Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 256–261
Bioorganic & Medicinal Chemistry Letters

Cyclic guanidines as dual 5-HT$_{5A}$/5-HT$_7$ receptor ligands: Structure–activity relationship elucidation

Jens-Uwe Peters,[a,*] Thomas Lübbers,[a] Alexander Alanine,[a] Sabine Kolczewski,[a] Francesca Blasco[b,†] and Lucinda Steward[c]

[a]*Discovery Chemistry, Pharma Division, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*
[b]*Safety Technical Services, Pharma Division, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*
[c]*Preclinical CNS Research, Pharma Division, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland*

Received 4 September 2007; revised 22 October 2007; accepted 25 October 2007
Available online 30 October 2007

Abstract—The optimisation of affinity and selectivity in a novel series of dual 5-HT$_{5A}$/5-HT$_7$ receptor ligands is described. Brain penetrant 2-aminodihydroquinazolines with low nanomolar affinities were identified.

Since the initial discovery of 5-hydroxytryptamine (5-HT) receptor (R) subtypes in 1957,[1] 14 different 5-HTR subtypes have been identified.[2] For many of these receptors, selective ligands have been discovered, and advanced compounds are in clinical development, or are marketed drugs. The physiological functions of the 5-HT$_{5A}$R and the 5-HT$_7$R, which were first cloned in 1993–1994,[3,4] are not yet well understood. Especially the investigation of the 5-HT$_{5A}$R has been hampered by the lack of selective ligands until very recently. The expression of the 5-HT$_{5A}$R[3,5] and the 5-HT$_7$R[4,6] in the hippocampus, thalamus, hypothalamus, amygdale and cerebral cortex suggests a potential role for these receptors in the pathology or modulation of mood disorders.[2a,f,7] Indeed, selective 5-HT$_7$R antagonists[2f,8–15] as well as recently discovered, selective 5-HT$_{5A}$R antagonists[16–18] have shown promising results in behavioural models. Also, both receptors are expressed in the suprachiasmatic nucleus of the hypothalamus, which functions as the circadian clock,[19] and are therefore a potential target for the treatment of sleep disorders.[2f] Together, these results suggest that selective or dual 5-HT$_{5A}$/5-HT$_7$R ligands might be useful for the treatment of psychiatric diseases and/or sleep disturbances.

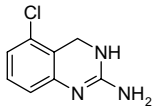
We originally set out to identify novel 5-HT$_{5A}$R ligands and screened the Roche compound library using recombinant, human 5-HT$_{5A}$Rs in a radioligand ([^{3}H]LSD) binding displacement assay. Although the screen delivered a large number of hits, most compounds were not selective against a panel of related monoaminergic receptors. 2-Aminodihydroquinazolines **1** and **2**[20] emerged as novel tractable hits, even though **1** and **2** were only partially selective over all other human 5-HTRs tested (Table 1).[21] A prominent feature of **1** and **2** is their guanidine motif, a feature also found in the recently disclosed, guanidine-based 5-HT$_{5A}$R antagonist A-843277[18] that was not known to us at this time.

For an initial SAR elucidation, we prepared single-substituted derivatives (Tables 2 and 3), whose 5-HT$_{5A}$R affinities were compared with that of the unsubstituted 3,4-dihydroquinazolin-2-ylamine (**3**-H, Table 2). A methyl or an ethyl substituent in the 4-position leads to a large improvement in 5-HT$_{5A}$R binding affinity (**4**, **5**), whereas a 4-propyl substituent is already too big (**6**); likewise, di-methyl substitution does not improve affinity (**7**). Mono- and bis-alkylation of the exocyclic 2-amino function leads to a progressive reduction of 5-HT$_{5A}$R affinity (NH_2 [**3**-H] > NHMe [**9**] > NMe_2 [**10**]), and alkylation of the 3-N leads to a complete loss of activity (**8**). A substituent in the 5-position (Cl or Me, but not F), or an 8-MeO substituent, improves the 5-HT$_{5A}$R affinity; other aromatic substituents have less pronounced effects or lead to an affinity reduction (Table 3, compare with **3**-H, Table 2).

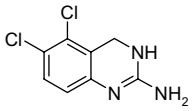
Keywords: 5-HT; GPCR; SAR; Guanidine.
* Corresponding author. Tel.: +41 61 6882636; e-mail: jens-uwe.peters@roche.com
† Present address: Novartis Institute for BioMedical Research, CH-4002 Basel, Switzerland.

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.080

Table 1. Binding profile of screening hits **1** and **2**



1

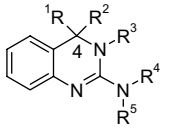


2

	K_i (nM)							
	5-HT_{5A}	5-HT_{1A}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3	5-HT_6	5-HT_7
1[a]	99	1701	3594	1164	658	1051	$>10^4$	793
2[a]	43	1080	1265	434	208	172	1157	294

[a] As HBr salt.

