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In vitro functional evaluation of isolaureline, dicentrine and glaucine enantiomers at 5-HT$_2$ and α_1 receptors

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Compounds with activity at serotonin (5-hydroxytryptamine) 5-HT$_2$ and α_1 adrenergic receptors have potential for the treatment of central nervous system (CNS) disorders, drug addiction or overdose. Isolaureline, dicentrine and glaucine enantiomers were synthesised and their *in vitro* functional activities at human 5-HT$_2$ and adrenergic α_1 receptor subtypes were evaluated. The enantiomers of isolaureline and dicentrine acted as antagonists at 5-HT$_2$ and α_1 receptors with (*R*)-isolaureline showing the greatest potency (pK_b = 8.14 at the 5-HT$_{2C}$ receptor). Both (*R*)- and (*S*)-glaucine also antagonised α_1 receptors, but they behaved very differently to the other compounds at 5-HT$_2$ receptors: (*S*)-glaucine acted as a partial

agonist at all three 5-HT_2 receptor subtypes, whereas (*R*)-glaucine appeared to act as a positive allosteric modulator at the 5-HT_{2A} receptor.

KEYWORDS

aporphines; enantiomers; functional studies; G protein-coupled receptors, serotonin 5-HT_2 receptors; adrenergic α_1 receptors; antagonist; partial agonist; allosteric modulator

1 INTRODUCTION

The serotonin (5-hydroxytryptamine) 5-HT_2 (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}) and α_1 (α_{1A}, α_{1B} and α_{1D}) adrenergic receptors are two families of G protein-coupled receptors (GPCRs) with significant clinical interest. In particular, the action of second generation (atypical) antipsychotics at these two sets of receptors is thought to contribute to the suppression of positive symptoms of schizophrenia and the reduction of extrapyramidal side effects compared to first generation (typical) antipsychotics.[1,2] Furthermore, compounds with 5-HT_2 and/or α_1 receptor activity have potential for the treatment of psychostimulant addiction[3,4] and as antidotes for 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) overdose.[5-7]

Aporphines are tetrahydroisoquinoline alkaloids which contain a chiral centre at position C6a and display a wide range of pharmacological properties.[8,9] In particular, there have been a number of reports on aporphines acting at dopamine[8] and 5-HT_{1A}[10] receptors and these have indicated that enantiomers tend to show contrasting functional activities, with (*R*)- and (*S*)-isomers acting as agonists and antagonists, respectively. However, in the first reported study on the actions of the individual enantiomers of an aporphine at 5-HT_{2A} receptors, both isomers of nantenine (**1**) (Figure 1) were found to act as competitive antagonists.[11] This pattern of behaviour has also been observed in studies carried out in our laboratory on the individual enantiomers of roemerine (**2a**) and nuciferine (**3a**).[12] Similarly, both enantiomers of these three compounds, as well as apomorphine (**4a**) and *N*-n-propylnorapomorphine (**4b**), have been found to antagonise α_1 receptors.[11-13] We therefore decided to investigate whether the structurally-related compounds, isolaureline (**2b**), dicentrine (**2c**) and glaucine (**3b**) follow the same pattern. Since (*S*)-glaucine is the only one of these compounds that is commercially-available with sufficient purity, we first performed a stereoselective synthesis of each enantiomer of the compounds prior to evaluating their functional activities at human 5-HT_2 and α_1 receptors.

2 MATERIALS AND METHODS

2.1 Chemicals

(*R*)-isolaureline (**2b**), (*R*)-dicentrine (**2c**) and (*R*)-glaucine (**3b**) were prepared via a synthetic route (Scheme 1) that is analogous to that previously reported by us[12] using (*S*,*S*)-**8** as the catalyst for the asymmetric hydrogenation step (see Additional Supporting Information). (*S*)-isolaureline and (*S*)-dicentrine were prepared in the same way, but using (*R*,*R*)-**8** as the catalyst. (*S*)-glaucine was obtained from Santa Cruz Biotechnology (Dallas, TX, U.S.A.) and its identity and purity were confirmed by ^{1}H NMR spectroscopy, polarimetry and chiral HPLC analysis. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

