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Design and synthesis of morpholine derivatives. SAR for dual serotonin & noradrenaline reuptake inhibition

Paul V. Fish,[a,*] Christopher Deur,[c] Xinmin Gan,[c] Keri Greene,[c] David Hoople,[b] Malcolm Mackenny,[a] Kimberly S. Para,[c] Keith Reeves,[b] Thomas Ryckmans,[a] Cory Stiff,[c] Alan Stobie,[a] Florian Wakenhut[a] and Gavin A. Whitlock[a]

[a]*Department of Genitourinary Chemistry, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK*
[b]*Department of Synthetic Services, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK*
[c]*Department of Chemistry, Pfizer Global Research & Development, Ann Arbor Laboratories, Ann Arbor, MI 48105, USA*

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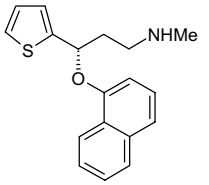
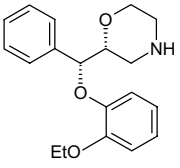
Abstract—Single enantiomer (*SS*) and (*RR*) 2-[(phenoxy)(phenyl)methyl]morpholine derivatives **5**, **8**–**23** are inhibitors of monoamine reuptake. Target compounds were prepared using an enantioselective synthesis employing a highly specific enzyme-catalysed resolution of racemic *n*-butyl 4-benzylmorpholine-2-carboxylate (**26**) as the key step. Structure–activity relationships established that serotonin and noradrenaline reuptake inhibition are functions of stereochemistry and aryl/aryloxy ring substitution. Consequently, selective SRI, selective NRI and dual SNRIs were all identified. One of these compounds, a potent and selective dual SNRI, (*SS*)-**5a** was selected as a candidate for further pre-clinical evaluation.

Selective inhibition of serotonin (5-HT) and noradrenaline (NA) reuptake (SNRI) has been shown to be an attractive dual pharmacology approach for the treatment of a number of diseases.[1,2] For example, dual 5-HT/NA reuptake inhibitor duloxetine (**1**) has shown clinical efficacy in the treatment of depression, pain, and urinary incontinence.[3,4]



1: duloxetine



2: (±)-reboxetine

As part of our research efforts to identify potential drug candidates, we have recently reported SNRIs derived from piperazine,[5] 3-amino-pyrrolidine,[6] and benzylamine[7] templates. In this letter, we disclose some factors that modulate 5-HT and NA reuptake inhibition in a morpholine scaffold as a surrogate for the biogenic amine.

Reboxetine (**2**), a selective NA reuptake inhibitor used clinically in the treatment of depression,[8] was selected as a suitable chemical starting point. As an initial venture, we elected to introduce a 3-Cl and a 4-Cl substituent onto the aryloxy ring of reboxetine to explore the effect upon SRI and NRI activity.[6,7] In addition, the influence of the two vicinal chiral centres on monoamine reuptake inhibition was explored by the preparation of these targets as racemic, single diastereoisomers **3**–**5**.[9]

The combination of the 2-OEt-4-Cl with the *syn* diastereoisomer **4ab** furnished a promising lead as SRI activity had been introduced and NRI activity retained giving a dual SNRI with 25-fold selectivity over dopamine (DA) reuptake inhibition (DRI) (Table 1). The 2-OEt-3-Cl, *syn* diastereoisomer **3ab** was also a balanced SNRI but with weaker affinity at both transporters. The SRI and NRI profiles of the corresponding 2-OMe-4-Cl analogues **5ab** and **5cd** were similar to those of **4ab** and

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* Corresponding author. Tel.: +44 (0) 1304 644589; fax.: +44 (0) 1304 651987; e-mail: paul.fish@pfizer.com

