Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4867–4871
Bioorganic & Medicinal Chemistry Letters

Bicyclic heteroarylpiperazines as selective brain penetrant 5-HT$_6$ receptor antagonists

Mahmood Ahmed,[a] Michael A. Briggs,[a] Steven M. Bromidge,[b] Tania Buck,[a] Lorraine Campbell,[c] Nigel J. Deeks,[a] Ashley Garner,[c] Laurie Gordon,[c] Dieter W. Hamprecht,[b] Vicky Holland,[c] Christopher N. Johnson,[a,*] Andrew D. Medhurst,[a] Darren J. Mitchell,[a] Stephen F. Moss,[a] Jenifer Powles,[c] Jon T. Seal,[a] Tania O. Stean,[a] Geoffrey Stemp,[a] Mervyn Thompson,[a] Brenda Trail,[a] Neil Upton,[a] Kim Winborn[c] and David R. Witty[a]

[a]*Neurology and GI Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b]*Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*
[c]*Discovery Research, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*

Received 4 April 2005; revised 4 April 2005; accepted 14 June 2005
Available online 6 September 2005

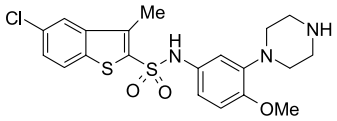
Abstract—Starting from the potent and selective but poorly brain penetrant 5-HT$_6$ receptor antagonist SB-271046, a successful strategy for improving brain penetration was adopted involving conformational constraint with concomitant reduction in hydrogen bond count. This provided a series of bicyclic heteroarylpiperazines with high 5-HT$_6$ receptor affinity. 5-Chloroindole 699929 combined high 5-HT$_6$ receptor affinity with excellent brain penetration and also had good oral bioavailability in both rat and dog.

5-HT$_6$ receptor mRNA is almost exclusively expressed within the brain, and many CNS drugs have high affinity for the 5-HT$_6$ receptor. Hence, there has been considerable interest in elucidating the role of the 5-HT$_6$ receptor in CNS disorders through the development of selective agents. Several classes of agonists and antagonists have been disclosed in recent years,[1] including the highly potent and selective 5-HT$_6$ receptor antagonist SB-271046 **1**.[2] Following the discovery that 5-HT$_6$ receptor antagonists have a beneficial effect on memory consolidation in animal models of cognitive enhancement such as the Morris water maze and Novel Object Recognition paradigms,[3,4] a possible role has been suggested for 5-HT$_6$ receptor antagonists in the treatment of learning and memory disorders.[5] The proposed involvement of 5-HT$_6$ receptor antagonism in memory consolidation is further supported by the effect of **1** on neuronal cell adhesion molecule (NCAM) polysialylation in rat brain.[6] NCAM polysialylation is a process that contributes to learning-associated dendritic remodelling in the adult central nervous system. Acute and chronic administration of SB-271046 has been shown to increase the frequency of NCAM polysialylated neurons activated in the entorhinal and perirhinal cortex, in response to water maze spatial learning.[7] These data imply that 5-HT$_6$ receptor antagonists may have a beneficial effect on synaptic plasticity, a property which may underpin their broad spectrum of activity in preclinical models of cognition enhancement.

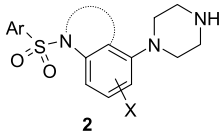
Although **1** had good oral bioavailability and showed activity in centrally mediated animal models of cognition, its brain–blood ratio in rat was low (0.05:1) and the compound was shown to be a substrate for the P-glycoprotein (P-gp) efflux pump. This combination of low brain penetration in rat and P-gp substrate liability was considered to give a high risk for poor brain penetration in human. Increased brain penetration and reduced P-gp liability were, therefore, key requirements for a back-up drug candidate.

* Corresponding author. Tel.: +44 1279 627735; e-mail: Christopher_N_Johnson@gsk.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.107



SB-271046, **1**

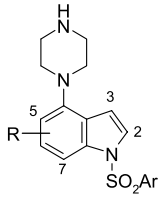


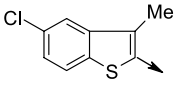
2

To identify compounds with improved brain–blood ratio, a strategy was adopted involving conformational constraint of the SB-271046 template with resultant removal of an acidic NH group as in **2**. In addition, a rat ex vivo binding assay[8] was used as a key early stage filter to identify compounds with inherently good oral exposure and high brain–blood ratio. This article describes the optimisation of a series of bicyclic heteroarylpiperazines related to **2**.

