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Structure–activity relationships of N-substituted piperazine amine reuptake inhibitors

M. Jonathan Fray,* Gerwyn Bish, Paul V. Fish, Alan Stobie, Florian Wakenhut and Gavin A. Whitlock

Sandwich Chemistry, Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

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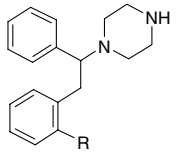
Abstract—We report the structure–activity relationships of further analogues in a series of piperazine derivatives as dual inhibitors of serotonin and noradrenaline reuptake, that is, with additional substitution of the phenyl rings, or their replacement by heterocycles. The enantiomers of compounds **1** and **2** were also profiled, and possessed drug-like physicochemical properties. In particular, compound (−)-**2** lacked potent inhibitory activity against any of the important cytochromes P_{450} and high selectivity over a wide range of receptors, which is unusual for a compound that inhibits human amine transporters.

In the preceding paper, we reported the discovery of a series of N-substituted piperazines, for example **1** and **2**, as inhibitors of serotonin (5-HT) and noradrenaline (NA) reuptake, with selectivity over the dopamine (DA) transporter.[1] Such compounds have potential utility in treating depression and stress urinary incontinence.[2]



1 R = Cl
2 R = OEt

We report here the structure–activity relationships of further analogues in the series: additional substitution of the phenyl rings, or their replacement by heterocycles (compounds **3**–**22**); the spatial requirements of the two aromatic rings in relation to the piperazine (compounds **23**, **24**); and replacement of the piperazine ring by homopiperazine (compounds **25**, **26**). The enantiomers of compounds **1** and **2** have also been separated and profiled.

Compounds **3**–**22** were conveniently prepared by the methods previously reported (Scheme 1).[1,3,4]

Thus, condensation of the appropriate aryl or heterocyclic aldehyde,[5] *N*-Boc-piperazine and benzotriazole in refluxing toluene with azeotropic removal of water afforded intermediates **27**, which were not isolated, but added directly as a solution in toluene to a solution of the relevant benzylic Grignard reagent (2 eq.) or zinc reagent.[6] The resulting *N*-Boc piperazines were then deprotected under standard conditions to give **3**–**22**. Compounds **23** and **24** were prepared in the same way, using phenethylmagnesium bromide as the Grignard component for the former and phenylacetaldehyde instead of benzaldehyde for the latter. Replacement of *N*-Boc piperazine with *N*-Boc homopiperazine afforded compounds **25** and **26**. The structures of all the compounds made are shown in Table 1 and Figure 1.

We also separated[7] and profiled the enantiomers of compounds **1** and **2**, and, for compound **2**, determined the absolute configuration of the (−) isomer to be (*R*).[8]

Compounds **3**–**26** were assayed for their ability to inhibit the uptake of [^{3}H]5-HT, [^{3}H]NA and [^{3}H]DA in HEK293 cells expressing a single human amine transporter.[9] Reference data were measured for fluoxetine, reboxetine, and duloxetine. Results for **1**–**22** are shown in Table 1 and will be discussed first; those for **23**–**26** and the enantiomers of **1** and **2** are in Figures 1 and 2, respectively.

In the series of analogues **3**–**10**, where the chlorophenyl ring of **1** was replaced, most of the structural changes

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* Corresponding author. Tel.: +44 0 1304 649175; fax: +44 0 1304 656776; e-mail: jonathan.fray@pfizer.com

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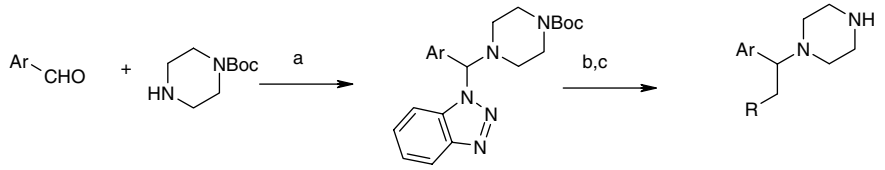
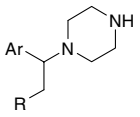


27 3-22

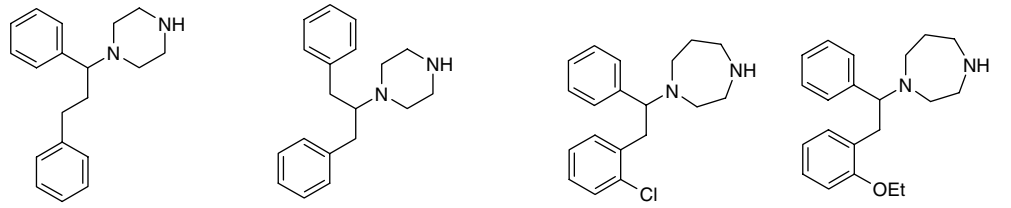
Scheme 1. Reagents and conditions: (a) benzotriazole, toluene, Dean–Stark apparatus, reflux 6–24 h; (b) RCH_2MgHal, THF, −78–0 °C, 30 min or RCH_2ZnHal, THF, 20 °C, 3 h, 35–80%; (c) 50% TFA, CH_2Cl_2, 20 °C, 80–95%.