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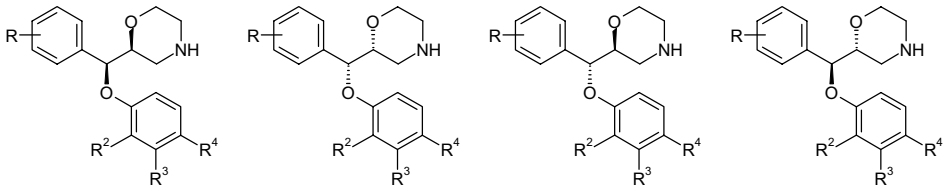
Table 1. In vitro inhibition of monoamine reuptake for racemic diasteroisomers (**3**–**5**) and single enantiomers (**5**, **8**–**23**)[a,b,c]



3-5, 8-23 (*SS*)-*syn*-**a** | **3-5, 8-23** (*RR*)-*syn*-**b** | **3-5, 8-23** (*RS*)-*anti*-**c** | **3-5, 8-23** (*SR*)-*anti*-**d**

Compound	R	R^2, R^3, R^4	Stereochemistry	5-HT K_i (nM)	NA K_i (nM)	DA K_i (nM)	*c*log *P*
duloxetine			*S*	5	45	435	4.26
reboxetine	H	2-OEt	*SS*/*RR*	1400	5	>10,000	3.26
3ab	H	2-OEt, 3-Cl	*SS*/*RR*	240	275	>10,000	3.82
3cd	H	2-OEt, 3-Cl	*RS*/*SR*	340	1490	>10,000	3.82
4ab	H	2-OEt, 4-Cl	*SS*/*RR*	60	15	1540	4.05
4cd	H	2-OEt, 4-Cl	*RS*/*SR*	220	390	1670	4.05
5ab	H	2-OMe, 4-Cl	*SS*/*RR*	28	36	5050	3.52
5cd	H	2-OMe, 4-Cl	*RS*/*SR*	85	320	2200	3.52
5a	H	2-OMe, 4-Cl	*SS*	110	8	1570	3.52
5b	H	2-OMe, 4-Cl	*RR*	11	420	1790	3.52
8a	H	2-OMe, 4-F	*SS*	740	6.0	3080	2.95
8b	H	2-OMe, 4-F	*RR*	22	610	NT	2.95
9a	H	2-Cl, 4-F	*SS*	260	12	1170	3.81
9b	H	2-Cl, 4-F	*RR*	20	830	760	3.81
10a	H	2-Me, 4-F	*SS*	185	18	1250	3.78
10b	H	2-Me, 4-F	*RR*	34	860	950	3.78
11a	H	2-F, 3-F	*SS*	3390	10	2600	3.20
11b	H	2-F, 3-F	*RR*	12	130	380	3.20
12a	H	2,3-OCH_2CH_2-	*SS*	1800	12	NT	3.22
12b	H	2,3-OCH_2CH_2-	*RR*	20	350	5750	3.22
13a	H	2-Cl, 3-Cl	*SS*	100	17	420	4.26
14a	H	2-OMe, 4-CN	*SS*	110	210	NT	2.48
15a	H	2-OMe, 4-Br	*SS*	30	12	NT	3.67
16a	H	2-OMe, 4-CF_3	*SS*	41	160	NT	3.82
17a	H	2-Cl, 4-OMe	*SS*	96	28	1090	3.64
18a	2-F	2-OMe, 4-Cl	*SS*	70	22	NT	3.66
19a	3-F	2-OMe, 4-Cl	*SS*	58	9	10,800	3.66
20a	4-F	2-OMe, 4-Cl	*SS*	62	29	NT	3.66
21a	2-Me	2-OMe, 4-Cl	*SS*	70	84	NT	3.97
22a	3-Me	2-OMe, 4-Cl	*SS*	69	16	NT	4.02
23a	4-Me	2-OMe, 4-Cl	*SS*	88	180	1650	4.02

[a] Nomenclature used throughout: **a** = *SS*; **b** = *RR*; **c** = *RS*; **d** = *SR*; **ab** is the racemic *SS*/*RR* *syn*-diastereoisomer.
[b] See Ref. 25 for details of assay conditions. Monoamine reuptake K_i values are geometric means of at least three experiments. Differences of <2-fold should not be considered significant.
[c] NT, not tested.