Novel compounds **5**–**22**, **26**–**29**, **32** and **35** (Tables 1 and 2) were prepared as described in Schemes 1–4. Compounds **5**–**10** were prepared in two steps from Boc-piperazinylindole **3a** by reaction with the appropriate arylsulfonyl chloride under basic conditions, followed by deprotection (Scheme 1). Introduction of substituents into the indole moiety could be carried out on either **3a** or on the arylsulfonated derivative **4a**.[9] Thus, chlorination of **3a** with *N*-chlorosuccinimide in 1,4-dioxane at room temperature gave a mixture of 3-, 5- and 7-chloro derivatives **3b–d**, from which the major component **3c** was separated by chromatography. The mixture of 3-chloro and 7-chloro isomers was treated with the appropriate arylsulfonyl chloride as described above and the resultant mixture of **4b** and **4d** was separated by chromatography. A 3-methyl substituent was introduced by treatment of

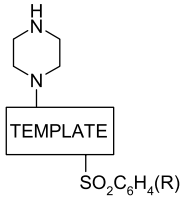
Table 1. 5-HT_6 receptor binding affinity (pK_i): 4-piperazinyl indoles[a]

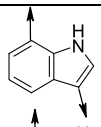
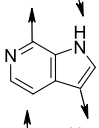
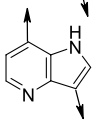
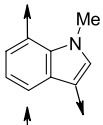
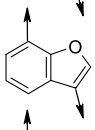
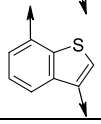


Compound[b]	Ar	R	pK_i
5		H	8.5
6	Ph	H	9.5
7	C_6H_4(2-Cl)	H	9.4
8	C_6H_4(3-Cl)	H	9.6
9	C_6H_4(4-Cl)	H	8.7
10	2-Pyridyl	H	9.0
11	C_6H_4(3-Cl)	3-Cl	9.5
12	C_6H_4(3-Cl)	5-Cl	8.6
13	C_6H_4(3-Cl)	7-Cl	9.5
14	C_6H_4(3-Cl)	3-Me	9.2
15	C_6H_4(3-Cl)	2-Me	9.4
16	C_6H_4(3-Cl)	5-CN	8.3
17	Ph	5-Cl	8.8
18	C_6H_4(2-Cl)	5-Cl	9.0
19	C_6H_4(4-Cl)	5-Cl	8.2
20	2-Pyridyl	5-Cl	9.3
21	1-Naphthyl	5-Cl	8.1
22	2-Naphthyl	5-Cl	7.6

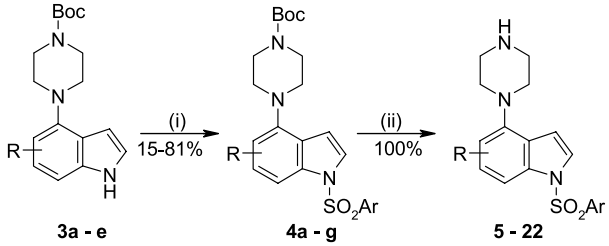
[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean. In this assay, SB-271046 **1** gave pK_i 8.9.
[b] All new compounds gave satisfactory analytical and/or mass spectral data.[16]

Table 2. 5-HT_6 receptor binding affinity (pK_i): alternative core templates[a]



Compound[b]	Template	R	pK_i
26		3-Cl	9.5
27		H	7.6
28		H	8.1
29		3-Cl	8.6
32		H	8.9
35		H	9.3

[a] All pK_i values represent the mean of at least three experiments, each within 0.3 of the mean. In this assay, SB-271046 **1** gave pK_i 8.9.
[b] All new compounds gave satisfactory analytical and/or mass spectral data.[16]



