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Synthesis and biological evaluation of novel hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines as potent and selective 5-HT$_{2C}$ receptor agonists

Hans G. F. Richter,[a,*] D. R. Adams,[b] A. Benardeau,[a] M. J. Bickerdike,[b] J. M. Bentley,[b,†] T. J. Blench,[b] I. A. Cliffe,[b] C. Dourish,[b] P. Hebeisen,[a] G. A. Kennett,[b] A. R. Knight,[b] C. S. Malcolm,[b] P. Mattei,[a] A. Misra,[b] J. Mizrahi,[a] N. J. T. Monck,[b] J.-M. Plancher,[a] S. Roever,[a] J. R. A. Roffey,[b] S. Taylor[a] and S. P. Vickers[b]

[a]*F. Hoffmann-La Roche Ltd, Discovery Research, 4070 Basel, Switzerland*
[b]*Vernalis Research Ltd, Oakdene Court, 613 Reading Road, Wokingham, RG41 5UA, UK*

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Abstract—Further lead optimization efforts on previously described 1,2,3,4,10,10a-hexahydro-1*H*-pyrazino[1,2-*a*]indoles led to the new class of 5,5a,6,7,8,9-hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines culminating in the discovery of (5a*R*,9*R*)-2-[(cyclopropylmethoxy)methyl]-5,5a,6,7,8,9-hexahydro-9-methyl-pyrido[3′, 2′:4,5]pyrrolo[1,2-*a*]pyrazine **18** as a potent, full 5-HT$_{2C}$ receptor agonist with an outstanding selectivity profile and excellent hERG and phospholipidosis properties.

Obesity is steadily increasing all over the world [1] and is a major risk factor in the development of hyperglycemia, hypertension, dyslipidemia, coronary artery disease, and certain cancers.[2]

The currently approved drugs for the long-term treatment of obesity are the appetite suppressant Sibutramine®, a centrally acting mixed noradrenaline/serotonin-reuptake inhibitor, and Xenical®, a non-systemic acting lipase inhibitor which increases the fecal loss of undigested triglycerides. Recent evidence of the utility of 5-HT$_{2C}$ receptor agonists as appetite suppressants in the management of obesity has led to a resurgence of interest in selective and safe 5-HT$_{2C}$ receptor agonists.

In preclinical studies, the involvement of the 5-HT$_{2C}$ receptor subtype in the control of feeding in animals has been established through the use of 5-HT$_{2C}$ receptor agonists, antagonists, and transgenic mouse models.[3]

The non-selective 5-HT$_{2C}$ receptor agonist *m*-chlorophenylpiperazine (mCPP) reduces food intake in rats and accelerates the appearance of the behavioral satiety sequence in rats. The anorectic action of mCPP is absent in 5-HT$_{2C}$ receptor knockout mutant mice[4] and is antagonized by the selective 5-HT$_{2C}$ receptor antagonist SB-242084.[5] In clinical studies, mCPP decreases food intake and body weight of obese subjects.[6] Phase IIa clinical trials have demonstrated significant body weight loss with the 5-HT$_{2C}$ receptor agonists BVT-933 (Biovitrum; development stopped) and APD356 (Arena; Phase IIb clinical trials initiated June 2005). The structures for both compounds have not been published yet. In the search for novel 5-HT$_{2C}$ receptor agonists, we recently reported the discovery and synthesis of 1,2,3,4,10,10a-hexahydro-1*H*-pyrazino[1,2-*a*]indoles as potent and selective 5-HT$_{2C}$ receptor agonists such as **1**.[7] Although compound **1** was a potent full agonist at the 5-HT$_{2C}$ receptor and showed >10-fold selectivity against both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors, it was also found to inhibit the hERG potassium channel in vitro with an IC$_{50}$ of 2.5 μM. Phospholipidosis was also induced by

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* Corresponding author. Tel.: +41 61 688 1330; fax: +41 61 688 6459; e-mail: hans.richter@roche.com
† Present address: GlaxoSmithKline S.p.A., Centre of Excellence for Drug Discovery, Via Fleming 4, 37135 Verona, Italy.