2.2 Functional assays at 5-HT$_2$ and α_1 receptors

Agonism and antagonism testing at human 5-HT$_2$ (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$) and α_1 (α_{1A}, α_{1B} and α_{1D}) receptors was carried out using a transforming growth factor-α shedding assay to assess GPCR activation in HEK293 cells (ATCC #CRL-1573) transiently transfected with the relevant receptor, as previously described.[12,14] For agonism testing, the cells were incubated for 1 hour at 37°C with varying concentrations (0.1 nM to 60 μM final concentration) of test compound or endogenous agonist (serotonin for 5-HT$_2$ receptors, noradrenaline for α_1 receptors) in stimulation buffer. For antagonism testing, the cells were pre-treated with varying concentrations (0.1 nM to 60 μM final concentration) of test compound or positive control (ketanserin for 5-HT$_2$ receptors, prazosin for α_1 receptors) in stimulation buffer for 15 minutes, followed by addition of a fixed concentration of serotonin (1 μM, 0.1 μM or 0.1 μM final concentration for 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$, respectively) or noradrenaline (0.1 μM, 1 μM or 1 μM final concentration for α_{1A}, α_{1B} and α_{1D} receptors, respectively) and the mixture was incubated for 1 hour at 37°C. Alkaline phosphatase activity in the cells and in the supernatant was determined by measuring the absorbance of *p*-nitrophenol (from hydrolysis of *p*-nitrophenyl phosphate) at 405 nm using a Cytation 3 cell imaging multi-mode microplate reader (Biotek Instruments, Inc., Winooski, VT, USA). Each assay was performed in triplicate. Receptor response was defined as the alkaline phosphatase activity of the supernatant/total alkaline phosphatase activity (cells + supernatant) and normalised against the maximal response obtained with the endogenous agonist. In order to obtain dose-response curves for serotonin at 5-HT$_{2A}$ receptors in the presence of different concentrations of (*R*)-glaucine, a similar procedure was followed as for antagonism testing, except that varying concentrations of serotonin (10 pM to 60 μM final

concentration) were added after pre-treatment of 5-HT_{2A} receptor-expressing cells with (*R*)-glaucine.

Data processing was performed using Prism 5 (GraphPad Software., San Diego, CA, USA) to generate relative E_{max} and EC_{50} (for agonists) or IC_{50} (for antagonists). Antagonist dissociation constant (K_b) values for the test compounds were calculated from pIC_{50} curves using a modified form of the Gaddum equation: $K_b = IC_{50}/([A]/EC_{50} - 1)$ where IC_{50} = the IC_{50} value of the antagonist, [A] = the concentration of the agonist and EC_{50} = the EC_{50} value of the agonist.[15]

2.3 Molecular modelling

The 3D structures of the test compounds were downloaded from the PubChem substances and compound database[16] and were prepared using LigPrep 2.3 (Schrödinger LLC, New York, USA), including formation of the protonated nitrogen species, which is expected to predominate under physiological conditions. The 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor structures previously generated by us were used for docking studies.[12] Receptor and ligand coordinate files were prepared using AutoDockTools 1.5.6.[17] Docking was performed with AutoDock Vina 1.0.2 with an exhaustiveness of 100.[18] The grid box was set to cover the extracellular half of the receptor with 1Å grid spacing. Images of the ligand-receptor complexes obtained were generated using PyMOL (Schrödinger LLC).

3 RESULTS AND DISCUSSION

3.1 Synthesis

The (*R*)-enantiomers of the test compounds were prepared using (*S*,*S*)-**8** as the catalyst for the asymmetric hydrogenation step (see Scheme 1).[12,19] Coupling of 2-(2-bromo-5-methoxyphenyl)acetic acid (**5a**) or 2-(2-bromo-4,5-dimethoxyphenyl)acetic acid (**5b**) with 2-(3,4-methylenedioxyphenyl)ethylamine (**6a**) or 2-(3,4-dimethoxyphenyl)ethylamine (**6b**) gave the corresponding amides (**7aa**–**7bb**). Bischler-Napieralski cyclisation, followed by asymmetric hydrogenation of the resulting imines, gave the (*R*)-1-benzyl-1,2,3,4-tetrahydroisoquinolines (**9aa**–**9bb**). Protection of the amino group as the methylcarbamate, followed by oxidative cyclisation and then reduction of the methylcarbonate group gave (*R*)-isolaureline (**2b**), (*R*)-dicentrine (**2c**) and (*R*)-glaucine (**3b**), in approximately 30% overall yield and with 95%, 98% and 90%, e.e., respectively, determined by chiral HPLC. In a similar

manner, but this time using (*R*,*R*)-**8** as the catalyst for the asymmetric hydrogenation step, (*S*)-isolaureline and (*S*)-dicentrine were obtained with 93% and 96% e.e.

