Synthesis of 3-Amino-2-(3-indolyl)propanol and Propanoate Derivatives and Preliminary Cardiovascular Evaluation in Rats

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Abstract

A series of tryptamine analogues has been prepared and tested for their 5-HT$_1$ receptor agonist properties.

The incorporation of an alkoxy group at the C-5 position of the indole nucleus resulted in a short-lived and dose-dependent immediate antihypertensive and bradycardic response in anaesthetized spontaneously hypertensive rats (SHR). In addition, a carbomethoxy function at the β-position of the side-chain of the tryptamines significantly increased the mean resting arterial blood pressure (MAP) in pithed rats and also produced contraction of the canine basilar artery in a dose-dependent fashion.

Structure–activity relationships (SAR) suggest that the 5-alkoxy group is an important pharmacophore in the production of the antihypertensive effect and that the introduction of a hydroxymethylene group on the side-chain, instead of the carbomethoxy group, changed the receptor affinity profile.

5-Hydroxytryptamine (5-HT), which plays an important role in the modulation of mood, nociception, motor behaviour, endocrine secretion, cardiovascular function and appetite (Glennon 1990), exerts its action via a multiplicity of binding sites that have been classified as 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, recombinant and so-called orphan receptors (Hoyer et al 1994; Saxena 1995).

The increasing availability of new compounds with high affinity and selectivity for subtypes of 5-HT receptors enables the development of drugs with potential therapeutic use in the treatment of some cardiovascular pathologies such as hypertension, migraine, some peripheral vascular diseases and heart failure (Saxena 1995). Much progress has been made during the last decade in understanding the role that 5-HT plays in the central regulation of blood pressure (McCall & Clement 1994). The identification of specific agonists and antagonists for 5-HT receptors has been responsible for the rapid progress in this area. It has recently become clear that 5-HT$_{1A}$ receptors play an important role in the mediation of the inhibitory effects of 5-HT (Routledge 1996). Activation of 5-HT$_{1A}$ receptors in the lower brain stem results in a vasodepressor response that is accompanied by sympathoinhibition and vagal bradycardia (Saxena & Villalón 1990).

In the context of cardiovascular pharmacology, 5-HT receptors are of particular interest as a target for novel antihypertensive drugs. Experiments with 8-hydroxy-2-(N,N-dipropylamino)tetralin (8-OH-DPAT) have shown that the drug reduces blood pressure via activation of medullary 5-HT$_{1A}$ receptors (Fozard et al 1987). Several other agonists at 5-HT$_{1A}$ receptors, e.g. flesinoxan (Doods et al 1988; Wouters et al 1988), N,N-dipropyl-5-carboxamidotryptamine and urapidil (Doods et al 1988) have been found to reduce arterial blood pressure and heart rate in several animal species.

The tryptamine derivative methyl 3-amino-2-(5-methoxy-3-indolyl)propionate (indorenate, compound 1a, Fig 1) is another

centrally acting agonist with high affinity for 5-HT$_{1A}$ receptors (Hong 1981; Hoyer et al 1985). Administration of indorenate through the cat left vertebral artery produced a rapid and long-lasting decrease in blood pressure (Hong et al 1983) and fourth ventricular application of indorenate caused a significant fall in arterial blood pressure (Shepheard et al 1994) and reduced renal, splanchnic and phrenic nerve activity. In addition to reducing blood pressure in normotensive and hypertensive animals (Hong 1981; Safdy et al 1982), the antihypertensive efficacy of indorenate has been confirmed clinically (Hong et al 1992).

In the search for new molecules with potential antihypertensive activity, we describe herein the synthesis of and SAR studies on 5-substituted tryptamine derivatives and the related reference indorenate. The effect of substitution in the side-chain on the biological activity of the compounds was also assessed by preparing the analogous compounds containing a hydroxymethylene group at the β-position of the side-chain in replacement of the carbomethoxyl group. The structures of the compounds investigated are presented in (Fig. 1).

Materials and Methods

Compounds 1a, 1b and 2a (Fig. 1) are known and were prepared as described in the literature (Safdy et al 1982; com-

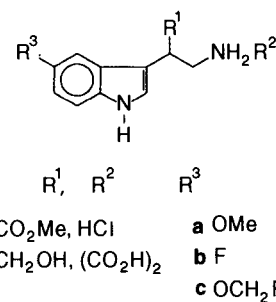| R^1,	R^2	R^3
1 CO$_2$Me, HCl		a OMe
2 CH$_2$OH, (CO$_2$H)$_2$		b F
		c OCH$_2$Ph

FIG. 1. The chemical structures of the compounds investigated.