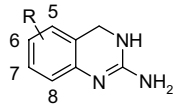
Table 2. SAR exploration of the dihydropyrimidine ring motif



	R^1	R^2	R^3	R^4	R^5	5-HT_{5A} K_i (nM)
3-H[a]	H	H	H	H	H	658
4[b]	Me	H	H	H	H	38
5	Et	H	H	H	H	24
6	Pr	H	H	H	H	805
7[b]	Me	Me	H	H	H	1172
8[b]	H	H	Me	H	H	>10,000
9[b]	H	H	H	Me	H	1791
10	H	H	H	Me	Me	2800

[a] As HBr salt.
[b] As HI salt.

Table 3. SAR exploration of aromatic ring substituents (5-HT_{5A} K_i [nM])



3-R

R	Cl	Me	MeO	F
5	99[a]	145[b]	N.D.	550[b]
6	807[a]	882[b]	N.D.	911[b]
7	347[b]	274[a]	1375[b]	647[a]
8	892	463[b]	97[b]	1990[a]

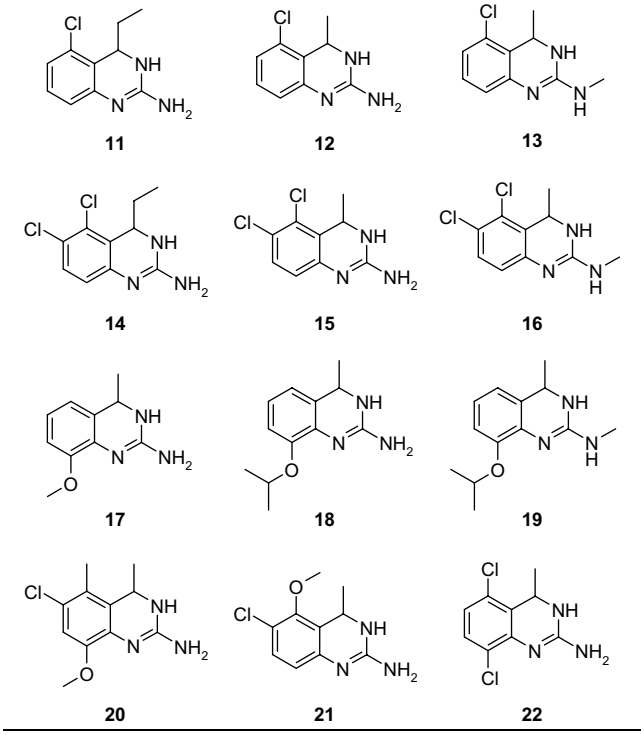
N.D., not tested.
[a] As HBr salt.
[b] As HI salt.

These findings led to a series of compounds which combined affinity-increasing 4-methyl/ethyl substitution with beneficial aromatic substituents (Table 4). Gratifyingly, the effects were in general additive, and high-affinity compounds were obtained (**12**, **13**, **15**–**22**). Exceptions were two derivatives **11** and **14** combining an ethyl substituent with a 5-Cl substituent, which were less active than expected. We speculate that the reduced affinity of these compounds might be due to a steric interaction between the 5-Cl and the ethyl group, which forces the ethyl group out of an optimal binding conformation. Compounds **11**–**22** were tested in a functional assay and were found to be antagonists at the human 5-HT_{5A}R, with the functional inhibition constants (Kb's) correlating with binding affinities (K_i's). Furthermore, **12** and **13** (pK_i 8.29 and 7.97, respectively) were evaluated in a [^{35}S]GTPγS assay, and were found to be competitive antagonists at the 5-HT_{5A}R with pA_2 values of 8.52 and 8.13, respectively.[22]

All compounds were cross-screened against a panel of other human 5-HTRs. We found that none of our compounds was more than 4-fold selective over the 5-HT_7R. However, due to the expression of the 5-HT_{5A} and the 5-HT_7Rs in similar brain regions,[3–6] and based on published preclinical results,[2f,11,12,15,16] we considered dual 5-HT_{5A}/5-HT_7R ligands to be a viable alternative worthy of investigation in addition to selective 5-HT_{5A}R ligands in our drug discovery programme. Whereas 5-HT_{5A}R selectivity over the 5-HT_{2A}, 5-HT_3, and 5-HT_6Rs was generally greater than 30-fold, few compounds had greater than 30-fold selectivity over the 5-HT_{1A} and 5-HT_{2C}Rs. Compounds **18** and **19** with an 8-isopropoxy substituent also had low selectivity (~7-fold) over the 5-HT_{1D}R. In general, it was observed that the selectivity profile improved with increasing 5-HT_{5A}/5-HT_7R affinity. **15** and **16** were of especially high affinity, however it was later found that all 5,6-dichloro derivatives also have a high affinity for the human H_1R. For instance, mono-chloro derivative **12** had only micromolar (K_i 1100 nM) hH_1R affinity, whereas the dichloro-derivative **15** had low nM affinity (K_i 8 nM) for this receptor. Thus, **12** emerged as the most selective compound and was further profiled at 1 μM at 75 targets (CEREP) including many aminergic GPCRs. Greater than 60% inhibition of binding was observed only for the α_2 adrenoR and the 5-HT_{2B}R. This finding was followed up by K_i/IC_{50} determination which was found to be >400 nM for both.

