Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2119–2128

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Pharmacological Activity of Metabolites of the 5-HT$_4$ Receptor Antagonist SB-207266

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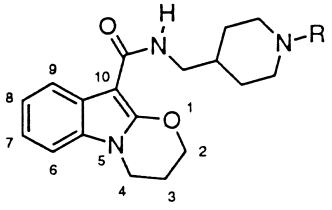
Received 18 December 2000; accepted 9 April 2001

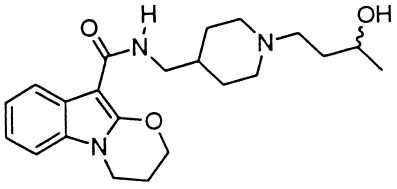
Abstract—Three metabolites of *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2*H*-[1,3]-oxazino[3,2_*a*]indole-10-carboxamide (SB-207266) (**1**) were synthesised and their pharmacological activity determined.

Introduction

SB-207266, *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-2*H*-[1,3]-oxazino[3,2-*a*]indole-10-carboxamide (**1**) is a potent and selective 5-HT$_4$ receptor antagonist with possible therapeutic indications proposed in a number of areas such as control of atrial arrhythmia or gastrointestinal disorders.[1,2a] This compound is metabolised by de-alkylation (loss of butyl side-chain), gamma hydroxylation on the butyl-side chain and hydroxylation at positions C-7 and C-4 to give **2**, **3**, **4** and **5** respectively. The synthesis and pharmacological activity of the 'des-butyl' metabolite **2** has been described previously.[2a] We now wish to report the synthesis of **3**, **4** and **5** together with their pharmacological activity as selective 5-HT$_4$ receptor antagonists.[2b,c]



1 R = $(CH_2)_3CH_3$ **SB-207266**
2 R = H



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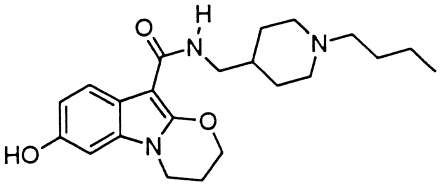

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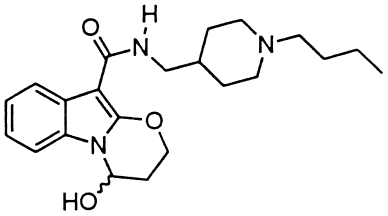

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0968-0896/01/$ - see front matter
PII: S0968-0896(01)00120-1

Results and Discussion

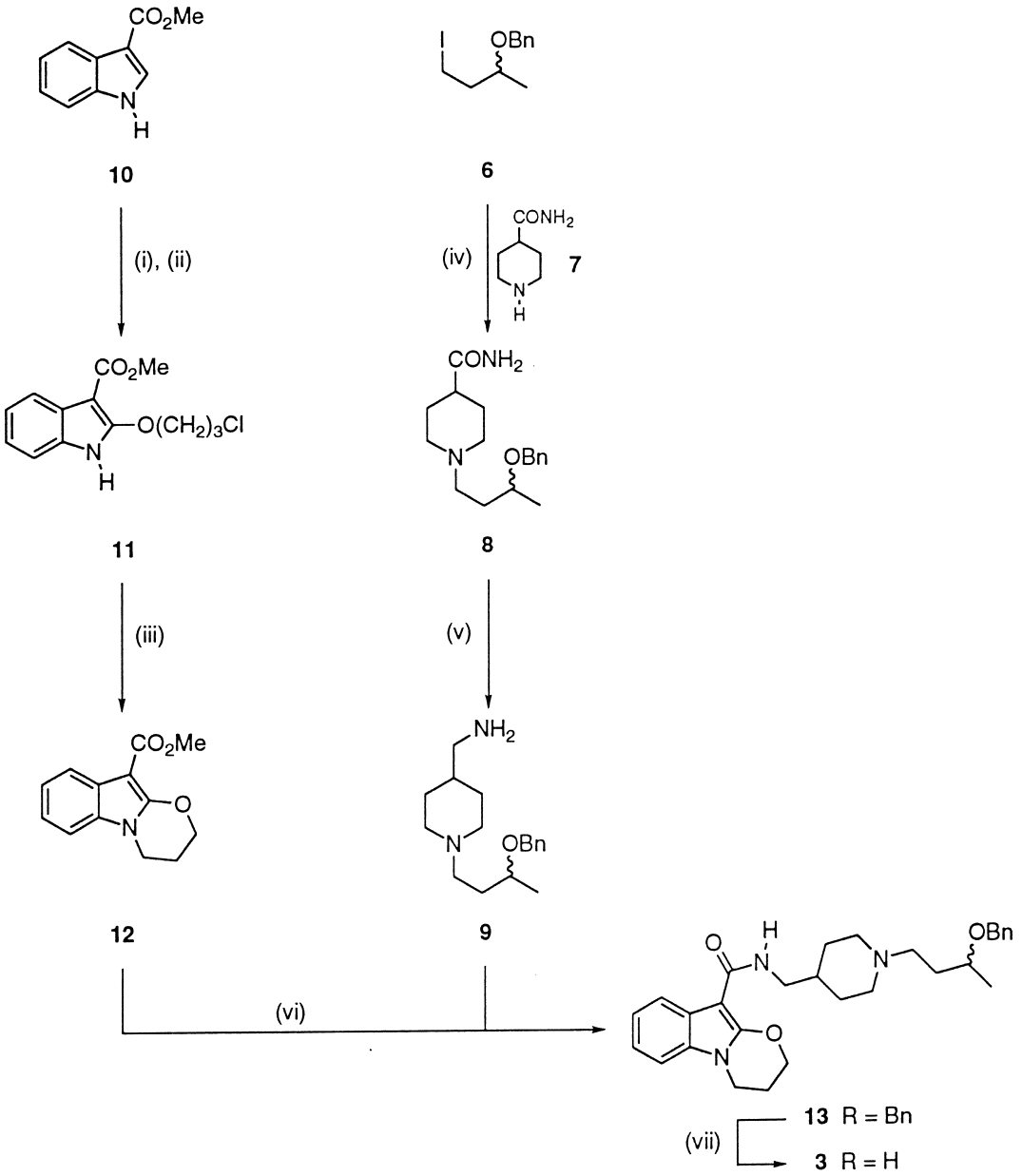
Synthesis

Synthesis of (±)-3,4-dihydro-*N*-[1-(3-hydroxybutyl)-4-piperidinyl]methyl] - 2*H* - [1,3]oxazino[3,2_*a*]indole - 10 - carboxamide (3)

The synthesis of the metabolite **3** is shown in Scheme 1.

The synthesis of the 3-benzyloxyiodobutane **6** has been described elsewhere.[3] Treatment of **6** with *iso*-nipecotamide **7** in toluene in the presence of potassium carbonate gave the amide **8** in 64% yield. This was reduced with lithium aluminium hydride in THF to furnish the key intermediate **9** in a yield of 96%. The second key intermediate, the oxazinoindole ester **12**, was prepared by treatment of methyl indole-3-carboxylate **10** with NCS in dichloromethane in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) followed by chloropropanol to give the intermediate **11** (64% yield) which was cyclised by treatment with aqueous sodium hydroxide solution in toluene giving **12** in 96% isolated yield.