4cd, with again a preference for the *syn* diastereoisomer **5ab**. This modification of the aryloxy ring (i.e., **4ab** → **5ab**) brought a 2-fold increase in SRI activity resulting in a potent, balanced SNRI with >100-fold selectivity over DRI. The SAR within these compounds **3**–**5** showed the *anti* diastereoisomers to be inferior in both SRI and NRI activity compared to the *syn* and were discontinued.

Compound **5ab** has physicochemical properties consistent with CNS target space[10] (m. wt. 333; *c*log *P* 3.52; log $D_{7.4}$ 1.9; pK_a 7.7; HBD 1; HBA 4; PSA 40 Å^2)[11] and was selected as a lead for our drug discovery programme.

Caution must be exercised when evaluating SAR from the biological activities of racemic compounds as the contribution to the observed effects may not be equal for each enantiomer; this is further complicated when seeking dual activities at two biological targets.[12] Hence, compound **5ab** was then prepared as the two single enantiomers (*SS*)-**5a** and (*RR*)-**5b** so as to determine the influence of absolute stereochemistry on the SRI and NRI activity.

Target compounds **5**, **8**–**23** (Table 1) were prepared using a new enantioselective synthesis starting from the racemic morpholine ester **7** (Fig. 1).[13] This route was attractive to us as it allowed for the late stage installation of both the aryl and aryloxy rings. The key (*R*) and (*S*) morpholine acids **6** were efficiently prepared on large scale by an enzyme-catalysed resolution of racemic ester **7**.

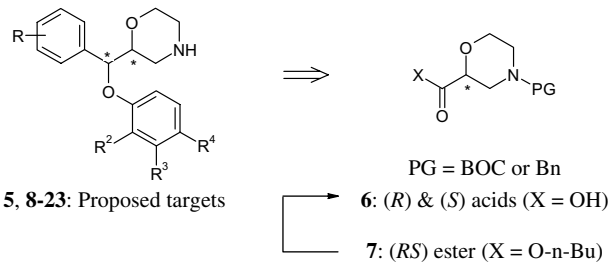


Figure 1. Target compounds, retrosynthesis and homochiral synthons.