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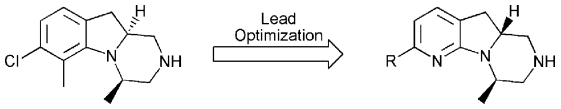
1 in cultured fibroblasts[8] at the lowest tested concentration of 2.5 μM (test range 2.5–20 μM). Striving for improved properties with respect to both the hERG and phospholipidosis flag we hypothesized that replacement of the indoline core by an azaindoline system would reduce lipophilicity and amphiphilicity and lead to molecules with improved safety profile with respect to these properties. This led us to the identification of 5,5a,6,7,8,9-hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines as a new class of potent human 5-HT_{2C} receptor agonists with improved binding selectivity profiles and good in vivo potency in rats after oral administration. In addition, several analogues in fact showed significantly reduced hERG inhibition and phospholipidosis induction in vitro.



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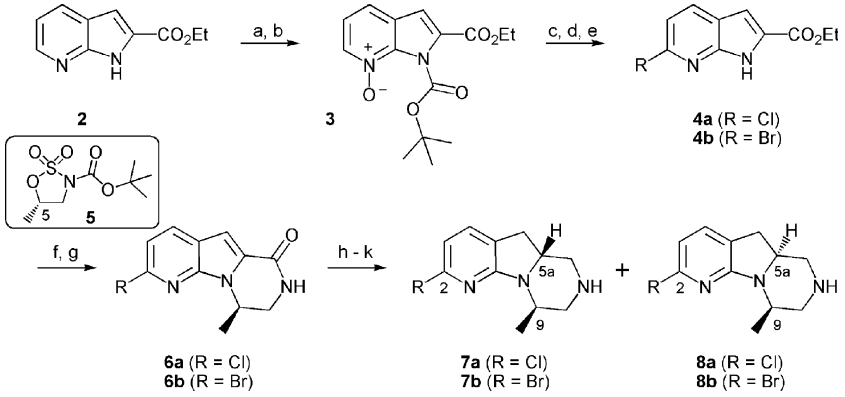
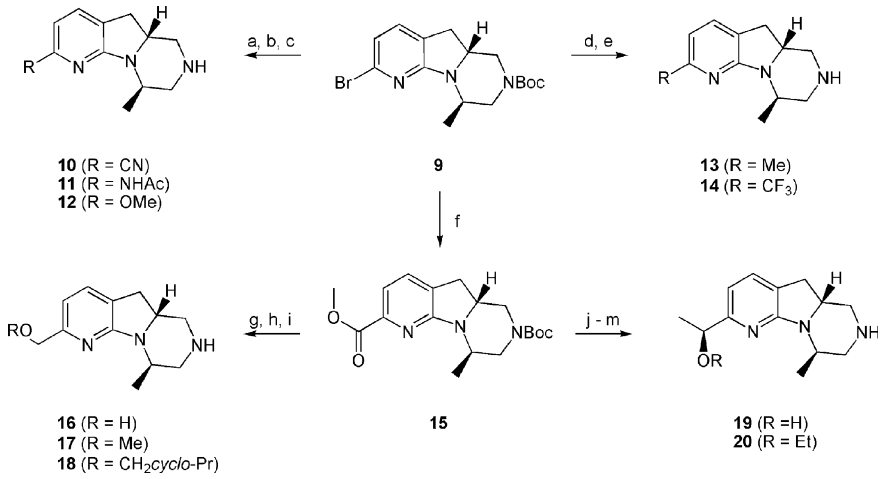
Synthesis of chiral 9-methyl-substituted hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines was accomplished using 1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylic acid ethyl ester **2**[9] and recently developed 5-methyl-2,2-dioxo[1,2,3]oxathiazolidine-3-carboxylic acid *tert*-butyl ester **5** as a new type of crystalline and stable chiral alkylating agent (Scheme 1).[10] Chlorination or bromination of **2**, *ortho*- to the pyridine nitrogen, was achieved after Boc-protection, oxidation with *m*-CPBA, and subsequent rearrangement of the *N*-oxide **3** with ethyl chloroformate and benzoylbromide, respectively, in THF with HMDS as a base. The 7-aza-indoles **4** were alkylated in excellent yields using the previously described sulfamidate **5**. In line with an S_N2 reaction this proceeded with inversion of configuration. Boc-deprotection of the 1-alkylated 7-azaindole-2-carboxylates with trifluoroacetic acid in dichloromethane was followed by cyclization to the dihydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazinones **6** using potassium carbonate in methanol as base. Reduction with $LiAlH_4$ gave the corresponding tetrahydro-9-methyl-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines, which after Boc-protection were further reduced with $NaCNBH_3$ in acetic acid to provide the (5a*R*,9*R*)-diastereomer[11] in an 8:1 excess over its (5a*S*,9*R*)-diastereomer. The stereoisomers were separated by conventional column chromatography on silica gel, which after deprotection gave the final compounds **7** and **8**. Similarly the (5a*S*,9*S*)- and (5a*R*,9*S*)-diastereomers as well as the 9-unsubstituted analogues could be synthesized from either the (*R*)-5-methyl- or the 5-unsubstituted 2,2-dioxo[1,2,3]oxathiazolidine-3-carboxylic acid *tert*-butyl esters (not shown).