Table 1. Inhibition of human amine reuptake by N-substituted piperazines



Compound	IC_{50} (nM)				
	Ar	R	5-HT	NA	DA
1	Ph	2-Cl phenyl	5.4	22	1300
2	Ph	2-OEt phenyl	13	16	>4000
3	Ph	2-Naphthyl	17	>400	NT
4	Ph	1-Naphthyl	12	>400	NT
5	Ph	2-Pyridyl	230	>400	NT
6	Ph	4-Pyridyl	38	>400	NT
7	Ph	2,3-Cl_2 phenyl	3.9	100	NT
8	Ph	2,4-Cl_2 phenyl	11	>400	>4000
9	Ph	2,5-Cl_2 phenyl	8.2	120	>4000
10	Ph	2,6-Cl_2 phenyl	12	82	970
11	3-Pyridyl	2-Cl phenyl	14	75	9200
12	3-Pyridyl	2-OEt phenyl	46	15	>4000
13	2-Thiazolyl	2-Cl phenyl	79	45	NT
14	2-Thiazolyl	2-OEt phenyl	39	340	NT
15	5-Thiazolyl	2-Cl phenyl	12	120	NT
16	5-Thiazolyl	2-OEt phenyl	40	21	NT
17	2-F phenyl	2-Cl phenyl	30	130	NT
18	2-F phenyl	2-OEt phenyl	10	38	NT
19	3-F phenyl	2-Cl phenyl	15	25	2300
20	3-F phenyl	2-OEt phenyl	28	22	NT
21	4-F phenyl	2-Cl phenyl	13	94	1600
22	4-F phenyl	2-OEt phenyl	17	28	NT
Fluoxetine			16	5200	4400
Reboxetine			590	11	>25,000
Duloxetine			6.0	19	870

Note: IC_{50} values are a geometric mean of at least $n = 4$. A difference of <2-fold should not be considered significant. NT, not tested.



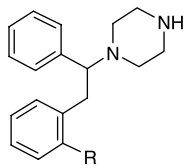
23 5-HT IC_{50} = 23 nM; NA IC_{50} >400 nM

24 5-HT IC_{50} = 23 nM; NA IC_{50} = 48 nM; DA IC_{50} = 400 nM

25 5-HT IC_{50} = 42 nM; NA IC_{50} = 76 nM

26 5-HT IC_{50} = 300 nM; NA IC_{50} = 8.8 nM

Figure 1. Inhibition of human amine reuptake by compounds **24**–**26**.



1 R = Cl
2 R = OEt

Comp.	IC_{50}s (nM) 5-HT	NA	DA
(±)-**1**	5.4	22	1300
(+)-**1**	13	50	1600
(−)-**1**	3.9	19	2900
(±)-**2**	13	16	>4000
(*S*)(+)-**2**	9.8	19	>4000
(*R*)(−)-**2**	13	16	>4000

Figure 2. Inhibition of human amine reuptake by compounds **1**, **2**, and their enantiomers.

(benzene annelation, replacement with pyridyl, additional chlorine atom) had a dramatically deleterious effect on potency against the NA transporter, whereas good potency against the 5-HT transporter was frequently retained. These data confirm, as we had observed in other analogues,[1] that the NA transporter cannot tolerate much steric bulk (as in naphthyl) or polarity (as in pyridyl) in the substituent R. Only compounds **7** and **9** possessed comparable potency to **1** against the 5-HT transporter, but were still 5- to 6-fold less potent than **1** against the NA transporter. The next part of Table 1 shows the results for compounds **11**–**22**, in which the 2-chlorophenyl or 2-ethoxyphenyl substituent was retained, and the other phenyl ring replaced by heterocycles or substituted with a single fluorine atom. Overall, these alterations generally led to a loss of potency against both the 5-HT and NA transporters. Compound **1** had an IC_{50} ratio 5-HT/NA 1:4, whereas for compound **2** this ratio is near unity. The nature of the heterocyclic group affected the relative potencies, as discussed below. For example, the 3-pyridyl and 5-thiazolyl analogues (**11**, **15**) with a chlorophenyl substituent retained a similar IC_{50} ratio to **1** (i.e. 5-HT/NA 1:4–6) at about 3-fold lower potency. In the same way that **1** was 3-fold more potent vs. the 5-HT transporter than **2**, **11**, and **15** were 3–4 more potent than **12** and **16**, respectively, against the 5-HT transporter; however, the corresponding ethoxy analogues (**12**, **16**) showed enhanced potency (5- to 6-fold) for the NA transporter compared with **11** and **15**. Consequently, **12** and **16** demonstrated 2- to 3-fold selectivity for the NA transporter compared to **11** and **15**, which were selective for the 5-HT transporter. In the series of fluorophenyl analogues **17**–**22**, the compounds were 2- to 6-fold less potent than **1** versus 5-HT; vs. the NA transporter, the ethoxy analogues (**18**, **20**, **22**) were $\leqslant$2-fold less potent than **2**, whereas in the chloro analogues (**17**, **19**, **21**) the fluoro substituent was clearly preferred in the 3-position of the phenyl ring. None of the analogues' profiles was superior to the parent analogues **1** and **2**, although all the compounds that were screened against the DA transporter had low potency.