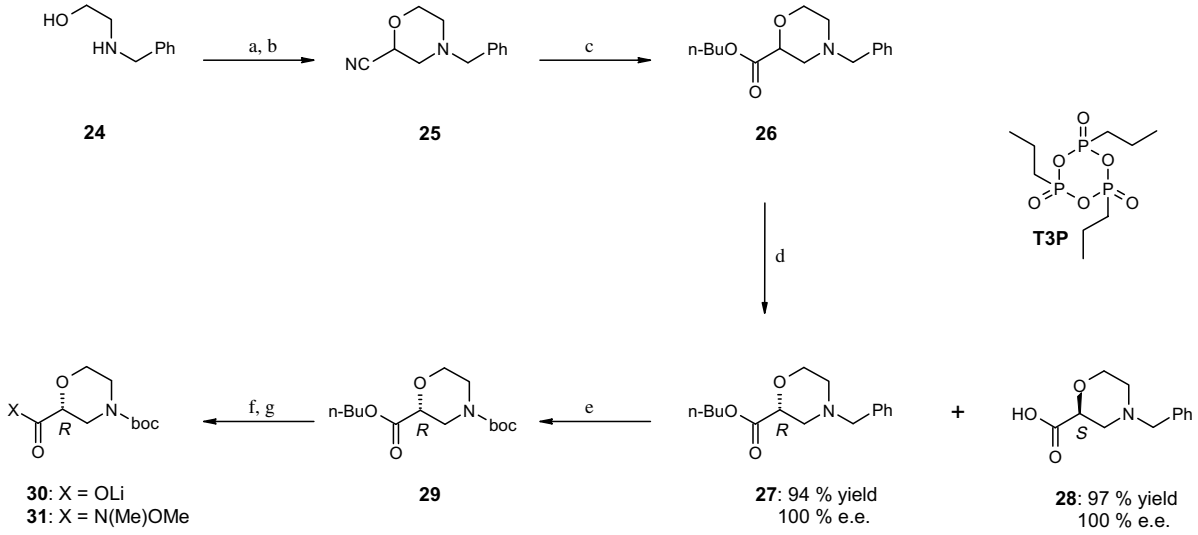
Racemic ester **26**, prepared in two steps from *N*-benzylethanolamine (**24**) and chloroacrylonitrile,[14] was screened against a panel of lipases (Scheme 1). Lipase Candida rugosa[15] was completely stereospecific, catalyzing the hydrolysis of the (*S*)-ester to give (*S*)-acid **28** whilst leaving the (*R*)-ester **27** untouched.[16,17] The *N*-benzyl protecting group of **27** was then exchanged for the *N*-BOC amine **29** as it had a beneficial effect on the yields of subsequent reactions and simplified deprotection at the final step. Base hydrolysis of the *n*-butyl ester **29** gave the acid as the Li salt **30**, and activation of **30** with 1-propanephosphonic anhydride (T3P) followed by the reaction with HN(Me)OMe gave Weinreb amide **31**. Treatment of **31** with Grignard reagents ArMgX gave the aryl ketones **32** with no detectable loss in *e.e.* (Scheme 2). Reduction of **32** with $Zn(BH_4)_2$ created the second stereocentre giving alcohol **33** with good diastereoselectivity (*RS*:*RR* 16:1), and then the reaction of **33** with $MeSO_2Cl$ gave mesylate **34**. The displacement of the MsO group of **34** with phenols ArOH gave the corresponding (*RR*)-aryloxy ethers **35** in good yield and with complete inversion of the benzylic stereocentre, and finally deprotection of the *N*-BOC amine with HCl afforded the (*RR*)-target compounds **5**,**8**–**23**. The (*SS*)-enantiomers were prepared by an identical sequence but starting with the (*S*)-acid **28**.

The absolute stereochemistry of **5**[17] was found to have a significant effect on activity (Table 1): (*SS*)-**5a** was a potent NRI with SRI activity, whereas (*RR*)-**5b** was a selective SRI. This split in SRI versus NRI activity was also observed with five additional pairs of enantiomers **8**–**12**. In some cases, selective SRI (e.g., **9b**) and selective NRI (e.g., **8a**, **11a**) were achieved. Overall, there was a broad range in both SRI and NRI activities and the ratios of activity (SRI:NRI, 350:1 to 1:40).

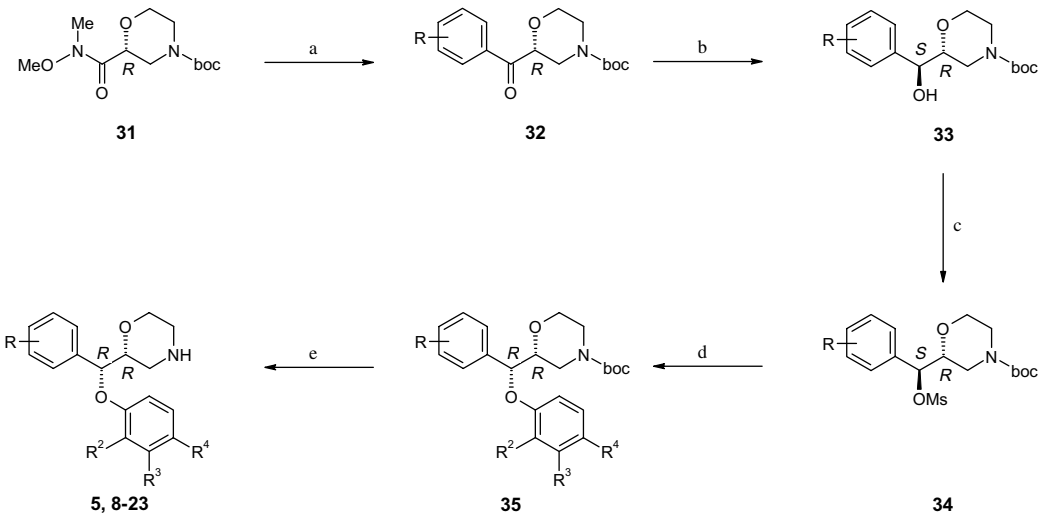
Both **5a** and **5b** were screened for off-target pharmacology against a panel of 70 receptors, enzymes and ion channels. Compound **5b** was found to have significant binding affinity for the histamine H1 receptor[18] (K_i = 26 nM), whereas **5a** was inactive at H1 (<20% at 10 μM). It is of note that neither **5a** or **5b** had confirmed functional activity against any other target in this panel at ⩽3 μM. It was subsequently determined that **8b**–**12b** also had affinity for the H1 receptor (K_i = 10–200 nM), whereas **8a**–**12a** were inactive. The modest NRI activity combined with H1 receptor affinity resulted in the discontinuation of the (*RR*)-series.