Brain concentrations of **12** were sustained at >200 nM over a time course of 0.8–3 h after an oral dose of 10 mg/kg (mice). This concentration might be sufficient to achieve an adequate receptor occupancy in-vivo. However, if the compound has similar protein binding in the brain as in plasma (84%), there will be an accordingly decreased free fraction. For a pharmacological tool to be used in proof-of-concept studies, an improved brain concentration would be desirable, to ensure sufficient receptor occupancy during in-vivo experiments. Further efforts focusing on the improvement of the brain concentration will be described in the following paper.

Table 4. Combination of beneficial substituents as identified in Tables 2 and 3



	K_i (nM)				
	5-HT_{5A}	5-HT_{1A}	5-HT_{1D}	5-HT_{2C}	5-HT_7
11[a]	84.4	1081	>10,000	380	97.4
12[b]	5.1	192	1272	152	15.9
13[b]	10.8	389	1457	235	15.3
14[a]	26.5	765	1275	155	73.0
15[b]	2.0	181	260	26	8.7
16[b]	6.3	246	454	94	13.2
17[b]	6.3	32	446	556	8.2
18[b]	10.0	265	70	488	1.0
19[b]	25.4	669	160	970	2.4
20[b]	4.7	105	143	19	2.5
21[a]	22.9	339	1156	74	12.4
22[b]	6.5	91	966	74	2.4

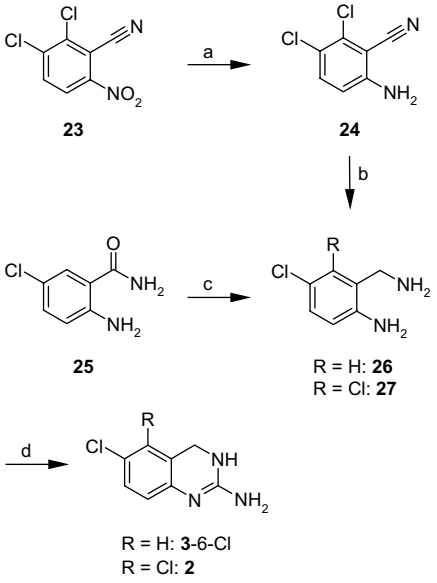
Compound **12** has a particularly high 5-HT_{5A}R affinity combined with ~30-fold selectivity over related receptors (except 5-HT_7).

[a] Low selectivity over other 5-HT subtypes.

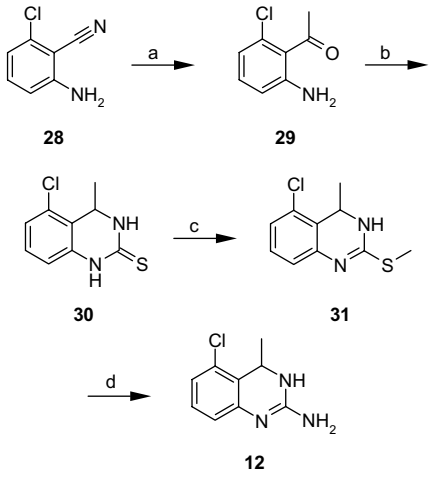
[b] >30-Fold selective over 5-HT_{2A}, 5-HT_3, 5-HT_6.

Dihydroquinazolinylamines without a substituent in the 4-position (e.g. Table 3) were prepared from aminobenzylamines such as **26** and **27** (Scheme 1). These aminobenzylamines are available, for example, by the method of Trinka et al.[23] from commercially available nitrobenzonitriles such as **23** or by LAH-reduction of aminobenzamides such as **25**. The aminobenzylamines can be converted into aminodihydroquinazolinylamines **2** and **3** in low yield by reaction with cyanogen bromide.[24] Alternatively, reaction with thiophosgene gives cyclic thioureas, which can be further converted in analogy to **30** (Scheme 2).