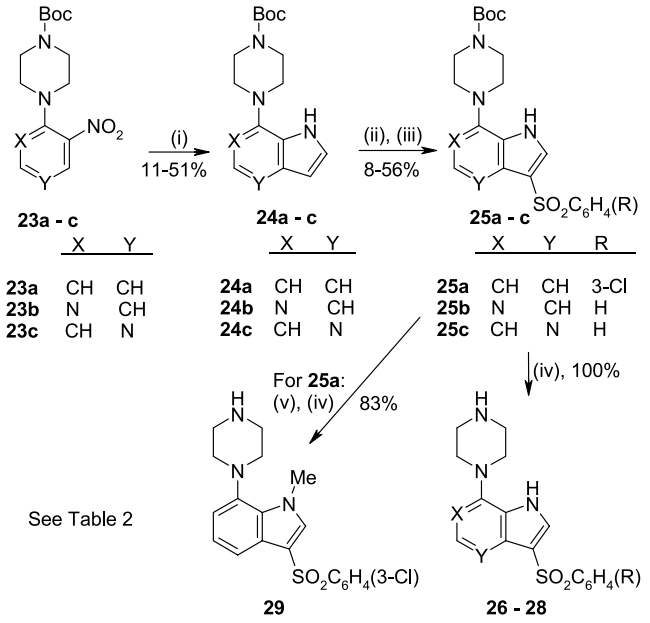
3a - e **4a - g** **5 - 22**

	R
3a	H
3b	3-Cl
3c	5-Cl
3d	7-Cl
3e	3-Me

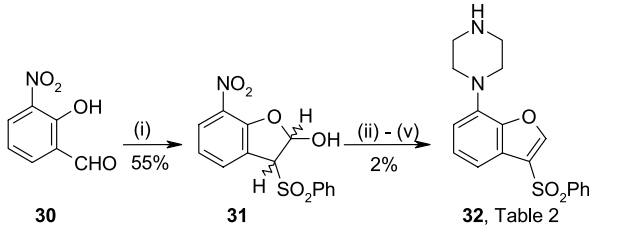
	R
4a	H
4b	3-Cl
4c	5-Cl
4d	7-Cl
4e	3-Me
4f	2-Me
4g	5-CN

See Table 1

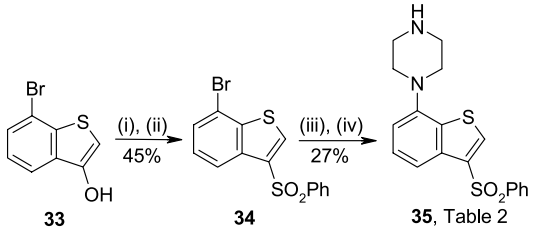
Scheme 1. Reagents and conditions: (i) 1. $KOBu^t$, THF, rt, 2 h; 2. $ArSO_2Cl$, rt, 15 h. (ii) CF_3CO_2H, CH_2Cl_2, rt, 4 h.



Scheme 2. Reagents and conditions: (i) CH_2=CHMgBr, THF, −40 °C, 0.7 h. (ii) 1. NaH, DMF, rt; 2. $[(R)C_6H_4S]_2$, rt, 2 h. (iii) 3-chloroperbenzoic acid, CH_2Cl_2, rt, 18 h. (iv) HCl, 1,4-dioxane, rt, 2 h. (v) Me_2SO_4, K_2CO_3, acetone, EtOH, reflux, 18 h.



Scheme 3. Reagents and conditions: (i) $ClCH_2SO_2Ph$, $KOBu^t$, DMF, rt, 2 h. (ii) concd H_2SO_4, AcOH, 60 °C, 10 h. (iii) H_2, Pd/C, DMF, EtOH, rt, 18 h. (iv) $MeN(CH_2CH_2Cl)_2 \cdot HCl$, Na_2CO_3, BuOH, 120 °C, 48 h. (v) 1. MeCH(Cl)OCOCl, $ClCH_2CH_2Cl$, 70 °C, 1 h; 2. MeOH, reflux, 1 h.



Scheme 4. Reagents and conditions: (i) PhSH, *p*-TsOH, C_6H_6, 80 °C, 16 h. (ii) monomagnesium peroxyphthalate, CH_2Cl_2, rt, 16 h. (iii) *N*-Boc-piperazine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, 1,4-dioxane, 100 °C, 16 h. (iv) HCl, 1,4-dioxane, rt, 2 h.