Further SAR was directed at improving SRI activity in the (*SS*)-series by exploring a broader set of substituents on the aryloxy ring **13a**–**17a**. Increasing the size of the group at the 4-position gave **15a** (4-Br) as a potent, balanced dual SNRI, although the introduction of 4-CF_3 **16a** inverted the SNRI profile with a greater affinity for the 5-HT transporter. Substitution on the aryl ring was investigated by the introduction of a F or Me at the 2-, 3-, or 4-positions of **18a**–**23a** (see Table 1).

From these experiments, **5a** emerged as having a desirable combination of dual NRI and SRI activity, good selectivity over DRI, and no significant off-target pharmacology. Furthermore, **5a** was one of the least lipophilic structures of those presented in Table 1. Reducing lipophilicity was an important selection criteria for this programme.[19] Compound lipophilicity was initially assessed by the calculation of partion coefficients (*c*log*P*) and then confirmed for selected examples



Scheme 1. Reagents and conditions: (a) CH_2=C(Cl)CN, Et_2O, 40 °C, quant.; (b) *t*-BuOK, DME, reflux 65%; (c) *n*-BuOH, *c*. H_2SO_4, reflux, 85%; (d) Candida rugosa, *t*-BuOMe–H_2O, rt, 42 h; (e) 2,5-dihydrotoluene, 10%-Pd/C, BOC_2O, EtOH, reflux, quant.; (f) LiOH (0.95 equiv), THF-H_2O, 0 °C, 90%; (g) T3P, HN(Me)OMe, NEt_3, CH_2CH_2, then aq K_2CO_3, 85%.



Scheme 2. Reagents and conditions: (a) ArMgBr, THF, rt, 98%; (b) $Zn(BH_4)_2$, Et_2O, rt, 87%; (c) $MeSO_2Cl$, NEt_3, CH_2Cl_2, 0 °C, quant.; (d) ArOH, Cs_2CO_3, microwave in THF or reflux in dioxane, 95%; (e) HCl in dioxane, CH_2Cl_2, 95%.

by the measurement of the octanol-buffer distribution coefficient (**5a**: $\log D_{7.4}$ 1.9).

Additional screening[20] in vitro showed **5a** to have good membrane permeability (CaCO-2 18/16) with low affinity for P-gp efflux transporters (MDCK-mdr1 22/35) suggesting the potential for good oral absorption and CNS penetration.[10] Compound **5a** had good metabolic stability in human liver microsomes ($t_{1/2}$ > 120 min) and human hepatocytes ($t_{1/2}$ 186 min) consistent with low predicted clearance. Compound **5a** had no significant inhibition of CYP450 enzymes (1A2, 2C9, 2C19, 2D6, 3A4; IC_{50} > 29 μM) and modest ion channel activity as measured by binding to potassium hERG ([^{3}H]-dofetilide, K_i 3.3 μM), sodium (site 2, K_i 1.2 μM) and calcium (L-type diltiazem site, K_i 2.0 μM) channels.