Dihydroquinazolinylamines with an alkyl substituent in the 4-position, such as **12**, were obtained as outlined in Scheme 2. The addition of a Grignard reagent to aminobenzonitrile **28** and hydrolysis of the resulting imine



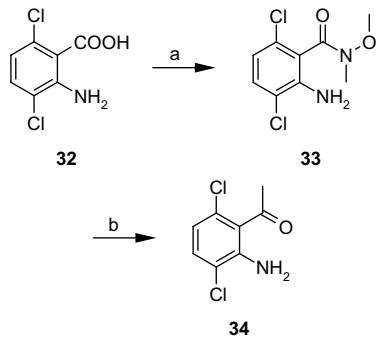
Scheme 1. Representative preparations of 4-unsubstituted dihydroquinazolinylamines, **2** and **3**-6-Cl. Reagents and conditions: (a) Pd/C (10%), H_2 (5 atm), THF/HCl_{aq}, 3 h rt, 82%; (b) KBH_4, TFA, THF, 3 h rt, 72%; (c) $LiAlH_4$, dioxane, 4 h reflux, 88%; (d) BrCN, toluene, reflux overnight, 15% (R = H).



Scheme 2. Representative preparation of a 4-alkyl-substituted dihydroquinazolinylamine, **12**. Reagents and conditions: (a) MeMgBr, Et_2O, 3 h reflux; then 6 N HCl, 4 h reflux, 67%; (b) $NaBH_4$, EtOH, 65 °C overnight; then KSCN, HCl, 3 h 65 °C, 43%; (c) MeI (3 equiv), acetone, weekend rt, 87%; (d) NH_4OH, H_2O/CH_3CN, microwave 30 min 170 °C, 62%.

gave an aminoacetophenone **29**.[25] This amino-ketone was converted in a one-pot reduction-HSCN sequence to a cyclic thiourea **30**. Methylation provided isothiourea **31** as its hydroiodide. Nucleophilic replacement of the methylsulfanyl leaving group by ammonia then gave the target compound **12**.[26]

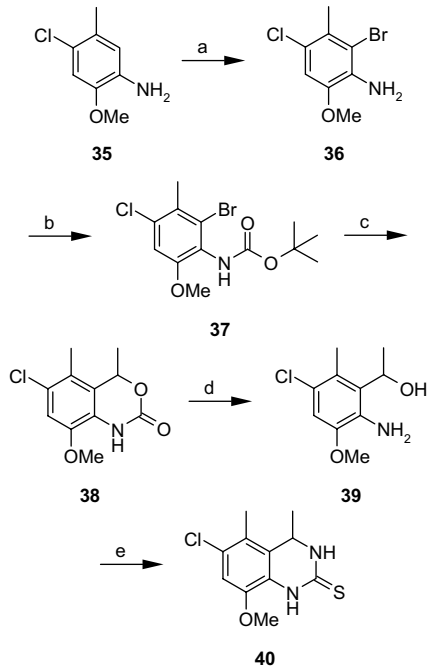
For the preparation of certain dihydroquinazolinylamines such as **20** and **22**, for which the corresponding highly substituted aminobenzonitriles were not commercially available, alternative methods were used.



Scheme 3. Alternative preparation of amino-ketone intermediate **34**. Reagents and conditions: (a) $CH_3NH–OCH_3$*HCl, *N*-methylmorpholine, HBTU, DMF, rt overnight, 100%; (b) MeLi, THF, −78 °C—rt, 29%.

Aminobenzophenone **34** (Scheme 3) was available from anthranilic acid **32** via Weinreb amide **33** and could be converted to dihydroquinazolinylamine **20** in analogy to **29** (Scheme 2).

The cyclic thiourea **40** was available by the reaction sequence outlined in Scheme 4: bromination of **35** and protection of **36** with Boc[27] led to **37**. Lithiation of **37** at low temperature and subsequent reaction with acetaldehyde was expected to give the corresponding benzyl alcohol, which was obtained as a cyclic carbamate, **38**. Hydrolysis led to the aminobenzylalcohol **39**, which is the analogue of a non-isolated intermediate in step b, Scheme 2. Following the conditions of Scheme 2, **39** was first converted into the cyclic thiourea **40** and subsequently into dihydroquinazolinamine **18**. Full experimental details can be found in Ref. 28.



Scheme 4. Alternative preparation of thiourea intermediate **40**. Reagents and conditions: (a) NBS, CH_3CN, rt overnight, 62%; (b) Boc_2O, DMAP, THF, 20 h reflux, then K_2CO_3, MeOH, 2 d reflux, 62%; (c) *n*-BuLi, then CH_3CHO, THF, −78 °C—rt, 79%; (d) KOH, H_2O, MeOH, 6 h reflux, 88%; (e) KSCN, HCl, H_2O, 2 h 95 °C, 78%.