The coupling of **9** and **12** was achieved by treatment of a mixture of the two intermediates in toluene with trimethylaluminium at reflux. The benzyl protected metabolite **13** was isolated in 60% yield after purification by column chromatography. Final de-protection was achieved by hydrogenation of the oxalate salt of **13** in



Scheme 1. Reagents and conditions: (i) NCS, DABCO, CH_2Cl_2, 0 °C, 10 min; (ii) $HO(CH_2)_3Cl$, CH_2Cl_2, $MeSO_3H$, 0 °C, 68%; (iii) NaOH(aq), toluene, 99%; (iv) K_2CO_3, toluene, 64%; (v) $LiAlH_4$, THF, 5 °C, 1 h, 96%; (vi) $AlMe_3$, toluene, reflux, 5 h, 60%; (vii) EtOH, AcOH, $(CO_2H)_2$, H_2, 10% Pd/C, 50 psi, 45 °C, 30 h, 40%.

ethanol/acetic acid at 45 °C and 50 psi for 30 h giving **3** in a yield of 40% after crystallisation from *iso*-propanol/diethyl ether.

Synthesis of *N*-[1-butyl-4-piperidinyl)methyl]-3,4-dihydro-7-hydroxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (4)

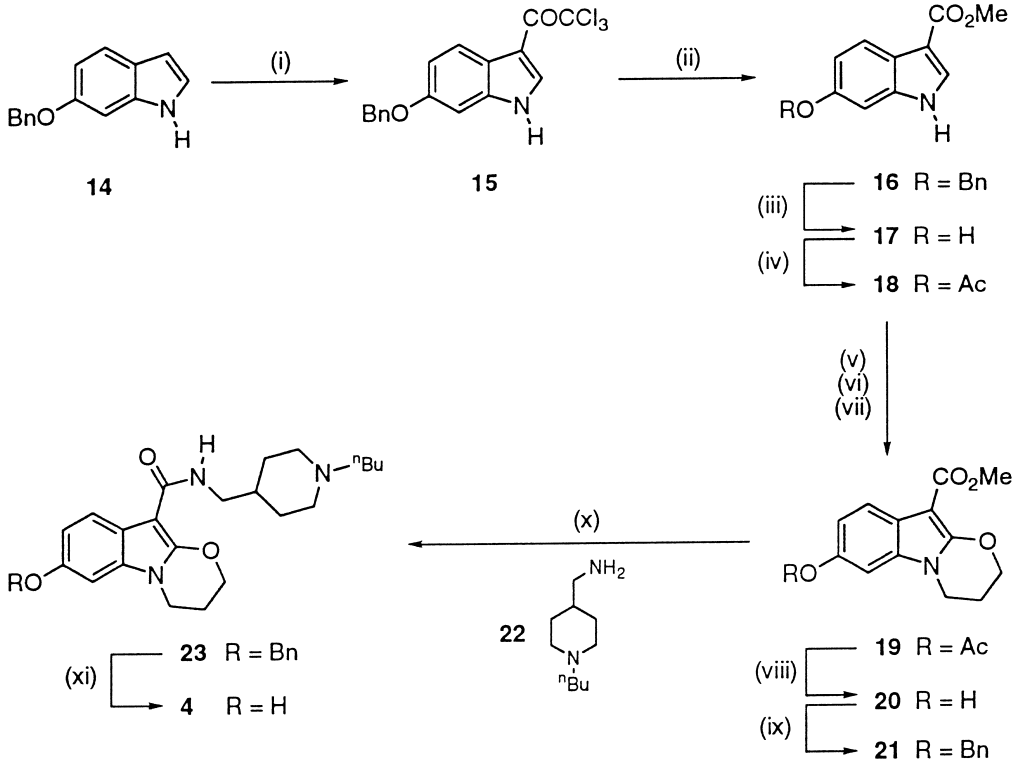
The synthesis of the 7-hydroxylated metabolite **4** is shown in Scheme 2.

The synthesis of 6-benzyloxyindole **14**, following Leimgruber chemistry has been described elsewhere.[4] Treatment of **14** with trichloroacetyl chloride in THF in the presence of pyridine gave the 3-trichloroacetylindole **15**, which was converted to the corresponding benzyloxy protected methyl ester **16** in 81% overall yield by treatment in methanol with a catalytic amount of potassium hydroxide.[5] Attempted formation of the oxazino ring from **16** failed, presumably due to the electron rich nature of the indole nucleus. However, exchanging the protecting group to acetoxy by hydrogenolysis in methanol/THF giving **17** in 81% followed by acetylation with acetic anhydride in THF/pyridine giving **18** in 99%, allowed successful construction of the oxazino ring. Thus, treatment of **18** with NCS in dichloromethane in the presence of DABCO followed by addition to a solution of 3-chloropropanol and methanesulphonic acid in dichloromethane gave **19**, contaminated with 3-chloropropanol, in a yield of 92%. As the acetoxy protecting group was thought unsuitable for the rest of the synthesis, benzyloxy protection was restored by hydrolysis to give **20** in 58%, followed by treatment with benzylchloride in DMF/K_2CO_3 to give **21** in 70%. Coupling with the amine **22** was achieved as described in part (a) above to give **23** in 54% yield after crystallisation from ethyl acetate. Final deprotection was achieved by hydrogenolysis over 10% Pd/C in methanol, giving the final product **4** in 84% after recrystallisation from ethyl acetate.

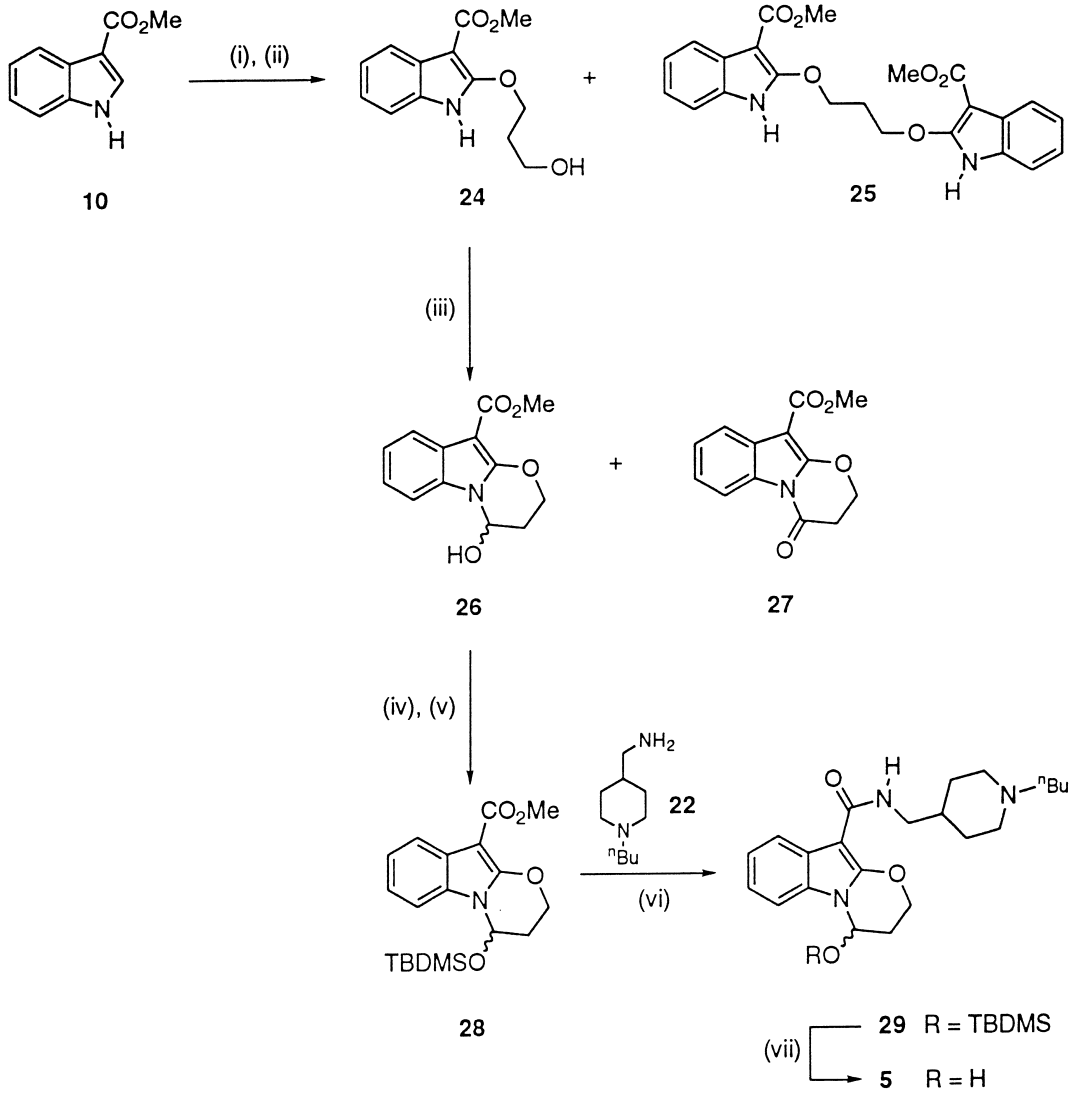
Synthesis of (±)-*N*-[1-butyl-4-piperidinyl)methyl]-3,4-dihydro-4-hydroxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (5)