Further pharmacological evaluation in vivo, in microdialysis experiments,[21,22] showed **5a** to increase both NA and 5-HT levels in interstitial fluid of the prefrontal cortex of conscious rats by 170% and 160%, respectively, above pre-drug baseline levels (10 mg/kg administered *s.c.*, $n = 6$).

Based on this profile, dual SNRI **5a** (PF-734629)[23] was selected as a candidate for further evaluation in pre-clinical disease models. The results of these studies will be reported in future publications.[24,25]

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References and notes

1. (a) Montgomery, S. *Int. J. Psych. Clin. Pract.* **2006**, *10*, 5; (b) Baldwin, D. S. *Int. J. Psych. Clin. Pract.* **2006**, *10*, 12; (c) Wernicke, J. F.; Iyengar, S.; Ferrer-Garcia, M. D. *Curr. Drug Therapy* **2007**, *2*, 161; (d) Jackson, S. *Curr. Med. Res. Opin.* **2005**, *21*, 1669.
2. For recent reviews of new chemical entities, see: (a) Walter, M. W. *Drug Dev. Res.* **2005**, *65*, 97; (b) Huang, Y.; Williams, W. A. *Expert Opin. Ther. Patents* **2007**, *17*, 889.
3. Thor, K. B.; Kirby, M.; Viktrup, L. *Int. J. Clin. Pract.* **2007**, *61*, 1349.
4. Steers, W. D.; Herschorn, S.; Kreder, K. J.; Moore, K.; Strohbehn, K.; Yalcin, I.; Bump, R. C. *BJU Int.* **2007**, *100*, 337.
5. (a) Fray, M. J.; Bish, G.; Brown, A. D.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4345; (b) Fray, M. J.; Bish, G.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4349.
6. Fish, P. V.; Fray, M. J.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2022.
7. Whitlock, G. A.; Blagg, J.; Fish, P. V. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 596.
8. Hajos, M.; Fleishaker, J. C.; Filipiak-Reisner, J. K.; Brown, M. T.; Wong, E. H. F. *CNS Drug Rev.* **2004**, *10*, 23.
9. Fish, P. V.; Mackenny, M. C.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. WO Patent 105100, 2005.
10. Mahar Doan, K. M.; Humphreys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-Singh, C. J.; Adkison, K. K.; Polli, J. W. *J. Pharm. Exp. Ther.* **2002**, *303*, 1029.
11. Definition of terms: m. wt.: molecular weight; *c*log *P*: calculated partion coefficient (BioByte software); $\log D_{7.4}$: measured octanol-buffer distribution coefficient; pK_a: measured ionisation constant; HBD: H-bond donor count (NH+OH); HBA: H-bond acceptor count (N+O); PSA: polar surface area.