In conclusion, we have investigated the SAR of a series of novel 2-aminodihydroquinazolines as dual 5-HT_{5A}/5-HT_7R ligands. We identified substitution patterns that lead to low nM affinities and reasonable selectivity profiles. **12** was the best compound with high affinity for the 5-HT_{5A} and 5-HT_7Rs, and with more than 30-fold selectivity for the 5-HT_{5A}R over related receptors. **12** distributes to the brain after oral administration; however, an improved brain penetration would be desirable for in-vivo pharmacological studies. Our efforts to improve our series of 2-aminodihydroqinazolines in this regard by a modulation of molecular properties will be the subject of the subsequent paper.

Acknowledgments

The excellent technical assistance of Philipp Ernst, Silvia Herzig, Stéphane Henriot, Stéphane Kritter, Nadine Marquardt, Catherine Muller, Gabrielle Py and Silja Weber is gratefully acknowledged.

References and notes

1. Gaddum, J. H.; Picarelli, Z. P. *Br. J. Pharmacol.* **1957**, *12*, 323.
2. (a) For reviews on 5-HT receptor subtypes, see issue *3*(1) of *Curr. Drug Targets: CNS & Neurological Disorders* **2004**: (a) Nelson, D. L., 53; (b) Bockaert, J.; Claeysen, S.; Compan, V.; Dumuis, A., 39; (c) Costall, B.; Naylor, R. J., 27; (d) Leysen, J. E., 11; (e) Lanfumey, L.; Hamon, M., 1.; (f) Thomas, D. R.; Hagan, J. J., 81; (g) Woolley, M. L.; Marsden, C. A.; Fone, K. C. F., 59.
3. (a) Erlander, M. G.; Lovenberg, T. W.; Baron, B. M.; de Lecea, L.; Danielson, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Foye, P. E.; Cannon, K.; Burns, J. E.; Sutcliffe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3452; (b) Rees, S.; den Daas, I.; Foord, S.; Goodson, S.; Bull, D.; Kilpatrick, G.; Lee, M. *FEBS Lett.* **1994**, *355*, 242.
4. (a) Plassat, J. L.; Amlaiky, N.; Hen, R. *Mol. Pharmacol.* **1993**, *44*, 229; (b) Lovenberg, T. W.; Baron, B. M.; Delecea, L.; Miller, J. D.; Prosser, R. A.; Rea, M. A.; Foye, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Danielson, P. E.; Sutcliffe, J. G.; Erlander, M. G. *Neuron* **1993**, *11*, 449; (c) Meyerhof, W.; Obermuller, F.; Fehr, S.; Richter, D. *DNA Cell Biol.* **1993**, *12*, 401; (d) Ruat, M.; Traiffort, E.; Leurs, R.; Tardivellacombe, J.; Diaz, J.; Arrang, J. M.; Schwartz, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8547; (e) Shen, Y.; Monsma, F. J.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. *J. Biol. Chem.* **1993**, *268*, 18200; (f) Tsou, A. P.; Kosaka, A.; Bach, C.; Zuppan, P.; Yee, C.; Tom, L.; Alvarez, R.; Ramsey, S.; Bonhaus, D. W.; Stefanich, E.; Jakeman, L.; Eglen, R. M.; Chan, H. W. *J. Neurochem.* **1994**, *63*, 456; (g) Bhalla, P.; Saxena, P. R.; Sharma, H. S. *Mol. Cell. Biochem.* **2002**, *238*, 81; (h) Bard, J. A.; Zgombick, J.; Adham, N.; Vaysse, P.; Branchek, T. A.; Weinshank, R. L. *J. Biol. Chem.* **1993**, *268*, 23422.
5. (a) Plassat, J. L.; Boschert, U.; Amlaiky, N.; Hen, R. *EMBO J.* **1992**, *11*, 4779; (b) Matthes, H.; Boschert, U.; Amlaiky, N.; Grailhe, R.; Plassat, J. L.; Muscatelli, F.; Mattei, M. G.; Hen, R. *Mol. Pharmacol.* **1993**, *43*, 313; (c)