The Boc-protection of **7** yielded intermediate **9**, which turned out to be a versatile building block applicable to various types of organometallic and transition metal-catalyzed reactions. Subsequent deprotection provided target molecules with more elaborate substitution patterns (Scheme 2). For example, carbonylation of **9** with carbon monoxide under Pd catalysis in methanol gave the corresponding methyl ester **15** which could be reduced with DIBAL-H to give the 2-hydroxymethyl compound which in turn could be further alkylated deprotected to yield compounds **16**–**18**. Alternatively the ester **15** could be transformed into the Weinreb amide using *N*,*O*-dimethylhydroxylamine and $AlMe_3$, which upon reaction with methyl magnesium bromide gave the acetyl compound. Borane mediated reduction of the acetyl derivative using chiral (*R*)-2-methyl-CBS-oxazaborolidine catalyst led to the (*S*)-1-hydroxyethyl intermediate in high diastereomeric excess (*de* > 99%). Alkylation and deprotection led to the final derivative **20**. Accordingly the (*R*)-1-hydroxyethyl diastereomer could be obtained by using the (*S*)-2-methyl-CBS-oxazaborolidine catalyst.

The compounds were screened at 1 μM for functional activity at human recombinant 5-HT_{2C} receptors expressed in CHO cells using a fluorimetric imaging plate reader (FLIPR). The maximum fluorescent signal was measured and compared with the response produced by 10 μM 5-HT (defined as 100%).[12] The active compounds from this functional assay were then compared



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, DMAP, CH_3CN, rt, 99%; (b) *m*-CPBA, CH_2Cl_2, rt, 60%; (c) $ClCO_2Et$, HMDS, THF, rt, 76% or (d) BzBr, HMDS, THF, rt, 51%; (e) TFA, CH_2Cl_2, 0 °C to rt, 96–99%; (f) **5**, NaH, DMF, 0 °C to rt, 93–98%; (g) TFA, CH_2Cl_2, 0 °C to rt, then MeOH, K_2CO_3, rt, 74–87%; (h) $LiAlH_4$, *t*-BuOMe, reflux, 15 min, 78–96%; (i) $(Boc)_2O$, CH_2Cl_2, rt, 85–98%; (j) $NaCNBH_3$, AcOH, rt, 66–88% for major diastereomer (5a*R*,9*R*); (k) TFA, CH_2Cl_2, rt, 96%.