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pound **2b** was prepared by reduction of **1b** with LiAlH₄ and compounds **1c** and **2c** were synthesized by use of a procedure similar to that reported for **1a** and **2a**, as is outlined in Scheme 1.

5-Substituted indoles **3a–c** were treated with Et₂NH and formaldehyde, according to the Mannich reaction, to afford **4a–c** in high yields. The tryptamines **4a–c** were reacted consecutively with CH₃I and KCN giving the 5-substituted-3-acetonitrilindoles **5a-c**. With (EtO)₂CO as reagent and solvent, **5a-c** reacted with two equivalents of Na leading to the twofold N- and C-carboalkoxylated derivatives **6a-c**. Treatment of **6a-c** with Raney-Ni W-2 (Ac₂O, 25°C, 8 h, 50 psi; Mozingo 1955) afforded amides **7a-c**. This reaction gives good yields, even for the amidoindole **7c** for which hydrogenolytic removal of the benzyl group could be an important side-reaction. The amides **7a-c** were converted into the amino acids **8a-c** by hydrolysis with aqueous NaOH. The target compounds **1b** and **1c** were obtained by esterification of the carboxyl group with thionyl chloride and methanol, followed by quaternization of the amino group with hydrogen chloride.

Basic treatment of **1a-c** afforded the respective free amines, which were converted into the desired γ-amino propanols **2a-c** by carbonyl reduction with LiAlH₄ (dry THF, 15–40°C, 15 h) then quaternization of the amino group with oxalic acid.
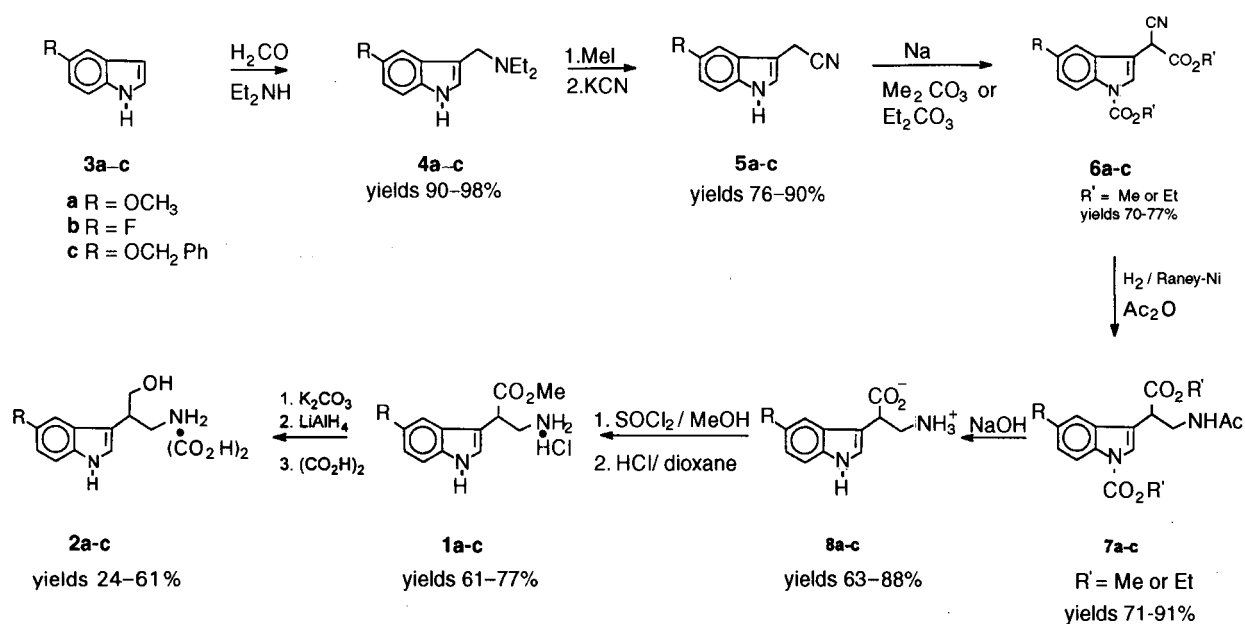
Chemistry: synthetic methods

Melting points were determined by means of a Fisher-Johns apparatus and are uncorrected. UV and IR spectra were obtained with Perkin-Elmer Lambda 12 and 727B spectrophotometers, respectively. ¹H and ¹³C NMR spectra δ (ppm) were measured with a Varian XL-300GS spectrometer at 300 and 75 MHz, respectively. Column chromatography was performed with 70–230 mesh silica gel. Thin-layer chromatography (TLC) was performed on silica gel 60F₂₅₄-coated aluminium plates and inspected under UV illumination (254 nm). Electron-impact mass spectra (EIMS) were recorded with a Hewlett-Packard 5989A spectrometer. High resolution

(HR) and fast atom bombardment mass spectra (FABMS) were obtained with a Jeol JMS-SX 102A spectrometer.