The synthesis of the 4-hydroxylated metabolite **5** is shown in Scheme 3.

Thus, treatment of methyl indole-3-carboxylate **10** with NCS in dichloromethane in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) followed by propane-1,3-diol gave the required 2-alkoxylated indole derivative **24** in 45% yield together with 12% of the dimeric indole **25**. TPAP[6] oxidation, which is usually selective for primary alcohols, gave the aminal **26** in 28% yield, but also resulted in some over-oxidation to give 11% of the amidoester **27**. The secondary hydroxyl function in **26** was protected as the TBDMS ether by treatment with TBDMS triflate and 2,6-lutidine in 69% yield, and the resulting ester **28** was coupled with the amine **22** to give



Scheme 2. Reagents and conditions: (i) $ClCOCCl_3$, pyridine, THF, 2 °C, 1 h, 20 °C, 16 h, 100%; (ii) KOH(aq), MeOH, reflux, 5 h, 20 °C, 16 h, 81%; (iii) MeOH, H_2, 10% Pd/C, 5 days, 81%; (iv) THF, pyridine, Ac_2O, 4 °C, 16 h, 99%; (v) NCS, DABCO, CH_2Cl_2, 0 °C, 10 min; (vi) $HO(CH_2)_3Cl$, CH_2Cl_2, $MeSO_3H$, 0 °C; (vii) Acetone, K_2CO_3, 20 °C, 16 h, 92%; (viii) MeOH, H_2O, $NaHCO_3$, 40 °C, 2 h, 58%; (ix) BnCl, K_2CO_3, DMF, 100 °C, 6 h, 70%; (x) $AlMe_3$, toluene, reflux, 5 h, 54%; (xi) MeOH, H_2, 10% Pd/C, 28 h, 84%.



Scheme 3. Reagents and conditions: (i) NCS, DABCO, $CHCl_3$, 5 °C, 30 min; (ii) $HO(CH_2)_3OH$, $MeSO_3H$, 16 °C, 30 min, 45% (24), 12% (25); (iii) CH_2Cl_2/MeCN, *N*-methylmorpholine-*N*-oxide, TPAP, 4 Å mol sieves, 20 °C, 16 h, 28% (26), 11% (27); (iv) CH_2Cl_2, lutidine, TBDMS triflate, −70 °C, warm to 20 °C; (v) MeOH, −70 °C, 69%; (vi) $AlMe_3$, toluene, reflux, 4 h, 89%; (vii) AcOH, H_2O, 75 °C, 6.5 h, 83%.

the TBDMS protected metabolite **29** in 89% yield. Having tried and failed to de-protect **29** using tetrabutylammomium fluoride in THF, final de-protection was achieved by treatment of **29** with aqueous acetic acid giving **5** in a yield of 94%.

Pharmacological Activity

Radioligand binding affinity and selectivity for the 5-HT_4 receptor

The affinity of each metabolite for recombinant human 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_7 receptors expressed in human embryo kidney (HEK) 293 cells; 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_7 and adrenergic alpha$_{1B}$ receptors expressed in Chinese hamster ovary (CHO) cells; and 5-HT_6 receptors expressed in HeLa cells, together with the 5-HT_4 receptor from guinea pig was determined using radioligand binding assays, as described by Kennett et al.[10] All incubations were carried out at 37 °C and K_i were derived from the IC_{50} as described by Cheng and Prusoff.[11] pK_i was defined as $-\log_{10}[K_i]$. The results are shown in Table 1.

The rank-order affinities of SB-207266-A and its metabolites for the 5-HT_4 receptor were: **5** = **3** = SB-207266-A > **2** > **4**. SB-207266-A and metabolites **5**, **4** and **3** displayed low affinity (pK_i < 6.0) for a range of human recombinant 5-HT receptors. For metabolite **2** the pK_i was < 6.0 for all of the tested receptors except 5-HT_{2A} [6.02], 5-HT_{2B} [6.27] and 5-HT_{2C} [6.11]).

5-HT_4 receptor antagonism

To evaluate their functional activity as 5-HT_4 receptor antagonists, we used the guinea-pig isolated distal colon longitudinal muscle-myenteric plexus (LMMP) preparation,[7] in which SB-207266-A has previously been shown to be a highly potent 5-HT_4 receptor antagonist.[1] In this model, 5-HT_4 receptor antagonism is assessed by measuring the ability of a compound to inhibit 5-HT-

Table 1. pK_i values obtained from inhibition of radioligand binding to human recombinant and guinea pig brain receptors. mean ±SEM (*n*)

Receptor	SB-207266-A	**2**	**3**	**4**	**5**
5-HT_{1A}	<5 (3)	<5 (3)	<5 (3)	<5 (3)	<5 (3)
5-HT_{1B}	<6 (3)	<5.2 (3)	<6 (3)	<6 (3)	<5.5 (3)
5-HT_{1D}	<6 (3)	<5.2 (3)	<5.5 (3)	<5.5 (3)	<5.5 (3)
5-HT_{1E}	<5 (3)	<5 (3)	<5 (6)	<5 (3)	<5 (3)
5-HT_{1F}	<5 (3)	<5 (3)	<5 (6)	<5 (3)	<5 (3)
5-HT_{2A}	<6 (6)	6.05±0.05 (4)	<6 (3)	5.5 (3)	<5 (3)
5-HT_{2B}	6.34±0.10 (5)	6.27±0.05 (3)	<6 (3)	<5 (3)	<5 (3)
5-HT_{2C}	<6 (6)	6.10±0.03 (6)	<6 (3)	5.5 (3)	<5 (3)
5-HT_4	8.75±0.05 (11)	7.17±0.05 (3)	9.08±0.09 (3)	6.05±0.05 (3)	9.09±0.03 (6)
5-HT_6	<5 (3)	<5 (3)	<5 (3)	<5 (3)	<5 (3)
5-HT_7	<5 (3)	<5 (3)	<5 (3)	<5 (3)	<5 (3)
Adrenergic α_{1B}	<5 (3)	<5 (3)	<5.5 (6)	<5 (3)	<5 (3)

evoked, neuronally-mediated contractions of the muscle, in the presence of methiothepin and granisetron, to block 5-HT_2/5-HT_1-like and 5-HT_3 receptors, respectively. The selectivity of such inhibition is then assessed by determining the ability of the compound to inhibit similar neuronally-mediated contractions evoked by the nicotinic receptor agonist 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP).