Scheme 2. Reagents and conditions: (a) CuI, $Pd_2(dba)_3$, dppf, Et_4NCN, dioxane, reflux; TFA, CH_2Cl_2, rt, 61% (**10**); (b) $Pd_2(dba)_3$, (±)-BINAP, NaO*t*-Bu, toluene, benzophenone imine, reflux; Pd/C, NH_4-formate, MeOH; AcCl, NEt_3, CH_2Cl_2, rt; TFA, CH_2Cl_2, rt, 48% (**11**); (c) $Pd_2(dba)_3$, BnOH, (±)-BINAP, NaOH, toluene, 70 °C; H_2, Pd/C, MeOH/EtOAc, rt; MeI, NaH, DMF, rt; TFA, CH_2Cl_2, rt, 39% (**12**); (d) $Pd[PPh_3]_4$, Na_2CO_3, $(MeBO)_3$, DME, reflux; TFA, CH_2Cl_2, rt, 41% (**13**); (e) CF_3CO_2Na, CuI, NMP, 180 °C; TFA, CH_2Cl_2, rt, 20% (**14**); (f) CO (40 bar), dppf, $Pd(OAc)_2$, NEt_3, MeOH, 80 °C, 74% (**15**); (g) DIBAL-H, THF, 0 °C to rt; TFA, CH_2Cl_2, rt, 17% (**16**); (h) DIBAL-H, THF, 0 °C to rt; MeI, NaH, DMF, rt; TFA, CH_2Cl_2, rt, 77% (**17**); (i) DIBAL-H, THF; *c*-$PrCH_2Br$, NaH, DMF, rt; TFA, CH_2Cl_2, rt, 41% (**18**); (j) HNMe(OMe), $AlMe_3$, THF, 0 °C to rt, 94%; (k) MeMgBr, THF, −78 °C to rt, 92%; (l) (*R*)-Me-CBS-oxazaborolidine, BMS, THF, 0 °C, 98%; (m) TFA, CH_2Cl_2, 0 °C to rt, 74% (**19**) or EtBr, NaH, DMF, 50 °C; TFA, CH_2Cl_2, 0 °C to rt, 52% (**20**).

in radioligand binding assays at recombinant human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors expressed in CHO cells. For the 5-HT_{2A} receptor [^{125}I]-DOI was used as the radioligand, whereas [^{3}H]-5-HT was used for the 5-HT_{2B} and 5-HT_{2C} receptor subtypes.[13] The results are shown in Tables 1 and 2. As described in Table 1 for the 2-chloro substituted analogues, introduction of a 9*R*-methyl substituent increased the 5-HT_{2C} receptor binding affinity. As exemplified for the 2-chloro and 2-bromo analogues (**11** and **12**, respectively), both the (5a*R*,9*R*) and (5a*S*,9*R*) stereochemistries provided full agonists. In contrast to the carba series[7] however, the (5a*R*,9*R*)-diastereomer provided both higher binding affinity for the 5-HT_{2C} receptor and significantly higher binding selectivity over the other 5-HT_2 receptor subtypes compared to the (5a*S*,9*R*)-diastereomer. The (5a*R*,9*R*)-7-bromo-9-methyl compound **7b** was an exceptional sub-nanomolar agonist with approximately 66-fold and 100-fold binding selectivity over the 5-HT_{2A} and 5-HT_{2B} receptor, respectively.

The influence of several other substituents in the 2-position of the 5,5a,6,7,8,9-hexahydro-9-methyl-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazine skeleton on receptor affinity and selectivity is exemplified in Table 2. Small lipophilic substituents in the 2-position (CN, CF_3, and Me), whether electron withdrawing or donating, all led to high affinity for the 5-HT_{2C} receptor, while polar substituents led to a comparative loss in affinity. Hydroxy (i.e., **16**, **19**) and especially alkoxy substituents (i.e., **17**, **18**, and **20**) were also tolerated, however, only if the oxygen atom was not directly attached to the core, as it is for compound **12**. From the branched ether analogues compound **20** had high binding affinity for the target receptor (K_i 2.3 nM), 28-fold binding selectivity over the 5-HT_{2A} receptor, and exceptionally high binding selectivity of 128-fold over the 5-HT_{2B} receptor. The corresponding (*R*)-1-ethoxyethyl diastereomer **21** was much less potent and selective, especially over the 5-HT_{2B} receptor.