3.2 Pharmacological evaluation

The functional activities of each enantiomer of the test compounds were evaluated at human 5-HT_2 and α_1 receptors in HEK293 cells transiently transfected with the relevant receptor.[12,14] The results are summarised in Tables 1–3.

The enantiomers of isolaureline (**2b**) and dicentrine (**2c**) all antagonised the response obtained at both 5-HT_2 and α_1 receptors in the presence of the corresponding endogenous agonist (serotonin and noradrenaline, respectively). The potency of (*R*)-isolaureline at the 5-HT_{2C} receptor (pK_b = 8.14) was even greater than that previously determined for (*R*)-roemerine (pK_b = 7.82).[12] Its selectivity ranged from 4- to 5-fold compared to the 5-HT_{2A} and 5-HT_{2B} receptors and from 25- to 63-fold compared to the three α_1 receptor subtypes. The results for (*S*)-dicentrine at α_1 receptors are consistent with previous studies.[20-24]

Both (*R*)- and (*S*)-glaucine (**3b**) were also observed to act as antagonists at α_1 receptors, in line with previous studies on (*S*)-glaucine.[13,25-28] However, they behaved very differently to the other test compounds at 5-HT_2 receptors: (*S*)-glaucine acted as a partial agonist (Figure 2A), whereas (*R*)-glaucine appeared to enhance the response obtained in the presence of serotonin (in antagonism testing), particularly at the 5-HT_{2A} receptor, suggesting that it may act as a positive allosteric modulator (Figure 2B). The former result is in agreement with a recent report of a radioligand binding study on (*S*)-glaucine that showed that it can displace [^{3}H]-ketanserin from 5-HT_{2A} receptors (K_i = 966 nM).[29] Interestingly, some analogues of nantenine with cycloalkyloxy substituents at position C1 of the A ring have also been reported to have weak (partial) agonist activities.[30]

In order to investigate further the positive allosteric modulatory behaviour of (*R*)-glaucine at the 5-HT_{2A} receptor, dose-response curves for serotonin in the presence of different concentrations of (*R*)-glaucine were obtained (Figure 3). The observed upward shift of the serotonin response (with minimal horizontal shift) is consistent with (*R*)-glaucine being able to increase the efficacy of serotonin. Although allosteric modulation of other GPCRs is relatively well known, to date, there have been very few reports of such behaviour at 5-HT_2 receptors.[31-33]

With regards to stereoselectivity, the most striking observation is the aforementioned contrast in the functional behaviour of (*R*)- and (*S*)-glaucine at 5-HT$_2$ receptors. Isolaureline also exhibited some stereoselectivity, albeit more moderate, with the (*R*)-enantiomer displaying 4-fold selectivity over the (*S*)-enantiomer at the 5-HT$_{2C}$ receptor and the (*S*)-enantiomer displaying 5- and 3-fold selectivity over the (*R*)-enantiomer at the α_{1B} and α_{1D} receptors, respectively. However, the two enantiomers of dicentrine were observed to have very similar activities at all 5-HT$_2$ and α_1 receptor subtypes.

3.3 Molecular modelling

An attempt was made to rationalise the difference in behaviour of (*R*)-glaucine at the 5-HT$_2$ receptors compared to the other test compounds by docking the compounds into structures generated from a crystal structure of the 5-HT$_{2B}$ receptor in complex with ergotamine (PDB code: 4IB4).[12,34] For all the test compounds, the majority of the top-ranked binding poses at the 5-HT$_{2A}$ receptor were at the orthosteric site as expected (results not shown). However, some of the top-ranked binding poses were at a possible allosteric site located adjacent to the extracellular ends of transmembrane (TM) helices IV and V. In particular, (*R*)-glaucine exhibited a binding pose which enabled the A ring to engage in π-π stacking interactions with Phe213, the protonated nitrogen atom to form a salt bridge with Asp232 and the D ring to engage in hydrophobic interactions with Leu215 and other residues in extracellular loop 2 (ECL2) (Figure 4). ECL2 links the ends of TM helices IV and V and has been found to be involved in the binding of allosteric ligands to other class A GPCRs.[35] However, there was no clear indication from the current study why only (*R*)-glaucine should preferentially bind to the proposed allosteric site of the 5-HT$_{2A}$ receptor rather than to the orthosteric site.