Male Dunkin Hartley guinea-pigs 200–300 g (Charles River U.K. Ltd) were used. The colon was removed approximately 10 cm from the anus and 2–3 cm long sections of LMMP were dissected as described previously[7]. The preparations were suspended under 1 g tension in tissue baths and were bathed in Krebs–Henseleit solution (mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, glucose 11.1, $NaHCO_3$ 25, $CaCl_2.6H_2O$ 2.5) containing granisetron (1×10^{-6} M) and methiothepin (1×10^{-7} M), bubbled with 5% CO_2 in oxygen and maintained at 37 °C. Muscle contractions were measured using isotonic transducers connected to Lectromed Multitrace 8 chart recorders. SB-207266-A was dissolved in double distilled water to a stock concentration of 1×10^{-3} M. Compound **2** was dissolved in DMSO. Compounds **4** and **5** were dissolved in methanol. Compound **3** was dissolved in 70% ethanol. All metabolite stock solutions were at 1×10^{-2} M. Further dilutions were carried out in double distilled water.

After preparation, tissues were left to stabilise for 30 min. Pargyline (1×10^{-4} M), a monoamine oxidase inhibitor, was added to the solution bathing the tissues and washed out 15 min later. Tissues were then sensitised to 5-HT as described,[7] or to DMPP (1×10^{-6} M, 30 s exposure, repeated at intervals of 15 min until a consistent response was obtained). Agonist concentration–effect curves were constructed non-cumulatively by adding increasing concentrations in half log unit increments at 15 min intervals. Each agonist was left in contact with the tissue until a maximum response was obtained but not for longer than 30 s. Two agonist concentration–effect curves were constructed in each tissue, the first in the absence of test compound and the second in the presence of the appropriate concentration of test compound (1×10^{-8} M for all experiments with DMPP). In all experiments, the test compound was left to equilibrate with the tissue for 45 min before construction of the second concentration–effect curve. Responses were expressed as mean±standard error (*n* animals). All agonist concentration–response curves were fitted to the following three parameter logistic equation:

$$E = \alpha/1\ ([A]/EC_{50})^n$$

using Robofit.[8] α, [A] and n represent the maximum response, agonist concentration and curve mid point slope factor, respectively. The EC_{50} is the concentration of agonist that produces 50% of the maximal response.

Where the maximum response to the agonist was not significantly reduced by the antagonist, affinity estimates for the antagonist were expressed as pK_B values calculated according to the method of Arunlakshana and Schild.[9] As previously reported,[1] higher concentrations of SB-207266-A reduced the maximum response to 5-HT and some of the metabolites had a similar effect. For this reason, the term 'apparent pK_B' is used.

In this model of the 5-HT_4 receptor, none of the compounds affected the tone of the tissue in the absence of 5-HT, indicating a lack of intrinsic activity at the 5-HT_4 receptor, at the concentrations tested. Further, when tested at the high concentration of 1×10^{-8} M, each compound had little-or-no ability to antagonise the neuronally-mediated contractions evoked by the nicotinic receptor agonist DMPP (Table 2).

However, low concentrations of SB-207266-A (1–30 nM) caused a parallel rightward displacement of the 5-HT concentration–effect curve, with some depression of the maximum response to 5-HT at the higher concentrations of SB-207266, as reported previously.[1] A Schild plot of these results yielded an apparent pA_2 of 9.0. The slope of this plot was 1.42, the difference from 1 probably reflecting the depression of maximum response to 5-HT seen at the higher concentrations of SB-207266-A. The apparent pK_B determined from the mean pEC_{50} values in the absence and presence of 1×10^{-8} M SB-207266-A was 9.6 (Table 3).

Compounds **2** and **4** each produced no significant effect on the 5-HT concentration–effect curve when tested at

Table 2. Effects of SB-207266-A and its metabolites on DMPP-evoked contractions of guinea-pig LMMP preparation[a]

Compound	pEC_{50},	
	1st concentration–effect curve	2nd concentration–effect curve
SB-207266-A	5.5±0.1	5.4±0.3 ($n=2$)
2	5.9±0.1	5.3±0.1 ($n=2$)
4	5.5±0.3	5.2±0.3 ($n=2$)
5	5.8±0.1	5.6±0.2 ($n=2$)
3	5.9±0.1	5.7±0.1 ($n=2$)

[a]pEC_{50} values for DMPP were derived from the first concentration–effect curve in the absence of test compound, and the second curve in the presence of test compound at 1×10^{-8} M.

1×10^{-8} M but caused small rightward shifts at 1×10^{-6} M [surmountable for (**2**)], corresponding to an apparent pK_B of 7.4 and 5.7, respectively (Table 3). Compound **5** at 1×10^{-8} M produced a rightward shift of the 5-HT concentration–effect curve, with an apparent pK_B of 8.5 (Table 3). Similar results were obtained using **3** 1×10^{-8} M, yielding an apparent pK_B of 9.3 (Table 3). Like SB-207266-A tested in the same experiment, **3** produced some depression of the maximum effect of 5-HT.

The present results confirmed that SB-207266-A is a potent antagonist of 5-HT_4 receptor-mediated contractions in the LMMP preparation, which, at concentrations up to 1×10^{-8} M, acted in an apparently competitive manner. Of the four human-derived metabolites of SB-207266-A tested here, one (**4**) was a very weak antagonist of 5-HT-evoked contractions in the LMMP preparation and two (**5** and **2**) were only moderately potent antagonists with apparent pK_B's 5–10-fold lower than that of the parent compound. Compound **3** was the most potent of the metabolites tested with an apparent pK_B similar to that of SB-207266-A when tested in the same experiments. Like SB-207266-A, **3** also caused some depression of the maximum response to 5-HT when present at 10^{-8} M. This lack of surmountability of the effect of SB-207266-A has been ascribed to pseudoirreversible antagonism.[1]

Conclusions

Two human-derived metabolites of SB-207266-A, **5** and **3**, were found to have high affinity for the 5-HT_4 receptor and as for SB-207266-A, acted as potent, surmountable and selective antagonists at this receptor; in the guinea-pig distal colon LMMP model of the receptor, the pK_i and apparent pK_B values were, respectively, 9.09 and 9.08, and 8.5 and 9.3. These values are of the same order as that of SB-207266-A. Two other metabolites tested, **2** and **4**, were weaker 5-HT_4 receptor antagonists (pK_i and pK_B values of 7.17 and 6.05, and 7.4 and 5.6, respectively). None of the compounds exhibited high affinity for a range of other 5-HT and non-5-HT receptors. Further, when tested in the guinea-pig colon LMMP preparation at 10^{-8} M, none of the compounds markedly inhibited the contractions induced by the nicotinic agonist DMPP, indicating that when 5-HT_4 receptor antagonism was observed in this preparation, this was unlikely to be mediated via a direct action on the cholinergic neurones.