Figure 1 shows the results for compounds **23**–**26**. Both compounds **23** and **24** were reasonably potent against the 5-HT transporter (IC_{50} = 23 nM). The former was very significantly less active versus NA (IC_{50} > 400 nM), and the latter was moderately potent versus DA, so neither analogue was considered a very interesting lead. Likewise, the homopiperazines **25** and **26** were not progressed further, as, although **25** possessed a balanced profile, it lacked potency, and **26** possessed significant selectivity for the NA transporter.

Figure 2 shows the potency of the enantiomers of compounds **1** and **2** compared with the racemates. The enantiomers in both series possessed significant activity. In the case of compound **1**, the (−)-isomer was about 3-fold more potent than the (+)-enantiomer against both 5-HT and NA transporters, but around 2-fold less potent versus the DA transporter. The differences in potency for the ethoxy enantiomers are probably not significant. It is interesting to note that the enantiomers of duloxetine both have similar potencies for 5-HT and NA reuptake inhibition.[10]

Further profiling was conducted on (+)-**1**, (−)-**1**, (+)-**2** and (−)-**2**. All four compounds possess moderate lipophilicity ($\log D_{7.4\ \text{octanol}}$ = 1.8 and 1.5 for the chloro and ethoxy analogues, respectively), and formed stable, high-melting, crystalline hydrochloride salts with good aqueous solubility (1–5 mg/ml at pH 7.4). Compounds (±)-**1**, (+)-**2**, and (−)-**2** demonstrated rapid flux rates across Caco2 cells (Table 2), without evidence for any affinity for efflux transporter proteins, suggesting that absorption in vivo should be complete. The set of enantiomers was profiled against a panel of cytochrome P_{450} (CYP) enzymes in comparison with known inhibitors (Table 2). Although (+)-**1** and (−)-**1** were weak inhibitors of CYPs 3A4, 2C9, 2C19, and 1A2, they were both potent inhibitors of 2D6 (comparable potency to fluoxetine). In contrast, (+)-**2** and (−)-**2** were both relatively weak inhibitors of 2D6 (comparable potency to venlafaxine), as well as the other CYPs. One interesting property of all the enantiomers was that they demonstrated good metabolic stability in human liver microsome preparations, but very poor stability when incubated with rat or dog liver microsomes (Table 2). The reason for these differences is not understood, but may indicate the compounds are metabolised by different enzymes in different species.

Further data obtained with (−)-**2** indicated it possessed >100-fold selectivity for 5-HT/NA transporters over a wide variety of G-protein coupled receptors, including adrenergic, dopaminergic, muscarinic, nicotinic, and opiate receptors. It did, however, demonstrate modest binding affinity for sodium channels (site 2, IC_{50} 0.41 μM), calcium channels (L-type, diltiazem site, IC_{50}

Table 2. Caco flux rates, metabolic half-lives, and CYP inhibition IC_{50} data

Compound	Caco flux[a]	HLM[b] $t_{1/2}$ (min)	RLM[c] $t_{1/2}$ (min)	DLM[d] $t_{1/2}$ (min)	CYP inhibition IC_{50} (μM)				
					2D6	3A4	1A2	2C9	2C19
(±)-**1**	20/17	120	NT[e]	NT	NT	NT	NT	NT	NT
(+)-**1**	NT	>120	2	NT	0.6	>30	>30	23	14
(−)-**1**	NT	>120	2	NT	0.5	>30	26	23	14
(+)-**2**	30/41	76	4	9	10	10	NT	NT	NT
(−)-**2**	33/36	96	5	4	30	30	>30	>30	>30

Notes to Table 2.

[a] Flux across Caco2 cells was measured at 25 μM concentration. Figures quoted correspond to the initial flux rates ($\times 10^{-6}$ cm^{-1}) for apical to basolateral (first figure) and basolateral to apical (second figure) sides of the cells.

[b] HLM, human liver microsomes; the maximum $t_{1/2}$ measurable was 120 min.

[c] RLM, rat liver microsomes.

[d] DLM, dog liver microsomes.