Further pharmacological characterization of **18** and **20** was performed using measurements of the stimulation of intracellular calcium mobilization in stable CHO cell lines expressing each of the human 5-HT_2 receptor subtypes[14] and the results are summarized in Table 3. Both

Table 1. 5-HT_2 receptor efficacy and binding of 5,5a,6,7,8,9-hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazines—initial SAR

Compound	Substituents	Stereochemistry	Relative efficacy[a] h5-HT_{2C} (%)	K_i[b] h5-HT_{2A} (nM)	K_i[c] h5-HT_{2B} (nM)	K_i[c] h5-HT_{2C} (nM)
25	2-Cl, 9-H	*rac*	ND[d]	217	221	131
7a	2-Cl, 9-Me	5a*R*,9*R*	98	42	48	1.6
8a	2-Cl, 9-Me	5a*S*,9*R*	81	54	217	52
7b	2-Br, 9-Me	5a*R*,9*R*	97	53	81	0.8
8b	2-Br, 9-Me	5a*S*,9*R*	87	58	166	21
1		4*R*,10a*S*	97	19	40	1.9
1		4*R*,10a*R*	98	2.6	3.2	0.3

[a] Efficacy at 1 μM ligand concentration relative to 10 μM of 5-HT (100%).
[b] Displacement of [^{125}I]-DOI.
[c] Displacement of [^{3}H]-5-HT.
[d] ND, not determined.

Table 2. 5-HT_2 receptor efficacy and binding of 2-substituted (5a*R*,9*R*)-9-methyl-5,5a,6,7,8,9-hexahydro-pyrido[3′,2′:4, 5]pyrrolo[1,2-*a*]pyrazines

Compound	2-Substituent	Relative efficacy[a] h5-HT_{2C} (%)	K_i[b] h5-HT_{2A} (nM)	K_i[c] h5-HT_{2B} (nM)	K_i[c] h5-HT_{2C} (nM)
10	CN	85	2547	1820	60
11	NHAc	0	ND[d]	ND[d]	ND[d]
12	MeO	77	511	440	104
13	Me	101	136	77	2.7
14	CF_3	96	43	68	14
16	CH_2OH	88	942	1139	54
17	CH_2OMe	97	565	379	16
18	CH_2OCH_2c-Pr	87	74	174	3.6
19	(*S*)-CH_3(OH)CH	101	392	835	39
20	(*S*)-CH_3(OEt)CH	83	65	295	2.3
21	(*R*)-CH_3(OEt)CH	86	2385	1306	93

[a] Efficacy at 1 μM ligand concentration relative to 10 μM of 5-HT (100%).
[b] Displacement of [^{125}I]-DOI.
[c] Displacement of [^{3}H]-5-HT.
[d] ND, not determined.

Table 3. Functional activities[a] in stable CHO cell lines expressing human 5-HT_2 receptor subtypes

Compound	h5-HT_{2A}		h5-HT_{2B}		h5-HT_{2C}	
	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}
18	N/A[b]	9	23	31	15	90
23	N/A[b]	6	26	56	6.2	93

[a] Efficacy was determined by receptor-induced mobilization of intracellular Ca^{2+} using fluorometric imaging plate reader (FLIPR^{TM}).
[b] N/A: insufficient efficacy for generating an EC_{50} value.

compounds were found to be potent and full 5-HT_{2C} receptor agonists, weak partial agonists at the 5-HT_{2B} receptor, and failed to activate 5-HT_{2A} receptors.

The lack of $5HT_{2A}$ receptor agonism prompted us to evaluate **18** for its ability to antagonize 5-HT-stimulated calcium mobilization in a 5-HT_{2A} receptor-expressing cell line. Indeed, **18** also possessed 5-HT_{2A} receptor antagonist activity and exhibited appreciable affinity with a pK_B of 6.7. Ancillary binding studies addressing selectivity of **18** toward a number of biogenic amine binding sites and a broad spectrum of receptor, transporter, and ion channel targets failed to reveal potent binding affinity for any of the targets examined, indicating the compound to be highly selective for the 5-HT_{2C} receptor subtype (Table 4).

Although the original lead compound **1** was a potent 5-HT_{2C} receptor agonist with favorable 5-HT_2 receptor subtype selectivity, it also had some undesired effects, as it induced phospholipidosis in vitro and inhibited the hERG potassium channel with an IC_{50} of 2.5 μM.