3a with sodium hydride in tetrahydrofuran followed by chlorotriisopropylsilane to give the 1-triisopropylsilyl indole, treatment of the latter with Eschenmoser's salt in acetonitrile and subsequent catalytic hydrogenation over palladium on charcoal to give **3e**, after chromatography. A 2-methyl substituent was introduced by treatment of **4a** with *tert*-butyllithium in hexane/tetrahydrofuran at −78 °C followed by quenching with iodomethane. 5-Cyano **4g** was obtained by treatment of **4a** with chlorosulfonyl isocyanate in acetonitrile at 0 °C followed by addition of triethylamine. Conversion of indole-substituted derivatives **3b–e**, **4f** and **4g** to final products **11–22** was carried out using methods analogous to those used for the synthesis of compounds **5–10**.[9]

To prepare 7-piperazinyl indole isomer **26**, routes were initially investigated involving palladium-catalysed reaction of a suitable 7-bromoindole derivative with *N*-Boc-piperazine.[10] This approach proved unsuccessful whether or not the indole NH was protected, for example, with a Boc group. A far more successful strategy involved use of the Bartoli indole synthesis,[11] involving reaction of an aromatic nitro compound with an alkenyl Grignard reagent (Scheme 2). We reasoned that a piperazine group, suitably protected on the distal nitrogen, should serve as an appropriate large moiety to force the nitro group out of plane, reportedly a prerequisite for a successful outcome.[12] Nitro derivative **23a**, obtained by reaction of 2-fluoronitrobenzene with *N*-Boc-piperazine, was treated with a solution of 3.2 molar equivalents of vinylmagnesium bromide in tetrahydrofuran at −40 °C to afford 7-piperazinyl indole **24a** in 11% yield.[13] The 3-arylsulfonyl group was introduced by reaction with the appropriate aryl disulfide under basic conditions, followed by oxidation with 3-chloroperbenzoic acid to give **25a**. Acid-mediated deprotection gave desired target **26**. Corresponding *N*-methyl indole **29** was obtained from **25a** by alkylation with dimethyl sulfate under basic conditions prior to removal of the Boc-protecting group. The success of this approach prompted us to investigate the Bartoli synthesis for the preparation of azaindoles **27** and **28**. Nitropyridines **23b** and **23c** (obtained by reaction of the appropriate fluoro compound with *N*-Boc-piperazine) were successfully treated with vinylmagnesium bromide as described above to afford desired azaindoles **24b** and **24c** in 17% and 51% yield, respectively, and these were converted into final products **27** and **28** as shown in Scheme 2.

Benzofuran **32** was prepared as shown in Scheme 3. Nitroaldehyde **30** was treated with the anion derived from chloromethylphenyl sulfone to give dihydrobenzofuran **31** in moderate yield, the reaction proceeding via rearrangement of an initially formed epoxide.[14] Dehydration of **31** followed by catalytic hydrogenation gave the 7-aminobenzofuran and subsequent piperazine ring formation with bis-(2-chloroethyl)methylamine hydrochloride gave, after demethylation with 1-chloroethylchloroformate, the desired NH-piperazine **32**. The low overall yield of this sequence was primarily due to low recovery from the piperazine ring forming stage, though this step was not optimised. Benzothiophene **35** was prepared as shown in Scheme 4. 7-Bromo-3-hydroxybenzothiophene **33** was condensed with thiophenol under acid conditions and then oxidised with monomagnesium peroxyphthalate to give sulfone **34**. Displacement of the 7-bromo substituent by *N*-Boc-piperazine was then carried out using palladium catalysis and a final deprotection gave the desired target

35. All final compounds were purified by chromatography and isolated as hydrochloride salts.

All compounds were evaluated by displacement of [^{3}H]LSD from human cloned 5-HT$_6$ receptors expressed in HeLa cells with data expressed as pK_i values (Tables 1 and 2).

Conformational constraint as described above led to a series of sulfonylated piperazinyl indoles **5–10** with high affinity for the 5-HT$_6$ receptor (Table 1). In all, over 130 Ar groups were investigated of which 96 had pK_i > 8, with 3-chlorophenyl **8** giving the highest affinity (pK_i 9.6). A selection of these was profiled in a steady-state CNS penetration assay in the rat[15] and, encouragingly, brain–blood ratios between 2:1 and 4:1 were obtained. However, all compounds evaluated suffered from high in vivo blood clearance due to poor metabolic stability. Substitution of the indolyl moiety was investigated for Ar = 3-chlorophenyl to block potential sites of metabolism. A range of substitution patterns was tolerated with respect to 5-HT$_6$ receptor affinity. Introduction of electron-withdrawing groups at C-5 reduced 5-HT$_6$ affinity to some extent (**12**, **16**) but improved selectivity against other 5-HT receptors to >100-fold. In a rat steady-state CNS penetration assay, 5-chloro derivative **12** had significantly reduced blood clearance (CLb 44 mL/min/kg) compared to the C-5 unsubstituted compound and, importantly, maintained a 3:1 brain–blood ratio. Variation of the arylsulfonyl group was investigated in the 5-chloroindole series and a selection is shown in Table 1.

