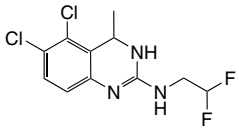
Compound	R=	log $D_{7.4}$	pK_a	MAB[a] (rat, %)	MAB[b] (human, %)	5-HT_{5A} K_i (nM)
2a[c]	H	−1.4	10.5	75	87	38
2j	Me	−0.6	10.6	77	100	103
2k[c]	Et	−0.9	10.8	68	93	196
2l	Pr	−0.8	10.7	58	82	153
2m	Bu	−0.3	10.7	17	74	189
2n	CH_2–CH_2F			Not determined		397
2o	CH_2–CHF_2	−0.2	9.7	78	100	87
2p	CH_2–CF_3	0.5	9.2	62	93	488

[a] Maximal achievable bioavailability estimated from incubations with rat liver microsomes.
[b] Human liver microsomes.
[c] As HI salt.

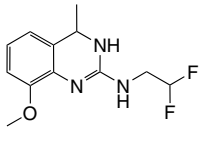
Table 4. Combination of aromatic substitution patterns leading to high affinity, with a difluoroethyl substituent leading to improved log D, pK_a



1b



3



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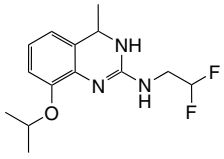

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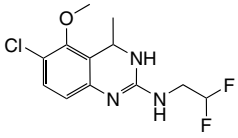

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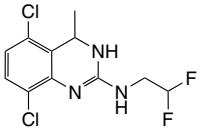


7

Compound	log $D_{7.4}$	pK_a	K_i (nM)				
			5-HT_{5A}	5-HT_{1A}	5-HT_{1D}	5-HT_{2C}	5-HT_7
1b[a]	1.5	8.9	10.1	85	1367	401	33.3
3[a,b]	2.7	8.5	5.7	124	630	162	23.3
(*S*)-**3**[a,c]	2.7	8.5	1.8	75	233	121	7.7
(*R*)-**3**	2.7	8.5	504	612	$>10^4$	238	1704
4[a]	−0.5	10.2	28.2	53	747	1658	38.9
5[a]	0.1	10.2	22.2	240	82	2145	4.2
6[a]	1.5	8.9	22.2	348	1810	233	17.6
7[a]	3.0	7.8	33.4	87	$>10^4$	675	366.3

[a] >30-fold selectivity over 5-HT_{2A}, 5-HT_3, 5-HT_6.
[b] hH_1 K_i = 18.3 nM.
[c] hH_1 K_i = 2.6 nM.

and low lipophilicities (**4**, **5**). Compound **1b** and **3** combined good physicochemical properties with a high 5-HT_{5A}R affinity. However, **3** carries the undesired 5,6-dichloro substitution pattern which we associated with high hH_1R affinity (see preceding publication).

Consequently, **1b** emerged as the best compound and was evaluated in a pharmacokinetic experiment in mice (Table 5). As anticipated, **1b** shows an improved brain-to-plasma ratio as compared to **1a** (20-fold increase), with otherwise similar pharmacokinetic properties, resulting in brain concentrations greater than 1 μM over 0.8–4 h after an oral dose of 6.6 mg/kg. However, this improvement was achieved at the expense of selectivity, as **1b** now had significant 5-HT_{1A} affinity (K_i: 85 nM, Table 4). Thus we had obtained two structurally related compounds **1a** and **1b**, the former having the better selectivity profile, the latter with substantially improved brain penetration.

The increased lipophilicity and relatively low basicity allowed a separation of the racemic **3** into enantiomers by chiral HPLC. An X-ray crystal structure was determined for the eutomer of **3**, which allowed the assignment of

Table 5. Pharmacokinetic properties of **1a** and **1b** (mice)

Compound	CL (ml/min/kg)	V_{ss} (l/kg)	$t_{1/2}$ (h)	C_{max} (ng/ml)	T_{max} (h)	F (%)	b/p	PB[a] (%)
1a	30[b]	9.0[b]	4.0[b]	660[c]	0.75[c]	59[c]	~0.2[c]	84
1b	31[b]	3.6[b]	1.4[b]	740[d]	0.25[d]	36[d]	~4[d]	92

Compound **1b** has an improved brain/plasma ratio (b/p), presumably due to its higher lipophilicity.
[a] Protein binding, determined in vitro.
[b] 5.0 mg/kg iv.
[c] 10.0 mg/kg po.
[d] 6.6 mg/kg po.

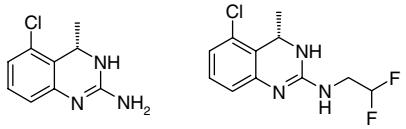
the (*S*)-configuration for this compound (Fig. 1). More polar compounds could not be separated by HPLC because of pronounced peak tailing and overlap. However, we were later able to circumvent this limitation by separation of the racemic thiourea precursors (e.g., **11**, Scheme 2) into their enantiomers, and the synthesis of enantiomerically pure target compounds from these intermediates. It was found that for all enantiomeric pairs, the 5-HT_{5A}R affinity resides principally in one of the enantiomers; the eudismic ratios were always greater than 100-fold (e.g., (*S*)-**3**, (*R*)-**3**, Table 4). In analogy to **3**, the configuration of the eutomers of **1a** and **1b** was assigned also as (*S*). The pharmacological profiles of (*S*)-**1a** and (*S*)-**1b** are summarised in Table 6.

Throughout these studies, numerous compounds were tested in functional assays and were found to be antagonists at the human 5-HT_{5A}R, with functional activities correlating with their binding constants. For instance, **1b** was found to be an antagonist in a [^{35}S]GTPγS assay, with a pA_2 value of 7.41 (pK_i 8.2). The binding affinities for the rat 5-HT_{5A}R were found to be similar to the affinities for the human receptor (e.g., Table 6).