*Preparation of 3-(diethylaminomethyl)-5-benzyloxyindole (**4c**)*
Et₂NH (8·4 mL; 0·081 mol) was added dropwise to pre-cooled (6°C) acetic acid (10·2 mL) over 30 min. Pre-cooled (6°C) 37% aqueous formaldehyde (5·7 mL; 0·070 mol) was added 5 min later and the mixture was poured over 5-benzyloxyindole (**3c**; 15 g, 0·067 mol). After being stirred for 17 h at room temp, the mixture was poured into a pre-cooled (10°C) 10% solution of NaOH (350 mL). The mixture was extracted with CH₂Cl₂ and washed with brine, dried over Na₂SO₄, filtered and concentrated at room temp in-vacuo. The product, **4c**, was obtained as an oil which was crystallized from AcOEt-n-hexane (19·5 g; 94·0%); mp 74–75°C (decomp.). UV (MeOH): λ(log ε) 211 (4·60), 273 (3·78), 294 (3·65), 305 (3·52). ¹H NMR (DMSO-d₆): δ11·07 (br s, 1H, NH), 7·47 (d, 2H, H-o), 7·39 (t, 2H, H-m), 7·36 (br s, 1H, H-2), 7·31 (t, 1H, H-p), 7·29 (d, 1H, H-7), 7·27 (d, 1H, H-4), 6·84 (dd, 1H, H-6), 5·10 (s, 2H, CH₂O), 4·01 (br s, 2H, CH₂N), 2·74 (q, 4H, 2CH₂), 1·11 (t, 6H, 2CH₃). ¹³C NMR (DMSO-d₆): δ152·3 (C-5), 137·7 (C-i), 131·4 (C-7a), 128·3 (C-m), 127·8 (C-3a), 127·5 (C-o, -p), 127·0 (C-2), 112·2 (C-7), 111·9 (C-6), 107·0 (C-3), 102·4 (C-4), 69·8 (CH₂O), 47·3 (CH₂N), 45·6 (CH₂CH₃), 10·3 (CH₃).

*Preparation of 5-benzyloxy-3-acetonitrilindole (**5c**)*
CH₃I (4·7 mL; 0·075 mol) was added to a pre-cooled (5°C) solution of **4c** (19·5 g; 0·063 mol) in THF and stirred overnight at room temp. The mixture was treated with a solution of KCN (5·1 g; 0·076 mol) in H₂O (2 mL) and stirred for 2 h at reflux under argon. After removal of the solvent in vacuo the residue was dissolved in CH₂Cl₂ (450 mL), washed successively with H₂O (2 × 150 mL), 5% aqueous HCl (150 mL) and brine, dried over Na₂SO₄, filtered and concentrated in-vacuo. Column chromatography (n-hexane-CH₂Cl₂, 1 : 4) afforded **5c**, which was crystallized from AcOEt-n-hexane (13·7 g; 82·7%); mp 78–79°C (Desaty & Keglevic 1965, 77–

3a–c

a R = OCH₃
b R = F
c R = OCH₂Ph

4a–c
yields 90–98%

5a-c
yields 76–90%

6a-c
R' = Me or Et
yields 70-77%

H₂ / Raney-Ni
Ac₂O

2a-c
yields 24–61%

1a-c
yields 61–77%

8a-c
yields 63–88%

7a-c
R' = Me or Et
yields 71-91%

SCHEME 1. General procedures for the synthesis of tryptamine derivatives **1a-c** and **2a-c**.