Oliver, K. R.; Kinsey, A. M.; Wainwright, A.; Sirinathsinghji, D. J. S. *Brain Res.* **2000**, *867*, 131.
6. (a) Schoeffter, P.; Ullmer, C.; Bobirnac, I.; Gabbiani, G.; Lubbert, H. *Br. J. Pharmacol.* **1996**, *117*, 993; (b) Gustafson, E. L.; Durkin, M. M.; Bard, J. A.; Zgombick, J.; Branchek, T. A. *Br. J. Pharmacol.* **1996**, *117*, 657; (c) To, Z. P.; Bonhaus, D. W.; Eglen, R. M.; Jakeman, L. B. *Br. J. Pharmacol.* **1995**, *115*, 107; (d) Neumaier, J. F.; Sexton, T. J.; Yracheta, Y.; Diaz, A. M.; Brownfield, M. *J. Chem. Neuroanat.* **2001**, *21*, 63.
7. Thomas, D. R. *Pharmacol. Ther.* **2006**, *111*, 707.
8. Suckling, C. J.; Murphy, J. A.; Khalaf, A. I.; Zhou, S.; Lizos, D. E.; Van Nhien, A. N.; Yasumatsu, H.; McVie, A.; Young, L. C.; McCraw, C.; Waterman, P. G.; Morris, B. J.; Pratt, J. A.; Harvey, A. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 264.
9. Pittala, V.; Salerno, L.; Modica, M.; Siracusa, M. A.; Romeo, G. *Mini-Rev. Med. Chem.* **2007**, *7*, 945.
10. Lattmann, E.; Merino, I.; Dunn, S.; Parveen, B.; Lattmann, P.; Billington, D. C.; Bunprakob, Y.; Sattayasai, J. *Lett. Drug Design Disc.* **2006**, *3*, 49.
11. Hedlund, P. B.; Huitron-Resendiz, S.; Henriksen, S. J.; Sutcliffe, J. G. *Biol. Psychiatry* **2005**, *58*, 831.
12. Guscott, M.; Bristow, L. J.; Hadingham, K.; Rosahl, T. W.; Beer, M. S.; Stanton, J. A.; Bromidge, F.; Owens, A. P.; Huscroft, I.; Myers, J.; Rupniak, N. M.; Patel, S.; Whiting, P. J.; Hutson, P. H.; Fone, K. C.; Biello, S. M.; Kulagowski, J. J.; McAllister, G. *Neuropharmacology* **2005**, *48*, 492.
13. (a) Wesolowska, A.; Tatarczynska, E.; Nikiforuk, A.; Chojnacka-Wojcik, E. *Eur. J. Pharmacol.* **2007**, *555*, 43; (b) Wesolowska, A.; Nikiforuk, A.; Stachowicz, K. *Eur. J. Pharmacol.* **2006**, *53*, 185; (c) Wesolowska, A.; Nikiforuk, A.; Stachowicz, K.; Tatarczynska, E. *Neuropharmacology* **2006**, *51*, 578.
14. Graf, M.; Jakus, R.; Kantor, S.; Levay, G.; Bagdy, G. *Neurosci. Lett.* **2004**, *359*, 45.
15. Sprouse, J.; Reynolds, L.; Li, X.; Braselton, J.; Schmidt, A. *Neuropharmacology* **2004**, *46*, 52.
16. Corbett, David F.; Heightman, Tom D.; Moss, Stephen F.; Bromidge, Steven M.; Coggon, Sara A.; Longley, Mark J.; Roa, Ana Maria; Williams, Jennifer A.; Thomas, David R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4014.
17. Thomas, D. R.; Soffin, E. M.; Roberts, C.; Kew, J. N. C.; de la Flor, R. M.; Dawson, L. A.; Fry, V. A.; Coggon, S. A.; Faedo, S.; Hayes, P. D.; Corbett, D. F.; Davies, C. H.; Hagan, J. *J. Neuropharmacol.* **2006**, *51*, 566.
18. Data presented at the *36th Annu. Meet. Soc. Neurosci.* (Oct 16–Oct 18, Atlanta) **2006**, (a) Garcia-Ladona, F.; Amberg, W.; Kling, A.; Lange, U. E. W.; Hornberger, W.; Wernet, W.; Netz, A.; Ochse, M.; Mezler, M.; Meyer, A. H.; Hahn, A.; Hillen, H.; Beyerbach, A.; Bowmik, S.; Schoemaker, H.; Sullivan, J. P., Abst. 33.1; (b) Drescher, K. U.; Amberg, W.; Kling, A.; Gross, G.; Schoemaker, H.; Sullivan, J. P.; Garcia-Ladona F. J., Abst. 33.2; (c) Rueter, L. E.; Wicke, K.; Basso, A. M.; Jongen-Relo, A. L.; van Gaalen, M. M.; Gross, G.; Decker, M. W.; Kling, A.; Schoemaker, H.; Sullivan, J. P.; Amberg, W.; Garcia-Ladona, F. J., Abst. 33.3; (d) Ebert, U.; Kling, A.; Amberg, W.; Garcia-Ladona, F. J.; Gross, G.; Schoemaker, H.; Sullivan, J. P., Abst. 529.25; (e) Jongen-Relo, A. L.; Bespalov, A. Y.; Rueter, L. E.; Freeman, A. S.; Decker, M. W.; Gross, G.; Schoemaker, H.; Sullivan, J. P.; van Gaalen, M. M.; Wicke, K. M.; Zhang, M.; Amberg, W.; Garcia-Ladona, F. J., Abst. 529.26.
19. Rusak, B.; Zucker, I. *Physiol. Rev.* **1979**, *59*, 449.
20. Interestingly, **2** is a metabolite of the PDE3 inhibitor, anagrelide, and has been proposed to be responsible for anagrelide's thrombocytopenic effect: Lane, W. J.; Hattori, K.; Dias, S.; Peerschke, E. I. B.; Moore, M. A. S.; Blanset, D. L.; Lang, P. C.; Petrone, M.; Rafii, S. *Exp. Hematol.* **2001**, *29*, 1417.