Interestingly, Phe213 and Asp232 are not present in either 5-HT$_{2B}$ or 5-HT$_{2C}$ receptors – the equivalent residues are Lys193 and Gly215 in the 5-HT$_{2B}$ receptor and Ile192 and Pro212 in the 5-HT$_{2C}$ receptor – and neither (*R*)-glaucine nor any of the other compounds were observed to bind to the corresponding regions of these receptor subtypes. This might account for (*R*)-glaucine producing its most noticeable positive allosteric effect at the 5-HT$_{2A}$ receptor.

4 CONCLUSIONS

The results described in this study confirm our previous observations that the presence of a methylenedioxy group at positions C1 and C2 in the A ring of aporphines increases antagonist activity at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors and that the configuration of the chiral centre at position C6a may confer stereoselectivity in their action at these two receptors.

Furthermore, it appears that the presence of alkoxy substituents at position C10 in the D ring of aporphines decreases antagonist activity at 5-HT_2 receptors and may even cause compounds to act as partial agonists or allosteric modulators. Overall, the results underline the potential of aporphines as pharmacological probes or agents for the treatment of psychotic disorders, drug addiction or overdose.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

LIST OF FIGURE LEGENDS

FIGURE 1 The structures of aporphines previously tested as individual enantiomers at 5-HT_2 and α_1 receptors and the aporphines used in this study

SCHEME 1 Aporphine synthesis. Reagents and conditions: a) $(COCl)_2$, CH_2Cl_2; b) **6**, Et_3N, CH_2Cl_2; c) $POCl_3$, CH_2Cl_2, reflux; d) (*S*,*S*)-**8** (3 mol-%), HCO_2H/NEt_3, DMF, rt; e) MeOCOCl, DIPEA, CH_2Cl_2, rt; f) $Pd(OAc)_2$ (5 mol-%), DavePhos **11** (10 mol-%), KOAc (2 eq.), DMA, 130 °C; g) $LiAlH_4$, Et_2O, 0 °C

FIGURE 2 A. Dose-response curves for (*S*)-glaucine and serotonin at the 5-HT_{2A} receptor. B. Dose-response curves from antagonism testing of (*R*)-glaucine and ketanserin at the 5-HT_{2A} receptor in the presence of 1 µM serotonin. Data points are normalised and shown as the mean ± SD from triplicate readings.

FIGURE 3 Dose-response curves for serotonin at the 5-HT_{2A} receptor in the presence of different concentrations of (*R*)-glaucine. Data points are normalised and shown as the mean ± SD from triplicate readings.

FIGURE 4 The docking poses of serotonin (green space-filling representation) at the orthosteric site of the 5-HT_{2A} receptor and (*R*)-glaucine (grey stick representation) at the proposed allosteric site. A. Global view showing the locations of the orthosteric and allosteric sites. B. Close-up view showing (*R*)-glaucine interacting with residues (yellow stick representation) in the allosteric site.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

TABLE 1 Antagonist potencies and selectivities of aporphine enantiomers at 5-HT_2 receptor subtypes

Compound	pK_b[a]			K_b (nM)			Subtype selectivity[b]		
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{2A}/ 5-HT_{2B}	5-HT_{2A}/ 5-HT_{2C}	5-HT_{2C}/ 5-HT_{2B}
(*R*)-isolaureline	7.48 ± 0.03	7.50 ± 0.09	8.14 ± 0.02	33.3	31.7	7.2	1	0.2	4
(*S*)-isolaureline	7.64 ± 0.01	7.44 ± 0.10	7.53 ± 0.16	22.7	36.0	29.8	2	1	1
(*R*)-dicentrine	7.12 ± 0.07	6.68 ± 0.03	6.11 ± 0.06	75.8	208	776	3	10	0.3
(*S*)-dicentrine	7.08 ± 0.11	6.35 ± 0.12	6.27 ± 0.23	83.9	444	536	5	6	0.8
(*R*)-glaucine	n.d.[c]	n.d.[c]	n.d.[c]	-	-	-	-	-	-
(*S*)-glaucine	n.d.[c]	n.d.[c]	n.d.[c]	-	-	-	-	-	-
(*R*)-roemerine[d]	7.97 ± 0.18	7.08 ± 0.43	7.82 ± 0.08	10.8	84.0	15.1	8	1	6
(*S*)-roemerine[d]	7.37 ± 0.10	7.03 ± 0.33	7.12 ± 0.13	42.9	92.7	76.9	2	2	1
(*R*)-nuciferine[d]	7.18 ± 0.03	7.51 ± 0.21	7.44 ± 0.01	65.3	31.0	36.4	0.5	0.6	1
(*S*)-nuciferine[d]	7.07 ± 0.17	7.38 ± 0.01	7.51 ± 0.22	84.3	41.7	41.7	0.5	0.4	1
Ketanserin[d,e]	8.69 ± 0.03	5.70 ± 0.04	6.95 ± 0.08	2.0	1980	112	1000	60	20