Experimental

All experiments were performed under dry nitrogen. THF was dried by distillation from sodium/benzophenone. All other solvents were used without further purification. Separations by column chromatography were achieved using conditions described by Still.[12] Thin layer chromatograms were run on 0.25 mm Merck precoated plates of silica gel 60 F254. High performance liquid chromatograms (HPLC) were obtained from C-18 reverse phase Hichrom RPB columns. Analytical samples were dried in a Buchi drying pistol at 40 °C. NMR spectra were obtained from a Bruker amx400 MHz spectrometer with tetramethylsilane as an internal standard. Mass spectra were recorded on a Micromass 70-VSEQ double focusing spectrometer. Melting points were obtained on a Mettler FP62 system.

Preparation of 1-[3-(phenylmethoxy)butyl]-4-piperidinecarboxamide (8). A suspension of *iso*-nipecotamide **7** (18 g, 140 mmol), anhydrous potassium carbonate (58.2 g, 420 mmol) and 3-benzyloxyiodobutane **6** (44.7 g, 150 mmol) was heated under nitrogen at reflux for 7 h. The resulting mixture was subjected to hot filtration and the filtrate evaporated under reduced pressure to give a solid. This was re-dissolved in ethyl acetate, washed with water and brine, and concentrated until crystallisation occurred. The solid was collected by filtration and washed with ethyl acetate and diethyl ether to give **8**

Table 3. Effects of SB-207266-A and its metabolites on 5-HT-evoked contractions in the guinea-pig LMMP preparation[a]

Compound (concentration)	pEC_{50}		Apparent pK_B
	1st concentration–effect curve	2nd concentration–effect curve	
None	8.8±0.1	9.0±0.1	na
SB-207266-A (1×10^{-8} M)	8.7±0.1	7.2±0.1 ($n=8$)	9.6±0.1
2 (1×10^{-6} M)	8.6±0.2	7.2±0.1 ($n=6$)	7.4±0.1
4 (1×10^{-6} M)	8.2±0.2	7.9±0.1 ($n=4$)	5.7±0.4
5 (1×10^{-8} M)	8.4±0.1	7.7±0.1 ($n=7$)	8.5±0.2
SB-207266-A (1×10^{-8} M)	8.4±0.2	7.4±0.2 ($n=8$)	9.0±0.1
3 (1×10^{-8} M)	8.2±0.1	6.9±0.1 ($n=10$)	9.3±0.1

[a]The apparent pK_B for each compound was calculated from the pEC_{50} values for 5-HT derived from the first concentration–effect curve, determined in the absence of test compound, and the second curve in the presence of test compound at the concentration indicated. The effects of SB-207266-A were tested twice, each in parallel with different metabolites (**2**, **4**, **5** and again to compare with **3**), as indicated.

26.1 g (64%). ^{1}H NMR ($CDCl_3$) δ 7.4–7.2 (5H + $CHCl_3$, m), 5.5 (2H, NH_2, br s), 4.5 (2H, AB quartet), 3.6° (1H, m), 2.9 (2H, m), 2.4 (2H, m), 2.15 (1H, m), 2.0–1.6 (8H, m), 1.2 (3H, d, J = 6.6 Hz); m/z 291 (ES^+, M + 1).

Preparation of 1-[3-(phenylmethoxy)butyl]-4-piperidinemethanamine (9). To a suspension of $LiAlH_4$ (16.82 g, 440 mmol) in THF (250 mL) at 5 °C was added the amide **8** (10.34 g, 36 mmol) dissolved in THF (150 mL) drop-wise over 1 h. The mixture was allowed to warm to ambient temperature, then stirred for 18 h under nitrogen. The mixture was cooled to 5 °C, aqueous sodium hydroxide solution (7.8 M, 33 mL) added drop-wise over 30 min, and the mixture filtered through Celite. The Celite was washed with THF (3×50 mL) and the combined filtrate and washings evaporated under reduced pressure to give **9** as an oil, 9.4 g (96%). ^{1}H NMR ($CDCl_3$) δ 7.4–7.2 (5H + $CHCl_3$, m), 4.5 (2H, AB quartet), 3.6 (1H, m), 2.9 (2H, m), 2.6 (2H, d, J = 6.6 Hz), 2.4 (2H, m), 2.0–1.2 (14H, m); m/z 277 (ES^+, M + 1).

Preparation of methyl 2-(3-chloropropoxy)indole-3-carboxylate (11). A suspension of methyl indole-3-carboxylate (240.4 g 1.37 mol) and DABCO (84.5 g, 0.75 mol) in dichloromethane (1200 mL) was cooled to 0 °C, treated in one portion with NCS (201.2 g, 1.51 mol) and the mixture stirred for 10 min. The resulting solution was added to a solution of 3-chloropropan-1-ol (142.5 g, 1.51 mol) in dichloromethane (1200 mL) containing methane sulphonic acid (10.6 mL) at such a rate as to maintain the temperature at about 0 °C. The resulting suspension was stirred for 30 min and then washed with 10% aqueous sodium carbonate solution (3×1250 mL). The organic phase was dried over sodium sulphate, filtered and concentrated on a rotary evaporator. The resulting oil was triturated with toluene (400 mL) at 0 °C for 1 h and the solid filtered, washed with a small amount of toluene and dried in vacuo to give **11** as an off-white solid, 249.5 g (68%). ^{1}H NMR ($CDCl_3$) δ: 9.6 (1H, NH, br s), 8.0 (1H, d, J = 7.9 Hz), 7.24–7.11 (3H, m), 4.43 (2H, t, J = 5.9 Hz), 3.9 (3H, s), 3.6 (2H, t, J = 5.9 Hz), 2.1 (2H, m); ^{13}C NMR ($CDCl_3$) δ 166, 157, 130, 126, 122.2, 121.9, 120.6, 111, 89, 69, 51, 41, 32; m/z 268 (ES^+, M + 1).

Preparation of methyl 3,4-dihydro-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (12). A mixture of **11** (5.0 g 18.7 mmol) and aqueous sodium hydroxide solution (3.8 mL, 5.4 M, 20.5 mmol) in toluene (50 mL) was heated at 40 °C for 4 h. The aqueous phase was separated and the organic phase was washed with water (3×25 mL) while maintaining the temperature at 60 °C. The organic solution was evaporated to dryness giving **12**, 4.29 g (99%). ^{1}H NMR (DMSO-d_6) δ 7.9 (1H, d, J = 7.1 Hz), 7.3 (1H, d, J = 7.7 Hz), 7.2–7.05 (2H, m), 4.5 (2H, t, J = 5.3 Hz), 4.1 (2H, t, J = 6.2 Hz), 3.75 (3H, s), 2.2–2.3 (2H, m); ^{13}C NMR (DMSO-d_6) δ: 164, 153, 131, 125, 121.7, 120.3, 119.2, 109, 85, 66, 50, 39, 20; m/z 232 (ES^+, M + 1).