For Ar = substituted phenyl, an *ortho* substituent, for example, 2-Cl **18**, was most beneficial for 5-HT$_6$ affinity, while a *para* substituent reduced affinity, for example, 4-Cl **19**. 2-Pyridylsulfonyl **20** gave the highest affinity in this series and also achieved >1000-fold selectivity over other 5-HT receptors. However, this compound gave poor exposure following oral dosing in the rat. Bicyclic arylsulfonyl groups were poorly tolerated in this series, for example, 1- and 2-naphthyl (**21** and **22**, respectively). An isomeric 7-piperazinyl indole series together with alternative 6,5-bicyclic replacements for the indole unit were investigated (Table 2).

Isomeric 7-piperazinyl 3-arylsulfonyl indole **26** retained good affinity (pK_i 9.5), which was reduced 10-fold by indole *N*-methylation **29**. The corresponding 6- and 4-azaindoles had markedly lower 5-HT$_6$ affinity (**27** and **28**, respectively). Replacement of indole by benzofuran **32** and benzothiophene **35** was, by contrast, well tolerated but these compounds had low in vitro metabolic stability as determined using rat and human liver microsomes. In the rat steady-state CNS penetration assay, 7-piperazinyl indoles **26** and **29** had moderate blood clearance (41 and 34 mL/min/kg, respectively). However, the brain–blood ratio of *N*-methyl **29** was significantly higher than that of NH derivative **26** (2.6:1 vs 0.7:1), reflecting the increased number of *H*-bond donors present in **26**. In the rat ex vivo binding assay,[8] **12** and **29** had ED$_{50}$ 3 and 5 mg/kg po, respectively. In comparison SB-271046 was significantly less potent, with ED$_{50}$ 11 mg/kg.

Compound **12** (699929) was selected for further profiling. The native tissue 5-HT$_6$ affinity of **12** in rat striatum (pK_i 7.9) and human caudate (pK_i 8.1) was lower in comparison with affinity at the human recombinant receptor (pK_i 8.6). The compound was shown to be >100-fold selective across a range of 50 receptors and ion channels. In a cAMP accumulation assay using human cloned 5-HT$_6$ receptors expressed in HeLa cells, **12** was an antagonist with apparent pK_b 7.2. The pharmacokinetic profile of **12** was further investigated and the compound was found to have 49% oral bioavailability in rat and 90% in dog, together with a long plasma half life (6 and 17 h, respectively). The P-gp substrate liability for **12** was assessed using MDCK cell monolayers expressing human multi-drug resistance (MDR) protein.[17] In this assay, **12** was shown to have reduced P-gp liability in comparison to SB-271046 **1**.

Conformational constraint of the SB-271046 template with concomitant reduction of hydrogen bond count proved to be a successful strategy for obtaining compounds with improved brain penetration. The resulting series of bicyclic heteroarylpiperazines had high affinity for the 5-HT$_6$ receptor and compounds such as **12** and **29** had improved potency in an ex vivo binding assay compared to SB-271046 **1**. From this series, 5-chloro-4-piperazinyl indole **12** (699929) was identified, with a brain–blood ratio of 3:1 in rat and good oral bioavailability in two species, and was selected for further progression.

Acknowledgment

The authors thank Kerry-Ann Garwood for assistance in the preparation of the manuscript.

References and notes

1. Glennon, R. A. *J. Med. Chem.* **2003**, *46*, 2795.
2. Bromidge, S. M.; Brown, A. M.; Clarke, S. E.; Dodgson, K.; Gager, T.; Grassam, H. L.; Jeffrey, P. M.; Joiner, G. F.; King, F. D.; Middlemiss, D. N.; Moss, S. F.; Newman, H.; Riley, G. J.; Routledge, C.; Wyman, P. *J. Med. Chem.* **1999**, *42*, 202.
3. Rogers, D. C.; Hagan, J. J. *Psychopharmacology* **2001**, *158*, 114.
4. King, M. V.; Sleight, A. J.; Woolley, M. L.; Topham, I. A.; Marsden, C. A.; Fone, K. C. F. *Neuropharmacology* **2004**, *47*, 195.
5. Reavill, C.; Rogers, D. C. *Curr. Opin. Investig. Drugs* **2001**, *2*, 104.
6. Regan, C. M.; Foley, A. G.; Murphy, K. J.; Hirst, W. D.; Gallagher, H. C.; Hagan, J. J.; Upton, N.; Walsh, F. S. *Abstracts of Papers*, Society for Neuroscience 33rd Annual Meeting, New Orleans, USA, Nov 8–12, 2003; 835.21.
7. Johnson, C. N.; Roland, A.; Upton, N. *Drug Discovery Today* **2004**, *1*, 13.
8. Rats received vehicle or 5-HT$_6$ receptor antagonist, dosed orally, 4 h pretreatment. Animals were then killed and striatum was removed. Tissue was homogenised and a binding assay was performed with the selective 5-HT$_6$