21. Radioligand binding assays to assess the affinity of compounds for 5-HT receptors: recombinant human 5-HT receptors (5-HT1A, 1D, 2A, 2C, 5A, 6 and 7) were expressed in HEK-293-EBNA cells using transient transfection then homogenised. All radioligand binding assays were carried out in 96-well plates in the presence of radioligand ([^{3}H]LSD for 5-HT_{1D} (2 nM), 5-HT_{5A} (1.3 nM), 5-HT_6 (1.6 nM), 5-HT_7 (2 nM); [^{3}H]-8-OH-DPAT for 5-HT_{1A} (1 nM); [^{3}H] ketanserin for 5-HT_{2A} (1 nM); [^{3}H]-mesulergine for 5-HT_{2C} (1.5 nM)) and 10 concentrations of compound (ranging from 10 μM to 0.03 nM). Non-specific binding was defined using 2 μM pindolol (5-HT_{1A}), sumatriptan (5-HT_{1D}), spiperone (5-HT_{2A}), mianserin (5-HT_{2C}), methiothepin (5-HT_{5A} and 5-HT_6) and 10 μM SB269970 (5-HT_7). Each well contained an aliquot of receptor membrane homogenate (varying concentrations), 0.5 mg of Ysi-poly-l-lysine SPA beads (Amersham; for all SPA assays (5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{5A}), not filtration assays (5-HT_{1A}, 5-HT_6, 5-HT_7)) in a final volume of 200 μl of buffer containing 50 mM Tris,10 mM $MgCl_2$, 1 mM EGTA and 10 μM pargiline (pH 7.4). All assays were conducted in duplicate and repeated at least twice. Assay plates were incubated for varying times at room temperature (5-HT_{1A} 30 min; 5-HT_{1D}, 5-HT_{2A} 60 min; 5-HT_{2C} 90 min, 5-HT_{5A}120 min) or at 37 °C (5-HT_7 60 min and 5-HT_6 90 min) before centrifugation (SPA) or filtration. For filtration assays these were terminated by rapid filtration under vacuum through GF/C filters, presoaked for at least 30 min with PEI (polyethylenimine; 0.3%), with 5×0.4 ml washes of ice-cold Tris buffer (50 mM, pH 7.4). For both SPA and filtration plates, bound ligand was determined using a Packard Topcount scintillation counter. XLfit, was used to iteratively plot the data and determine IC_{50} and Hill coefficient values. K_i was determined using the Cheng-Prussoff calculation.
22. Determination of competitive action of guanidines at the 5-HT_{5A} receptor using [^{35}S]GTPγS Schild analysis: 5-HT agonist EC_{50} curves were determined in the presence of different antagonist concentrations. Experiments for each compound at different concentrations were repeated three times and then a pA_2 Schild analysis performed. Experiments were conducted in 96-well plates, each well containing 0.2 nM [^{35}S]GTPγS (25 μl), 100 μl of buffer (50 mM Tris, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM EGTA, 10μM pargiline and dithiothreitol 100 μM (pH 7.4)) alone or containing 5-HT (11 concentrations ranging from 100 μM to 0.1 nM) or non-specific definer GTPγS (100 μM), and GDP (3 μM). Compound (25 μl) was tested at three different concentrations per experiment (ranging from 1 μM to 1 nM). Each well contained an aliquot (50 μl) of receptor membrane homogenate (5-HT_{5A} cell homogenates prepared for the radioligand binding were used) premixed with (1.5 mg/well) PVT-WGA SPA beads (Amersham). Plates were incubated at 30 °C for 30 min, then centrifuged for 5 min, before rapidly (within 15 min) counting the radioactivity using a Packard Topcount scintillation counter.
23. Trinka, P.; Slegel, P.; Reiter, J. *J. Prakt. Chem.* **1996**, *338*, 675.
24. Stalder, H. *Helv. Chim. Acta* **1986**, *69*, 1887.
25. Sikkar, R.; Martinson, P. *Acta Chem. Scand. B* **1980**, *B34*, 551.
26. Preparation of **12**: (a) 1-(2-amino-6-chloro-phenyl)-ethanone (**29**): a suspension of 2-amino-6-chlorobenzonitrile (**28**, 3.67g, 24 mmol) in diethyl ether (60 ml) was added slowly to methylmagnesium bromide (56 ml, 3 M in Et_2O,