[a] Values are expressed as mean ± SEM from at least 2 separate experiments. [b] Selectivities for receptor X/receptor Y were calculated as the ratio of K_b receptor Y/K_b receptor X. [c] Not determined - (*R*)-glaucine appeared to act as a positive allosteric modulator; (*S*)-glaucine is a partial agonist. [d] Data from ref.12 [e] Positive control.

TABLE 2 Antagonist potencies and selectivities of aporphine enantiomers at α_1 receptor subtypes

	pK_b[a]			K_b (nM)			Subtype selectivity[b]		
Compound	α_{1A}	α_{1B}	α_{1D}	α_{1A}	α_{1B}	α_{1D}	α_{1A}/α_{1B}	α_{1A}/α_{1D}	α_{1B}/α_{1D}
(*R*)-isolaureline	6.73 ± 0.16	6.34 ± 0.01	6.71 ± 0.01	185	453	193	2	1	2
(*S*)-isolaureline	6.88 ± 0.04	7.02 ± 0.04	7.20 ± 0.06	132	96.4	62.5	0.7	0.5	2
(*R*)-dicentrine	6.96 ± 0.03	6.69 ± 0.01	6.66 ± 0.07	109	202	218	2	2	1
(*S*)-dicentrine	6.79 ± 0.02	6.81 ± 0.03	6.74 ± 0.06	163	155	181	1	1	1
(*R*)-glaucine	7.00 ± 0.05	6.00 ± 0.14	6.42 ± 0.11	100	1010	383	10	4	3
(*S*)-glaucine	6.64 ± 0.03	6.34 ± 0.02	6.38 ± 0.01	230	454	418	2	2	1
(*R*)-roemerine[c]	6.47 ± 0.08	6.57 ± 0.05	7.34 ± 0.15	338	266	46.0	0.8	0.1	0.2
(*S*)-roemerine[c]	7.07 ± 0.01	6.81 ± 0.03	7.04 ± 0.07	86.0	154	92.2	2	1	0.6
(*R*)-nuciferine[c]	7.42 ± 0.07	7.22 ± 0.01	6.78 ± 0.01	37.8	60.6	165	2	4	3
(*S*)-nuciferine[c]	7.17 ± 0.08	6.50 ± 0.01	6.94 ± 0.01	67.1	316	116	5	2	0.4
Prazosin[c,d]	9.69 ± 0.07	11.41 ± 0.09	10.60 ± 0.02	0.20	0.004	0.025	0.02	0.1	6

[a] Values are expressed as mean ± SEM from at least 2 separate experiments. [b] Selectivities for receptor X/receptor Y were calculated as the ratio of K_b receptor Y/K_b receptor X. [c] Data from ref.12 [d] Positive control.

TABLE 3 Agonist potencies and relative efficacies of (*S*)-glaucine at 5-HT_2 receptor subtypes

	5-HT_{2A}		5-HT_{2B}		5-HT_{2C}	
Compound	pEC_{50} [a]	E_{max} [a]	pEC_{50} [a]	E_{max} [a]	pEC_{50} [a]	E_{max} [a]
(S)-glaucine	6.18 ± 0.01	42 + 1	n.d. [b]	n.d. [b]	6.35 ± 0.11	52 ± 1
Serotonin [c]	7.51 ± 0.09	100 ± 1	8.16 ± 0.01	100 ± 1	8.35 ± 0.04	100 ± 1

[a] Values expressed as mean ± SEM from at least 2 separate experiments. [b] Not determined - % of AP-TGFα release < 3.[14]

[c] Positive control.