Preparation of 3,4-dihydro-*N*-[1-[3-(phenylmethoxy)butyl]-4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (13). Trimethylaluminium (2 M in toluene, 18 mL) was diluted with toluene (18 mL) and the solution cooled to 0 °C. The amine **9** (9.2 g, 33 mmol) dissolved in toluene (30 mL) was added over 3 min, followed by the ester **12** (7.6 g, 33 mmol). The mixture was heated at reflux for 5 h, then allowed to cool, and sodium hydroxide solution (10% w/w, 80 mL) added drop-wise. The toluene layer was washed with water, brine and evaporated under reduced pressure to give an oil (14.7 g). This was purified by flash chromatography on silica (eluting with 0–20% MeOH/CH_2Cl_2) obtaining **13**, 9.3 g (60%) as an oil. ^{1}H NMR ($CDCl_3$) δ 8.3 (1H, d, J = 7.9 Hz), 7.4–7.1 (8H + $CHCl_3$, m), 6.5 (1H, NH, t, J = 5.9 Hz), 4.5 (4H, t, J = 5.3 Hz), 4.1 (2H, t, J = 6.6 Hz), 3.9 (1H, m), 3.3 (2H, t, J = 6.6 Hz), 3.0 (2H, m), 2.5–2.3 (4H, m), 2.1–1.6 (7H, m), 1.5–1.2 (5H, m); R_f 0.7 (SiO_2, 5:1:1 EtOAc/MeOH/NH_4OH); m/z 476 (ES^+, M + 1).

Preparation of 3,4-dihydro-*N*-[1-(3-hydroxybutyl)-4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (3). To a solution of **13** (8.2 g, 17 mmol) in ethanol (150 mL) and glacial acetic acid (8 mL) was added oxalic acid dihydrate (2.4 g, 19 mmol), and the mixture hydrogenated over 10% Pd/C (4.8 g) at 45 °C, 50 psi for 30 h. The mixture was allowed to cool, filtered through Celite, and the filtrate evaporated under reduced pressure. The residue was partitioned between chloroform (150 mL) and concentrated aqueous potassium carbonate solution (100 mL). The chloroform layer was washed with water (3×25 mL), brine (25 mL), dried over sodium sulphate, filtered, and the filtrate evaporated under reduced pressure to give an oil (4.7 g). This was crystallised from *iso*-propanol/diethyl ether obtaining (**3**), 3 g (40%). ^{1}H NMR ($CDCl_3$) δ 8.3 (1H, d, J = 6.6 Hz), 7.26–7.08 (3H, m), 6.5 (1H, NH, t, J = 5.9 Hz), 4.5 (2H, t, J = 5.3 Hz), 4.1 (2H, t, J = 5.9 Hz), 3.9 (1H, m), 3.3 (2H, m), 3.2 (1H, m), 2.9 (1H, m), 2.6–2.5 (2H, m), 2.4 (2H, m), 2.1 (1H, m), 1.8–1.3 (10H, m), 1.26 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 165, 149, 131, 126, 122, 121, 120.7, 108, 89, 70, 67, 58, 55, 52, 44, 39, 36, 33, 30.4, 29.9, 23, 21; m/z 385.2353 (calcd 385.2365); R_f 0.5 (SiO_2, 5:1:1 EtOAc/MeOH/NH_4OH); HPLC Retention time 15.8 min, purity 97.2% (PAR), column: Hichrome RPB 150×4.6 mm id, mobile phase: (A) 0.15M NH_4OAc adjusted to pH 4.0 with TFA, (B) methanol gradient: 25 to 60% B over 40 min, then 5 min at 60% B, detection: UV at 265 nm.

Preparation of 6-benzyloxy-3-trichloroacetylindole (15). To a solution of 6-benzyloxyindole **14** (32.6 g, 140 mmol) in THF (320 mL) was added pyridine (14.8 mL, 180 mmol) and the solution cooled to 2 °C. Trichloroacetyl chloride (20.1 mL, 180 mmol) dissolved in THF (320 mL) was added drop-wise over 1 h. The mixture was allowed to warm to ambient temperature over 16 h, then evaporated under reduced pressure and the residue partitioned between ethyl acetate (1400 mL) and hydrochloric acid (1M, 260 mL). The ethyl acetate layer was washed with further hydrochloric acid (1M, 2×260 mL), dried over sodium sulphate, filtered, and the filtrate evaporated under reduced pressure to give **15** as a solid. This was used in the next stage without

purification. ^{1}H NMR ($CDCl_3$) δ 8.65 (1H, br s), 8.22 (1H, d, J = 9.2 Hz), 8.18 (1H, d, J = 3.3 Hz), 7.4–7.1 (5H + $CHCl_3$, m), 7.04 (1H, dd, J = 11.8 Hz, 1.8 Hz), 6.9 (1H, d, J = 2.6 Hz), 5.05 (2H, s); m/z 370 (M + 1).

Preparation of methyl 6-benzyloxyindole-3-carboxylate (16). To a solution of **15** (obtained above) in methanol (2800 mL) was added aqueous potassium hydroxide (50% w/w, 10 mL). The solution was heated at reflux for 5 h, then stirred at ambient temperature for 16 h. The reaction mixture was concentrated until crystallisation occurred. The solid was collected by filtration, and washed with methanol to give **16**, 31.7 g (81%). A sample recrystallised from methanol melted at 169–170 °C (dec.). ^{1}H NMR ($CDCl_3$) δ 8.4 (1H, br s), 8.0 (1H, d, J = 9 Hz), 7.7 (1H, d, J = 3 Hz), 7.4–7.3 (5H, m), 5.0 (2H, s), 3.8 (3H, s); m/z 282 (M + 1); R_f 0.42 (SiO_2, $CHCl_3$).

Preparation of methyl 6-hydroxyindole-3-carboxylate (17). A solution of **16** (31.7 g, 110 mmol) in THF (250 mL) and methanol (200 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (15.9 g) for 5 days. The reaction mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure to an oil which was crystallised from chloroform, obtaining **17**, 17.5 g (81%). ^{1}H NMR (CD_3OD) δ 7.75 (0.5H, s), 7.70 (1.5H, m), 6.7 (1H, d, J = 2 Hz), 6.6 (1H, dd, J = 2.2 Hz, 8.6 Hz), 3.8 (3H, s); m/z 192 (M + 1).

Preparation of methyl 6-acetoxyindole-3-carboxylate (18). To a solution of **17** (15.1 g, 79 mmol) in THF (200 mL) at 3 °C was added pyridine (5.5 mL, 68 mmol) followed by acetic anhydride (22 mL, 230 mmol). The solution was stood for 16 h at 4 °C, then evaporated under reduced pressure and the residue partitioned between ethyl acetate (200 mL) and cold dilute hydrochloric acid (1 M, 100 mL). The organic layer was washed with further cold dilute hydrochloric acid (1 M, 100 mL), water (3×35 mL), brine (2×50 mL), dried over sodium sulphate, filtered and the filtrate evaporated under reduced pressure to give **18**, 8.4 g (99%) as a solid. ^{1}H NMR ($CDCl_3$) δ 8.82 (1H, NH, br s), 8.1 (1H, d, J = 8.7 Hz), 7.7 (1H, d, J = 3.0 Hz), 7.1 (1H, d, J = 2.0 Hz), 6.95 (1H, dd, J = 2.0 Hz, 8.7 Hz), 3.9 (3H, s), 2.35 (3H, s); ^{13}C NMR ($CDCl_3$) δ 170, 165.3, 147, 136, 132, 124, 122, 116, 109, 105, 51, 21; m/z 234 (M + 1).