[e] NT, not tested.

0.73 μM) and the hERG potassium channel (dofetilide binding IC_{50} 16 μM).

We have described the structure–activity relationships for a series of N-alkylated piperazine derivatives. Although none of the new analogues were superior to either compound **1** or **2**, the profiles of the enantiomers of **2** were very promising. It is notable that both enantiomers had very similar profiles and possessed good, drug-like physicochemical properties. Compound (−)-**2** lacked potent inhibitory activity against any of the important $CYPs_{450}$ and possessed high selectivity over a wide range of receptors, which is unusual for a compound that inhibits human amine transporters.

Acknowledgments

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References and notes

1. Fray, M. J.; Bish, G.; Brown, A. D.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A.; *Bioorg. Med. Chem. Lett.*, preceding paper.
2. (a) Reith, M. E. A. *Neurotransmitter Transporters: Structure, Function and Regulation*, 2nd ed., Humana Press: Totowa, NJ, 2002; (b) Iversen, L. *Mol. Psychiatry* **2000**, *5*, 357; (c) Schatzberg, A. F. *J. Clin. Psychiatry* **2000**, *61*(Suppl. 11), 9; (d) Barker, E. L.; Blakely, R. D. In *Psychopharmacology: The Fourth Generation of Progress*; Bloom, F. E., Kupfer, D. J., Eds.; Raven Press: Philadelphia, 1995; p 21; (e) Bump, R. C.; Norton, P. A.; Zinner, N. R.; Yalcin, I. *Obstet. Gynecol.* **2003**, *102*, 76.
3. Katritzky, A. R.; Yannakopoulou, K.; Lue, P.; Rasala, D.; Urogdi, L. *J. Chem. Soc. Perkin Trans. 1* **1989**, 225.
4. Katritzky, A. R.; Strah, S.; Belyakov, S. A. *Tetrahedron* **1998**, *54*, 7167.
5. Dondoni, A.; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P. *Synthesis* **1987**, 998, now available commercially.
6. *N*-Boc piperazine and *N*-Boc homopiperazine were purchased from Sigma–Aldrich. The organometallic reagents used were either obtained commercially or generated from the commercial benzyl/naphthylmethyl/picolyl chloride or bromide immediately prior to use.
7. HPLC conditions: for (+)-**1**, (−)-**1**: Chiralcel OJ column, eluting with hexane/ethanol/diethylamine 80:20:0.2, detection at 254 nm; for (+)-**2** and (−)-**2** Chiralcel OD column, eluting with hexane/isopropanol/diethylamine 70:30:0.3, detection at 254 nm.
8. Absolute configuration was determined by a single crystal X-ray diffraction study of the edisylate salt, grown by slow evaporation from methanol.
9. The assays were a modification of those described by Blakely, R. D.; Clark, J. A.; Rudnick, G.; Amara, S. G. *Anal. Biochem.* **1991**, *194*, 302, HEK293 cells expressing a single human amine transporter protein (7500 cells/well in Millipore 96-well filter bottom plates) were pre-incubated at 25 °C for 5 min with assay buffer containing vehicle (DMSO in water) or test compound. Uptake of neurotransmitter into the cells was initiated by the addition of tritiated 5-HT (50 nM), NA (200 nM) or DA (200 nM) substrates, the samples were shaken in an incubator at 25 °C for 5 min (5-HT, DA) or 15 min (NA). The assays were stopped by an ice-cold buffer wash followed by filtration. The filters were then dried before measuring the amount of radioactivity taken up into the cells by scintillation counting. Potency of test compounds was quantified as IC_{50} values, i.e. concentration required to inhibit the specific uptake of radiolabelled substrate into the cells by 50% relative to maximum (vehicle only) over a 10-point dose response range. A minimum of four measurements of the IC_{50} were made. Typically, the IC_{50}s did not fit a normal distribution. Thus, geometric means were used to minimise the distorting effect of outliers. From validation data sets on all three transporter assays, we established that the 95% confidence intervals were 1.3- (5-HT, DAT) to 1.5-fold (NA) of the IC_{50}, i.e. IC_{50} = 4 nM would have 95% confidence intervals of 1.4–6.6 nM.
10. (a) Boot, J. R.; Brace, G.; Delatour, C. L.; Dezutter, N.; Fairhurst, J.; Findlay, J.; Gallagher, P. T.; Hoes, I.; Mahadevan, S.; Mitchell, S. N.; Rathwell, R. E.; Richards,

S. J.; Simmonds, R. G.; Wallace, L.; Whatton, M. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5395; (b) Bymaster, F. P.; Beedle, E. E.; Findlay, J.; Gallagher, P. T.; Krushinski, J. H.; Mitchell, S. N.; Robertson, D. W.; Thompson, D. C.; Wallace, L.; Wong, D. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4477.