receptor antagonist radioligand [^{125}I]-SB-258585. An ED_{50} value was determined from the dose–response curve.
9. Bromidge, S. M. WO2002041889; *Chem. Abstr.* **2002**, *136*, 401783.
10. Hartwig, J. F. In Negishi, E., Ed.; Handbook of Organopalladium Chemistry; Wiley: Hoboken, NJ, 2002; Vol. 1, pp 1051–1096.
11. Bartoli, G.; Palmieri, G.; Bosco, M.; Dalpozzo, R. *Tetrahedron Lett.* **1989**, *30*, 2129.
12. Bosco, M.; Dalpozzo, R.; Bartoli, G.; Palmieri, G.; Petrini, M. *J. Chem. Soc. Perkin Trans. 2* **1991**, 657.
13. Procedure for Bartoli reaction: A solution of **23a** (33.1 g, 0.108 mol) in dry THF (750 mL) under argon was cooled to −45 °C and then treated with a solution of vinylmagnesium bromide (1 M, 345 mL) over 0.3 h. The mixture was stirred at −40 °C for 0.7 h and then quenched with saturated aqueous ammonium chloride (30 mL). The resulting mixture was extracted with dichloromethane (500 mL), then the organic phase was washed with water (500 mL), dried ($MgSO_4$), and evaporated in vacuo to give an oil. Chromatography on silica gel with 3–8% acetone–toluene gradient elution gave **24a** (3.55 g, 11%) δ_H ($CDCl_3$) 1.50 (9H, s), 3.06 (4H, t, J = 5.0 Hz), 3.65 (4H, t, J = 4.8 Hz), 6.56 (1H, m), 6.83 (1H, d, J = 7.5 Hz), 7.07 (1H, t, J = 7.7 Hz), 7.20 (1H, m), 7.39 (1H, d, J = 7.9 Hz), 8.28 (1H, br s).
14. Makosza, M.; Ziobrowski, T.; Kwast, A. *Tetrahedron* **1998**, *54*, 6811.
15. CNS penetration was determined at steady state in rat. Compounds were dissolved in 2% (v/v) DMSO in 5% (w/v) dextrose aq and administered at a constant infusion rate over 12 h at a target dose rate of 0.3 mg free base/kg/h. Blood samples were removed during the latter part of the infusion to confirm steady-state blood concentrations. Blood and brain samples were analysed by LC/MS/MS.
16. ^{1}H NMR spectra were recorded at 250 MHz in $CDCl_3$ as solvent. Compound **12**, mp 240–241 °C (dec.) (hydrochloride salt); δ_H 3.4 (4H, br s), 3.6 (4H, br s), 4.8 (2H, br s), 6.9 (1H, d, J = 3.8 Hz), 7.23 (1H, d, J = 8.8 Hz), 7.52 (1H, t, J = 4.0 Hz), 7.65–7.76 (2H, m), 7.82 (1H, d, J = 8.8 Hz), 7.90 (1H, d, J = 8.4 Hz), 7.96 (1H, t, J = 1.8 Hz).
17. Confluent MDR-MDCK monolayers on collagen-coated, microporous polycarbonate filters were used in a test medium of Hank's balanced salt solution containing 10 mM HEPES and 15 mM glucose at pH 7.4. Apical-to-basal and basal-to-apical permeability (P_{app}A-B and P_{app}B-A, respectively) of test compounds (3 μM) was assessed in the presence and absence of the P-gp inhibitor GF120918A (2 μM). P-gp substrates were defined as having P_{app}B-A/P_{app}A-B ratio >2, which reverted to unity in the presence of GF120918A.