Preparation of methyl 3,4-dihydro-7-acetoxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (19). To a solution of **18** (18.4 g, 80 mmol) in chloroform (250 mL) at 2 °C was added DABCO (4.9 g, 43 mmol) followed by *N*-chlorosuccinimide (11.6 g, 87 mmol). The mixture was stirred for 30 min, then 3-chloropropanol (14.9 g, 160 mmol) was added, followed by methanesulphonic acid (1.1 g, 11 mmol). The mixture was stirred for 75 min, then sodium carbonate solution (10% aqueous, 200 mL) was added. The phases were separated and organic layer was washed with water (2×100 mL), brine (100 mL), dried over sodium sulphate, filtered, and the filtrate evaporated under reduced pressure. The residue was re-dissolved in acetone (570 mL), anhydrous potassium carbonate (69 g, 0.5 mol) added and the mixture stirred for 16 h. The reaction mixture was filtered, and the filtrate evaporated under reduced pressure to give **19**, 20.9 g (92%) as a solid, contaminated with excess 3-chloropropanol (1 equiv). This material was used in the next stage without any further purification. ^{1}H NMR ($CDCl_3$) δ 8.0 (1H, d, J = 9 Hz), 7.0 (2H, m), 4.6 (2H, t, J = 5.2 Hz), 4.1 (2H, t, J = 6.2 Hz), 3.9 (3H, s), 2.3 (5H, m), plus resonances for 3-chloropropanol at δ 3.8 (2H, t, J = 5.9 Hz), 3.7 (2H, t, J = 6.4 Hz), 2.0 (2H, t, J = 6.2 Hz); m/z 290 (AP^+, M + 1).

Preparation of methyl 3,4-dihydro-7-hydroxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (20). To a suspension of crude **19** (20.9 g) obtained above, in methanol (1000 mL) and water (500 mL) was added saturated aqueous sodium bicarbonate solution (500 mL). The mixture was stirred at 40 °C for 2 h, concentrated to about 300 mL by evaporation under reduced pressure, diluted with ethyl acetate (400 mL) and cooled to 2 °C. The solution was neutralised with dilute hydrochloric acid and the organic layer collected. The aqueous layer was extracted with further ethyl acetate (2×400 mL) and the combined organic layers washed with water (3×250 mL), brine (250 mL), dried over sodium sulphate. The mixture was filtered and evaporated under reduced pressure to give a solid which was recrystallised from *iso*-propanol, obtaining **20**, 11.3 g (58%). ^{1}H NMR (DMSO-d_6) δ 9.07 (1H, OH, s), 7.6 (1H, d, J = 8.3 Hz), 6.6 (2H, m), 4.45 (2H, t, J = 5.3 Hz), 3.91 (2H, t, J = 6.6 Hz), 3.7 (3H, s), 2.2 (2H, m); ^{13}C NMR ($CDCl_3$) δ: 169, 157.9, 157.6, 137, 125, 122, 116, 101, 89, 71, 55, 44, 26; m/z 248 (M + 1).

Preparation of methyl 3,4-dihydro-7-benzyloxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (21). A mixture of **20** (11.2 g, 45.3 mmol), anhydrous potassium carbonate (6.3 g, 46 mmol), benzyl chloride (6 mL, 52 mmol) and DMF (125 mL) was heated at 100 °C with stirring under nitrogen for 6 h. The mixture was allowed to cool, evaporated to dryness under reduced pressure and the residue partitioned between ethyl acetate (600 mL) and water (150 mL). The organic layer was washed with further water (2×100 mL) and brine (100 mL), then dried over sodium sulphate. The mixture was filtered and the filtrate evaporated under reduced pressure to give a solid (16.5 g) which was recrystallised from methanol (250 mL) obtaining **21**, 11.9 g (70%) which melted at 101–102 °C. ^{1}H NMR ($CDCl_3$) δ 7.9 (1H, d, J = 8.6 Hz), 7.5–7.3 (5H, m), 6.93 (1H, dd, J = 2.3 Hz, 8.6 Hz), 6.7 (1H d, J = 2.2 Hz), 5.1 (2H, s), 4.5 (2H, t, J = 5.2 Hz), 4.0 (2H, t, J = 6.3 Hz), 3.9 (3H, s), 2.3 (2H, m); ^{13}C NMR ($CDCl_3$) δ: 165, 155, 153, 137, 132, 128.5, 128.3, 128, 127.5, 121, 119, 111, 95, 86, 71, 66, 51, 39, 30, 21; m/z 338 (M + 1).

Preparation of *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-7-benzyloxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (23). Trimethylaluminium (19.3 mL, 2 M in toluene) was diluted with toluene (18 mL) and the solution cooled to 2 °C under nitrogen. Amine **22** (6.03 g, 35.4 mmol) dissolved in toluene (55 mL) was added drop wise, allowing the temperature to rise to 10 °C. Compound **21** (11.9 g, 35.3 mmol) was added as a solid and

the mixture heated at reflux for 5 h. The solution was allowed to cool to 70 °C, then sodium hydroxide solution (10% w/w, 85 mL) was added cautiously over 10 min. The phases were separated and the toluene layer was washed with further sodium hydroxide solution (10% w/w, 85 mL), water (2×120 mL) and brine (120 mL). The solution was dried over sodium sulphate, filtered, and the filtrate evaporated under reduced pressure to give a solid (16.9 g) which was recrystallised from ethyl acetate, obtaining **23**, 9.1 g (54%). ^{1}H NMR ($CDCl_3$) δ 8.2 (1H, d, J=8.6 Hz), 7.5–7.3 (5H, m), 6.95 (1H, dd, J=2.2 Hz, 8.7 Hz), 6.7 (1H, d, J=2.2 Hz), 6.51 (1H, NH, t, J=5.9 Hz), 5.1 (2H, s), 4.5 (2H, t, J=5.1 Hz), 4.0 (2H, t, J=6.2 Hz), 3.3 (2H, t, J=6.1 Hz), 2.9 (2H, m), 2.3 (4H, m), 2.0–1.2 (11H, m), 0.9 (3H, t, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 165, 155, 149, 143, 137, 132, 128, 127.8, 127.5, 122, 120, 111, 95, 94.5, 89, 71, 67, 59, 57, 54, 44, 39, 36, 30, 29, 21, 20.9, 14, 12; m/z 476 (M + 1); R_f 0.6 (SiO_2, 5:1:1 EtOAc/MeOH/NH_4OH).

Preparation of *N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-7-hydroxy-2*H*-[1,3]oxazino[3,2-*a*] indole-10-carboxamide (4). A solution of **23** (9.1 g, 19 mmol) in methanol (190 mL) was hydrogenated over 10% Pd/C (0.99 g) at atmospheric pressure for 28 h. The reaction mixture was filtered through Celite and the filtrate evaporated under reduced pressure to give a solid which was recrystallised from ethyl acetate obtaining **4**, 6.2 g (84%) which melted at 125–126 °C. ^{1}H NMR (CD_3OD) δ 7.85 (1H, d, 8.4 Hz), 6.66 (1H, dd, J=2.3 Hz, 8.4 Hz), 6.58 (1H, d, J=2.2 Hz), 4.48 (2H, t, J=5.0 Hz), 3.92 (2H, t, J=6.2 Hz), 3.25 (2H, d, J=6.7 Hz), 2.95 (2H, m), 2.29 (4H, m), 1.95 (2H, m), 1.73 (2H, m), 1.47 (1H, m), 1.34 (2H, m), 1.30 (4H, m), 0.93 (3H, t, J=7.3 Hz); ^{13}C NMR (CD_3OD) δ 168, 154, 151, 134, 122, 120, 112, 96, 89, 68, 60, 55, 45, 40, 38, 31, 30, 22.3, 22, 14; nOe experiments confirmed 7-OH substitution; m/z 385.2353 (calcd 385.2365); HPLC retention time 12.1 min, purity 99.1% (PAR), column: Hichrome RPB 150×4.6 id mm, Mobile Phase: (A) 0.15 M NH_4OAc adjusted to pH 4.0 with TFA, (B) Methanol, Gradient: 25 to 60% B over 40 min, then 5 min at 60% B, detection: UV at 265 nm.