All compounds were obtained in analogy to the methods described in the preceding publication. For instance, 2-aminoacetophenone (**8**) was converted via **9** into isothiourea **10**; nucleophilic replacement of the methylsulfanyl leaving group by amines then afforded dihydroquinazolines **2** (Scheme 1).

Compound **3** could be separated into enantiomers by preparative, chiral HPLC (chiralpak AD, ethanol/ heptane), whereas **1a**, **1b**, **4** and **5** could not be separated by this method because of their high polarity. However,

Table 6. In vitro pharmacological profile of the two best compounds, (*S*)-**1a** and (*S*)-**1b**



(*S*)-**1a** (*S*)-**1b**

	(*S*)-**1a**[a]		(*S*)-**1b**[a]	
	K_i (nM)	Selectivity	K_i (nM)	Selectivity
h5-HT_{5A}	1.6		6.8	
r5-HT_{5A}	1.3		2.9	
h5-HT_{1A}	95.2	58-Fold	56.0	8-Fold
h5-HT_{1D}	1097.9	669-Fold	1437.4	210-Fold
h5-HT_{2A}	257.0	157-Fold	491.0	72-Fold
h5-HT_{2C}	55.0	34-Fold	351.7	52-Fold
h5-HT_3	1062.3	648-Fold	N.D.	
h5-HT_6	1028.4	627-Fold	4476.2	655-Fold
h5-HT_7	6.0	4-Fold	24.8	4-Fold

[a] Affinities of *R*-enantiomers, h5-HT_{5A}: K_i ([*R*]-**1a**) = 415 nM; K_i ([*R*]-**1b**) = 1472 nM; N.D., not determined.

the cyclic thioureas (e.g., **11**, Scheme 2) as their synthetic precursors are less polar and were conveniently separated. The conversion of the enantiomerically pure thioureas, for example, (*S*)-**11** into the dihydroquinazolines according to Scheme 2, provided the enantiomerically pure final compounds, for example, (*S*)-**1b**. Racemisation during this conversion was not found to take place by analytical chiral HPLC.

In summary, the introduction of a difluoroethyl side chain improved the physicochemical properties of a no-

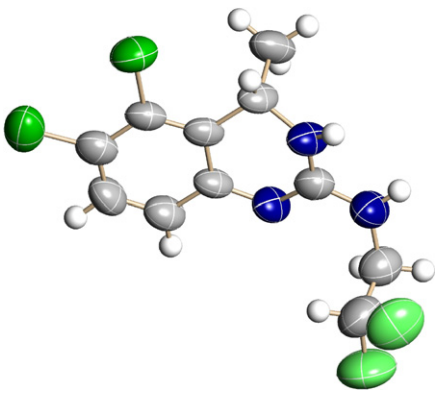
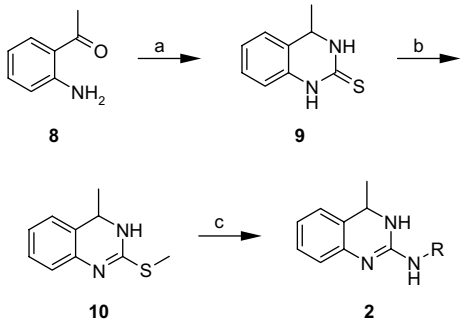
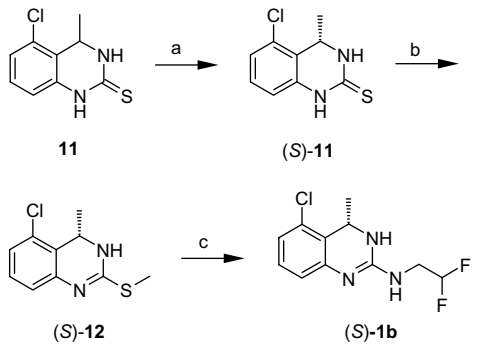


Figure 1. An X-ray crystal structure determination of the eutomer of **3** established its (*S*)-configuration.



Scheme 1. Preparation of dihydro-quinazolinylamines, **2**. Reagents and conditions: (a) $NaBH_4$, EtOH, 65 °C overnight; then KSCN, HCl, 3 h, 65 °C, 31%; (b) MeI (3 equiv), acetone, o.n. rt, 67%; (c) R–NH_2, CH_3CN, 80 °C, or microwave.



Scheme 2. Preparation of (*S*)-**1b**. Reagents and conditions: (a) chiral HPLC, separation of enantiomers; (b) MeI (3 equiv), acetone, weekend rt, 87%; (b) $H_2N–CH_2–CHF_2$, CH_3CN, **2d**, 80 °C, 46%.

vel series of guanidine-based 5-HT_{5A}R antagonists and led to a high brain-to-plasma ratio. (*S*)-**1a** and (*S*)-**1b** emerged as the two best compounds from our studies. (*S*)-**1a** is a high affinity 5-HT_{5A}R antagonist with more than 30-fold selectivity over related receptors (except 5-HT_7). The structurally related **1b** shows a high brain-to-plasma ratio but (*S*)-**1b** is somewhat less selective than (*S*)-**1a**. Compounds in this series were so far not found to be selective over the 5-HT_7R and are thus dual 5-HT_{5A}/5-HT_7R ligands. Additional structural modifications will be required to identify 5-HT_{5A}R antagonists with an improved selectivity profile, including selectivity towards 5-HT_7. Such compounds would be valuable pharmacological tools to further elucidate the physiological role of the 5-HT_{5A} receptor.

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