Table 4. Ancillary receptor binding affinities determined for **18**

Target	K_i (nM)
5-HT_{1D}	>360
5-$HT_{1A/1B/5A/6}$; D4	>720
5-$HT_{3/7}$	>1000
α1, β1, μ	>1000
5-HT-, NA-, DA-transporter	>1000
Na channel	>1000

Several of the newly synthesized aza-analogues were profiled under the same assay conditions and the results are summarized in Table 5. Compared to **1**, several of the tested aza-analogues in fact showed substantially attenuated hERG activity and exhibited a markedly reduced phospholipidosis potential that we attribute to their reduced lipophilicity and amphiphilicity. Compound **18** did not induce phospholipidosis up to the highest test concentration of 20 μM and had a clean hERG profile with an IC_{50} > 30 μM.

Several representatives showed good in vivo efficacy after per os (po) application in a rodent food-deprived model. For example, compound **18** was tested for its ability to reduce food intake in 23 h food-deprived Lister Hooded rats after an acute administration.[15] The compound significantly reduced food intake in a dose-dependent manner with a minimum effective dose (MED) of 10 mg/kg po. This effect was antagonized by the 5-HT_{2C} receptor antagonist, SB-242084 (0.3, 1, 3 mg/kg sc), suggesting that the effects of **18** on food intake are mediated solely through activation of 5-HT_{2C} receptors.

In conclusion, we have developed a novel series of chiral 5,5a,6,7,8,9-hexahydro-9-methyl-pyrido[3′,2′:4,5]pyrrolo-[1,2-*a*]pyrazines as potent, selective, and in vivo active

Table 5. In vitro hERG and phospholipidosis results for selected (5a*R*,9*R*)-9-methyl-5,5a,6,7,8,9-hexahydro-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]-pyrazines

Compound	**2**-Substituent	hERG[a] IC_{50} (μM)	Phospholipidosis[b]
1(4*R*,10a*S*)		2.5	+++ (7.5 μM)
13	Me	21	Negative (20 μM)
7a	Cl	40	+ (20 μM)
18	CH_2OCH_2c-Pr	>30	Negative (20 μM)
20	(*S*)-CH_3(OEt)CH	20% inhibition at 10 μM[c]	+ (20 μM)

[a] Inhibition of hERG K channel determined by whole-cell patch-clamp experiments on a transfected CHO cell line.
[b] Induction of phospholipidosis in cultured fibroblasts: strong (+++), moderate (++), and weak (+).
[c] Insufficient inhibition for generating an IC_{50} value.

5-HT_{2C} receptor agonists. From this series, compound **18** emerged as a potent, full agonist with an outstanding selectivity profile. Moreover, **18** did not induce phospholipidosis in vitro and had a clean hERG profile with an $IC_{50} > 30$ μM.

Acknowledgments

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11. Enantiomeric excess in the S_N2 reaction was determined by means of chiral HPLC using 2% ethanol in heptane as mobile and Chiralpak-AD as stationary phase. Confirmation of the absolute configuration of the C5a- and C9 carbon was carried out by crystallization and X-ray of (5a*R*,9*R*)-2-chloro-9-methyl-5a,6,8,9-tetrahydro-(5H)-pyrido[3′,2′:4,5]pyrrolo[1,2-*a*]pyrazine-7-carboxylic acid 1,1-dimethylethyl ester, which was synthesized according to Scheme 2. CCDC 288646 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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14. The functional activity was assayed using a Fluorimetric Imaging Plate Reader (FLIPR). CHO cells expressing either the human 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptor subtype were counted and plated into standard 96-well microtiter plates on the day before testing to give a confluent monolayer. The cells were then loaded with the calcium-sensitive dye, Fluo-3-AM. Unincorporated dye was removed using an automated cell washer to leave a total volume of 100 μL/well of assay buffer (Hanks, balanced salt solution containing 20 mM Hepes and 2.5 mM probenecid). The drug (dissolved in 50 μL of the assay buffer) was added at a rate of 70 μL/s to each well of the FLIPR 96-well plate during fluorescence measurements. The measurements were taken at 1 s intervals and the maximum fluorescent signal was measured (approx 10–15 s after drug addition) and compared with the response produced by 10 μM 5-HT (defined as 100%) to which it was expressed as a percentage response (relative efficacy). Dose–response curves were constructed using Graphpad Prism (Graph Software Inc.).
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