78°C). $R_F = 0.31$ (n-hexane-AcOEt, 7 : 3). UV (MeOH): λ (log ε) 208 (4·50), 273 (3·94), 295 (3·76), 307 (3·59). IR (KBr, cm^{-1}): 2255 (CN). ^{1}H NMR (CDCl$_3$): δ 8·16 (br s, 1H, NH), 7·45 (d, 2H, H-o), 7·36 (t, 2H, H-m), 7·30 (t, 1H, H-p), 7·19 (d, 1H, H-7), 7·06 (d, 1H, H-4), 7·02 (m, 1H, H-2), 6·94 (dd, 1H, H-6), 5·06 (s, 2H, CH$_2$O), 3·69 (d, 2H, CH$_2$). ^{13}C NMR (CDCl$_3$): δ 153·6 (C-5), 137·4 (C-i), 131·6 (C-7a), 128·5 (C-m), 127·8 (C-p), 127·6 (C-o), 126·4 (C-3a), 123·6 (C-2), 118·2 (CN), 113·7 (C-6), 112·4 (C-7), 104·1 (C-3), 101·5 (C-4), 71·0 (CH$_2$O), 14·3 (CH$_2$).

Preparation of ethyl 2-cyano-2-(5-benzyloxy-1-ethoxycarbo-nyl-1H-indol-3-yl)acetate (6c)

Na (1·0 g; 43·4 mmol) was added in small portions over a period of 30 min to a stirred solution of **5c** (4·6 g; 17·5 mmol) in (EtO)$_2$CO (50 mL) while the temperature was kept at 90°C. The mixture was stirred for 2 h at 105°C and the solvent was removed in-vacuo. The remaining residue was cautiously treated with a cold solution of 10% aqueous AcOH (200 mL) and the crude product extracted into AcOEt. The organic layer were combined, washed with brine, dried over Na$_2$SO$_4$, filtered and concentrated in-vacuo. Column chromatography (n-hex-ane-AcOEt, 1 : 1) afforded **6c**, which was crystallized from MeOH (5·36 g; 75·6%); mp 95–96°C, $R_F = 0·53$ (n-hexane-AcOEt, 7 : 3). UV (MeOH): λ (log ε) 200 (4·73), 238 (4·37), 301 (3·74), 309 (3·69). IR (KBr, cm^{-1}): 2250 (CN), 1735 (CO). ^{1}H NMR (CDCl$_3$): δ 8·09 (br d, 1H, H-7), 7·79 (br s, 1H, H-2), 7·47 (d, 2H, H-o), 7·39 (t, 2H, H-m), 7·32 (t, 1H, H-p), 7·18 (d, 1H, H-4), 7·08 (dd, 1H, H-6), 5·11 (s, 2H, CH$_2$O), 4·88 (d, 1H, CH), 4·48 and 4·35 (2q, 4H, 2CH$_2$), 1·46 and 1·27 (2t, 6H, 2CH$_3$). ^{13}C NMR (CDCl$_3$): δ 164·2 (CO, ester), 155·6 (C-5), 150·4 (CO, carbamate), 136·9 (C-i), 130·4 (C-7a), 128·6 (C-m), 128·3 (C-3a), 128·0 (C-p), 127·6 (C-o), 125·6 (C-2), 116·4 (C-7), 115·2 (C-6), 114·8 (CN), 110·1 (C-3), 103·1 (C-4), 70·7 (CH$_2$O), 63·6 and 63·5 (2CH$_2$CH$_3$), 35·7 (CH), 14·4 and 14·0 (2CH$_3$).

Preparation of ethyl 3-acetamido-2-(5-benzyloxy-1-ethoxycar-bonyl-1H-indol-3-yl)propanoate (7c)

A mixture of **6c** (4·0 g; 10·4 mmol) and Raney-Ni W-2 (approximately 4·0 g) in Ac$_2$O (55 mL) was placed in a hydrogenation flask which was filled with H$_2$ at 50 psi. The mixture was shaken for 8 h at room temp and the catalyst was then removed by filtration and washed with AcOEt. The filtrate was concentrated in-vacuo and the residue was dissolved in AcOEt, washed with 6% aqueous K$_2$CO$_3$ (5 × 80 mL) and brine. After drying over Na$_2$SO$_4$ and filtering, the solvent was removed in-vacuo to give an oil. Column chromatography (n-hexane-AcOEt, 6 : 4) afforded **7c**, which was crystallized from AcOEt-n-hexane (3·57 g; 76·5%); mp 90–92°C, $R_F = 0·61$ (AcOEt). UV (MeOH): λ (log ε) 216 (4·77), 274 (4·01), 296 (3·86), 307 (3·73). IR (KBr, cm^{-1}): 1725 (CO ester), 1660 (CO amide). ^{1}H NMR (CDCl$_3$): δ 8·05 (br d, 1H, H-7), 7·53 (br s, 1H, H-2), 7·48 (d, 2H, H-o), 7·38 (t, 2H, H-m), 7·33 (t, 1H, H-p), 7·23 (d, 1H, H-4), 7·03 (dd, 1H, H-6), 6·06 (br t, 1H, NH), 5·12 (s, 2H, CH$_2$O), 4·45 (q, 2H, CH$_2$), 4·15 (m, 2H, CH$_2$), 4·11 (dd, 1H, CH), 3·80 and 3·70 (AB, 2H, CH$_2$N), 1·95 (s, 3H, COCH$_3$), 1·44 (t, 3H, CH$_3$), 1·20 (t, 3H, CH$_3$). ^{13}C NMR (CDCl$_3$): δ 172·5 (COCH$_3$), 170·3 (CO, ester), 155·3 (C-5), 155·3 (C-5), 150·6 (CO, carbamate), 137·1 (C-i), 130·2 (C-7a), 130·0 (C-3a), 128·5 (C-m), 127·9 (C-p), 127·6 (C-o), 123·9