170 mmol), and the mixture was heated to reflux, until all starting material was consumed (3 h, HPLC control). The mixture was then placed in an ice bath, and HCl (6 M, 58 ml) was slowly added (vigorous reaction). The mixture was then again heated to reflux, cooled and made alkaline by addition of solid Na_2CO_3. The mixture was extracted several times with ethyl acetate, the combined organic layers were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. Purification of the residue by column chromatography (silica gel, solvent gradient *n*-heptane/ethyl acetate = 100/0–60/40) gave the title compound (2.72 g, 67%). ^{1}H NMR ($CDCl_3$): δ 2.65 (3H, s), 4.91 (2H, br s), 6.58 (1H, d), 6.73 (1H, d), 7.07 (1H, t); (b) 5-chloro-4-methyl-3,4-dihydro-1*H*-quinazoline-2-thione (**30**): at a temperature of 65 °C, sodium borohydride (411 mg, 10.9 mmol) was added to a solution of 1-(2-amino-6-chloro-phenyl)-ethanone (3.07 g, 18.1 mmol) in ethanol (10 ml), and the mixture was heated overnight (65 °C). Water (8 ml), KSCN (1.93 g in 4 ml H_2O, 19.9 mmol) and HCl (7 ml, 20%) were then added subsequently, and the mixture was again heated (3 h, 65 °C). The majority of the title compound precipitated upon cooling and could be isolated in sufficiently pure form by filtration. A small additional amount of the product was obtained by workup of the mother liquor (evaporation of solvent, column chromatography [silica gel, solvent gradient *n*-heptane/ethyl acetate = 100/0–60/40]). The title compound was obtained in a combined yield of 2.08 g (52%). ^{1}H NMR (d^6-DMSO): δ 1.26 (3H, d), 4.58 (1H, q), 6.97 (1H, d), 7.09 (1H, d), 7.23 (1H, t), 8.99 (1H, br s), 10.73 (1H, br s); (c) 5-chloro-4-methyl-2-methylsulfanyl-3,4-dihydroquinazoline hydroiodide (**31**): methyl iodide (1.16 ml, 19 mmol) was added to a suspension of 5-chloro-4-methyl-3,4-dihydro-1*H*-quinazoline-2-thione (**30**, 1.32 g, 6.2 mmol) in acetone (15 ml) and the mixture was stirred at rt over the weekend (the reaction is usually complete after 12 h). The precipitated product (2.08 g, 87%) was sufficiently pure for the next step. ^{1}H NMR (d^6-DMSO): δ 1.42 (3H, d), 2.76 (3H, s), 4.97 (1H, q), 7.11–7.14 (1H, m), 7.39–7.43 (2H, m), 10.58 (1H, br s), 12.38 (1H, br s); (d) 5-chloro-4-methyl-3,4-dihydroquinazolin-2-ylamine (**12**): 5-chloro-4-methyl-2-methylsulfanyl-3,4-dihydroquinazoline hydroiodide (**31**, 100 mg, 0.28 mmol) was suspended in a mixture of ammonium hydroxide (1 ml , 25% in H_2O) and acetonitrile (1 ml), and heated in a microwave oven to 130 °C (15 min) and subsequently to 170 °C (30 min). The title compound (35 mg, 62%) was isolated from the reaction mixture by preparative, reverse-phase HPLC (YMC CombiPrep C18 column 50×20 mm, solvent gradient 5–95% CH_3CN in 0.1% TFA (aq) over 6.0 min, λ = 230 nm, flow rate 40 ml/min) as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, d), 4.83 (1H, q), 6.85 (1H, d), 6.92 (1H, d), 7.06 (3H, t).

27. Darnbrough, S.; Mervic, M.; Condon, S. M.; Burns, C. J. *Synth. Comm.* **2001**, *31*, 3273.
28. (a) Alanine, A.; Gobbi, L. C.; Kolczewski, S.; Luebbers, T.; Peters, J.-U.; Steward, L. U.S. Pat. Appl. US 2006293349, 2006; (b) Alanine, A.; Gobbi, L. C.; Kolczewski, S.; Luebbers, T.; Peters, J.-U.; Steward, L. U.S. Pat. Appl. US 2006293350, 2006.