12. Boot, J.; Cases, M.; Clark, B. P.; Findlay, J.; Gallagher, L. H.; Man, T.; Montalbetti, C.; Rathmell, R. E.; Rudyk, H.; Walter, M. W.; Whatton, M.; Wood, V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 699.
13. For racemic syntheses, see: (a) Melloni, P.; Carniel, G.; Della Torre, A.; Bonsignori, A.; Buonamici, M.; Pozzi, O.; Ricciardi, S.; Rossi, A. C. *Eur. J. Med. Chem.* **1984**, *19*, 235; (b) reference 12; (c) Henegar, K. E.; Ball, C. T.; Horvath, C. M.; Maisto, K. D.; Mancini, S. E. *Org. Proc. Res. Dev.* **2007**, *11*, 346; For an asymmetric synthesis of (+)-(*S*,*S*)-reboxetine, see: (d) Brenner, E.; Baldwin, R. M.; Tamagnan, G. *Org. Lett.* **2005**, *7*, 937.
14. King, F. D.; Hadley, M. S.; Joiner, K. T.; Martin, R. T.; Sanger, G. J.; Smith, D. M.; Smith, G. E.; Smith, P.; Turner, D. H.; Watts, E. A. *J. Med. Chem.* **1993**, *36*, 683.
15. Lipase Candida rugosa is commercially available from Sigma (catalogue number: L-1754; 1140 U/mg).
16. Enantioselectivities were determined by chiral HPLC (Chiralcel OJ-H, 250 × 4.6 mm; hexane/0.1% TFA in EtOH, 80:20 isocratic, 1 mL/min).
17. The absolute stereochemistry was determined by conversion of **28** to an authentic sample of (*S*,*S*)-reboxetine and confirmed by single crystal X-ray structure determination of **5a** $PhSO_3H$ salt (mp 182 °C). Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC680331. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK.
18. Walsh, G. M. *Exp. Opin. Drug Safety* **2002**, *1*, 225.
19. For an analysis of the influence of drug lipophilicity on off-target pharmacology and in vivo toxicological outcomes, see: (a) Leeson, P. D.; Springthorpe, B. *Nature Rev. Drug Dis.* **2007**, *6*, 881; (b) Hughes, J. D.; Blagg, J.; Bailey, S.; De Crescenzo, G. A.; Dalvie, D.; Devraj, R. V.; Doubovetzky, M.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Grant, D.; Greene, N.; Huang, E.; Kreiger-Burke, T.; Lee, L.; Loesel, J.; Nahas, K.; Price, D. A.; Wager, T.; Whiteley, L.; Zhang, Y. Correlations between compound physicochemical properties and in vivo toxicological outcomes: Comprehensive analysis of a 245 compound data set. *Nat. Biotech.*, **2008**, in press.
20. CaCO-2: human colon adenocarcinoma cell line. MDCK-mdr1: Madin-Darby canine kidney cell line expressing the P-glycoprotein transporter (P-gp). Flux across cells was measured at 10 μM substrate concentrations. Figures quoted correspond to the flux rates ($P_{app} \times 10^{-6}$ cm^{-1}) for apical to basolateral (AB) and basolateral to apical (BA) directions. The maximum measurable half-life in human liver microsomes was 120 min, whereas the human hepatocyte assay was able to accurately determine half-lives of up to 240 min. See: van de Waterbeemd, H.; Smith, D. A.; Beaumont, K.; Walker, D. K. *J. Med. Chem*. **2001**, *44*, 1313 and references therein. Ion channel screening (Na^+, Ca^{2+}) was performed by CEREP, France.
21. Deecher, D. C.; Beyer, C. E.; Johnston, G.; Bray, J.; Shah, S.; Abou-Gharbia, M.; Andree, T. H. *J. Pharm. Exp. Ther.* **2006**, *318*, 657.
22. Rat 5-HT and NA transporter inhibition was measured in HEK293 cells expressing the rat 5-HT and NA transporters. For **5a**: rSRI, K_i = 59 nM (n = 2); rNRI, K_i = 6 nM (n = 2).
23. Data for **5a** HCl salt: mp 148 °C; $[\alpha]_D$ = +14.4° (MeOH; c = 0.20); ^1H NMR (CD_3OD, 400 MHz) δ 3.05–3.20 (m, 3H), 3.25 (d, 1H), 3.78–3.87 (m, 4H), 4.08–4.20 (m, 2H), 5.31 (d, 1H), 6.70 (m, 2H), 6.95 (s, 1H), 7.28–7.44 (m, 5H); LRMS APCI *m*/*z* 334 (MH^+); 100% *e.e.* by chiral HPLC.
24. For an accompanying paper, see: Xu, W.; Gray, D. L.; Glase, S. A.; Barta, N. S. *Bioorg. Med. Chem. Lett*. **2008**, submitted for publication.
25. Target compounds were tested for their ability to inhibit specific binding of selective radioligands at the human 5-HT, NA and DA transporters utilising scintillation proximity assay (SPA) technology and cellular membrane preparations generated from recombinant HEK293 cells expressing a single monoamine transporter. For experimental details of the assay conditions, see: Andrews, M. D.; Brown, A. D.; Fish, P. V.; Fray, M. J.; Lansdell, M. I.; Ryckmans, T.; Stobie, A.; Wakenhut, F.; Gray, D. L. F. WO Patent 064351, 2006, p 56–59.