Preparation of methyl 2-(3-hydroxypropoxy)-1*H*-indole-3-carboxylate (24). To a suspension of methyl indole-3-carboxylate **10** (25.3 g, 144 mmol) and DABCO (8.7 g, 78 mmol) in chloroform (250 mL) at 5 °C was added *N*-chlorosuccinimide (21 g, 157 mmol). After 30 min, 1,3-propanediol (110 g, 1.45 mol) was added, followed by methanesulphonic acid (3 g). The solution was allowed to warm to room temperature and stirred at room temperature for 1 h, then washed with 10% aqueous Na_2CO_3 solution, water, brine and dried over sodium sulphate. The mixture was filtered and evaporated under reduced pressure to leave an oil. Chromatography (5–50% ethyl acetate/dichloromethane) gave **24**, 16.2 g (45%), and **25**, 3.7 g (12%). ^{1}H NMR (**24**) (CD_3OD) δ 7.85 (1H, m), 7.25 (1H, m), 7.1 (2H, m), 4.45 (2H, t, J=5.9 Hz), 3.85 (5H, m), 2.1 (2H, m); m/z 250 (EI, M + 1); ^{1}H NMR (25) (DMSO-d_6) δ: 7.8 (2H, m), 7.26 (2H, m), 7.06 (4H, m), 4.55 (4H, t, J=6.0 Hz), 3.68 (6H, s), 2.3 (2H, m); m/z 421 (AP^-, M−1).

Preparation of methyl 3,4-dihydro-5-hydroxy-4-oxo-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (26). To a mixture of **24** (4.8 g, 190 mmol), powdered 4 Å molecular sieves (9.6 g), *N*-methyl morpholine-*N*-oxide (3.39 g, 29 mmol) in dichloromethane/acetonitrile (10:1, 44 mL) was added tetrapropylammonium perruthenate (TPAP) (0.34 g, 0.97 mmol). The mixture was stirred for 18 h, filtered through Celite, and the filtrate evaporated. The residue was chromatographed (SiO_2, 0–10% ethyl acetate/dichloromethane) obtaining (**26**), 1.23 g (28%) and **27**, 0.52 g (11%). ^{1}H NMR **26** ($CDCl_3$) δ 7.8 (1H, d, J=7.2 Hz), 7.3–7.0 (3H, m), 5.85 (1H, br s), 4.73–4.4 (2H, m), 3.7 (3H, s), 2.1–2.4 (2H, m); ^{13}C NMR (**26**) ($CDCl_3$) δ 165, 153, 130, 125, 123, 121, 120.6, 108, 87, 71, 62, 51, 29; m/z 248 (AP^+, M + 1); ^{1}H NMR (**27**) ($CDCl_3$) δ: 8.2 (1H, m), 7.95 (1H, m), 7.4–7.16 (2H, m), 4.66 (2H, t, J=6.7 Hz), 3.86 (3H, s), 3.0 (2H, t, J=6.7 Hz); m/z 246 (AP^+, M + 1).

Preparation of (±)-methyl 3,4-dihydro-4-[dimethyl-(1,1-dimethylethyl)silyl]oxy]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxylate (28). To a solution of **26** (2 g, 8.1 mmol) and 2,6-lutidine (4.2 mL, 36 mmol) in dichloromethane (64 mL) at −70 °C was added TBDMS triflate (4 mL, 174 mmol) drop-wise over 3 min. The solution was stirred for 1 h, then allowed to warm to room temperature. The solution was re-cooled to −70 °C, then methanol (9 mL) was added drop-wise over 5 min. The solution was diluted with dichloromethane (200 mL), washed with water, brine and dried (Na_2SO_4) to give an oil. This was chromatographed (SiO_2, 10% ethyl acetate/dichloromethane to give **28**, 2 g (69%). ^{1}H NMR ($CDCl_3$) δ: 8.1 (1H, m), 7.31–7.2 (3H + $CHCl_3$, m), 6.0 (1H, m), 4.86–4.7 (2H, m), 3.95 (3H, s), 2.44–2.37 (1H, m), 2.22–2.14 (1H, m), 0.91 (9H, s), 0.29 (3H, s), 0.22 (3H, s); m/z 362 (AP^+, M + 1).

Preparation of (±)-*N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-4-[dimethyl-(1,1-dimethylethyl)silyl]oxy]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (29). To a solution of 1-*n*-butyl-4-piperidinylmethylamine (**22**) (0.95 g, 47 mmol) in toluene (2.5 mL) was added trimethylaluminium (2.3 mL, 2 M in toluene, 46 mmol) followed by **28** (1.62 g, 45 mmol) in toluene (5 mL). The solution was heated under reflux for 4 h, then allowed to cool to room temperature and stirred with 10% aqueous NaOH solution (2 mL) for 30 min. The organic layer was washed with 10% aqueous NaOH solution, water, brine, dried over sodium sulphate, filtered and the filtrate evaporated to leave an oil. This was filtered through silica, washing with ethyl acetate, then methanol obtaining **29**, 2.0 g (89%). ^{1}H NMR (CD_3OD) δ: 8.1 (1H, m), 7.3 (1H, m), 7.1 (2H, m), 6.17 (1H, m), 4.7 (2H, m), 3.0 (2H, m), 2.6–0.9 (9H, m), 0.3 (3H, s), 0.2 (3H, s); m/z 500 (AP^+, M + 1).

Preparation of (±)-*N*-[(1-butyl-4-piperidinyl)methyl]-3,4-dihydro-4-hydroxy-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (5). A solution of **29** (0.15 g, 0.3 mmol) in water (5 mL) and glacial acetic acid (10 mL) was heated at 75 °C for 6.5 h. The solution was evaporated under reduced pressure and the residue partitioned between ethyl acetate (25 mL) and saturated aqueous $NaHCO_3$

solution (3 mL). The ethyl acetate layer was washed with further saturated aqueous $NaHCO_3$ solution, then with brine and dried over sodium sulphate. Evaporation under reduced pressure gave **5**, 0.096 g (83%) as a foam. ^1H NMR (CD_3OD) δ 8.16 (1H, m), 7.5 (1H, m), 7.2 (2H, m), 6.05 (1H, m), 4.8 (2H, m), 3.4 (2H, m), 3.1 (2H, m), 2.6–1.35 (15H, m), 1.03 (3H, t, $J = 7.2$ Hz); ^{13}C NMR (CD_3OD) δ 168, 151, 132, 127, 123, 122, 121, 110, 90, 72, 65, 62, 60, 45, 38, 31.3, 31, 30, 22, 21, 15, 14.5; *m/z* 385.2353 (calcd 385. 2365); HPLC retention time 14.5 min, purity 97.4% (PAR), column: Hichrome RPB 150×4.6 mm id, mobile phase: (A) 0.15 M NH_4OAc adjusted to pH 4.0 with TFA, (B) methanol, gradient: 25 to 60% B over 40 min, then 5 min at 60% B, detection: UV at 265 nm.

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