(C-2), 116·3 (C-3), 116·1 (C-7), 114·5 (C-6), 103·4 (C-4), 70·6 (CH$_2$O), 63·2 and 61·3 (2CH$_2$CH3), 42·5 (CH), 41·0 (CH$_2$N), 23·2 (COCH$_3$), 14·4 and 14·1 (2CH$_3$).

Preparation of 3-amino-2-(5-benzyloxy-1H-indol-3-yl)propa-noic acid (8c)

A mixture of **7c** (4·0 g; 8·8 mmol) and 10 N NaOH (11·2 mL) was heated under reflux for 8 h under an argon atmosphere, then diluted with H$_2$O (70 mL) and acidified to pH 6·12 with AcOH. The precipitate which formed was collected by vacuum filtration, dissolved in water (115 mL) and decolorized with activated charcoal. The solution was concentrated in-vacuo until solid material was seen, (approximately 25 mL) and cooled to approximately 4°C. After standing for 2 h the solid was removed by filtration and dried in a vacuum oven for 48 h at 40°C to yield the product **8c** as white solid (1·74 g; 63·2%); mp 166–169°C. UV (MeOH): λ (log ε) 200 (4·53), 205 (4·51), 276 (3·78), 296 (3·67) 308 (3·51). IR (KBr, cm^{-1}): 3050 (NH$_3^+$), 1580 (CO$_2^-$). ^{1}H NMR (AcOD-d$_3$): δ 9·76 (br d, 1H, NH), 7·53 (d, 2H, H-o), 7·42 (t, 2H, C-m), 7·37 (d, 1H, H-7), 7·35 (t, 1H, H-p), 7·35 (d, 1H, H-2), 6·99 (dd, 1H, H-6), 5·17 (s, 1H, CH$_2$O), 4·47 (t, 1H, CH), 3·78 and 3·57 (AB, 2H, CH$_2$N). ^{13}C NMR (AcOD-d$_3$): δ 178·6 (CO), 154·8 (C-5), 139·4 (C-i), 133·7 (C-7a), 129·9 (C-m), 129·2 (C-o, -p), 128·0 (C-3a), 126·5 (C-2), 114·6 (C-6), 114·0 (C-7), 109·2 (C-3), 104·0 (C-4), 72·3 (CH$_2$O), 42·6 (CH$_2$N), 42·1 (CH).

Preparation of methyl 3-amino-2-(5-benzyloxy-1H-indol-3-yl)propanoate hydrochloride (1c)

SOCl$_2$ (0·7 mL; 8·2 mmol) was added dropwise to a pre-cooled (−10°C) suspension of **8c** (2·0 g; 6·4 mmol) in MeOH (50 mL) and stirred 18 h at room temp under argon. After removal of the solvent in-vacuo the residue was partitioned between AcOEt (75 mL) and 6% aqueous K$_2$CO$_3$ (30 mL). The organic layer was separated and washed with brine, dried over Na$_2$SO$_4$, filtered and decolorized with activated charcoal. The solution was treated with 4 N HCl in dioxane to give the corresponding hydrochloride. This was collected by vacuum filtration and dried in a vacuum oven for 48 h at 40°C to yield the product **1c** as a white crystals (1·8 g; 77·6%); mp 232–234°C (decomp.). UV (MeOH): λ (log ε) 201 (4·54), 240 (4·31), 262 (3·99), 300 (3·64), 309 (3·59). IR (KBr, cm^{-1}): 3000 (NH$_3^+$), 1730 (CO). EIMS m/z: 324 (M$^+$, 14%), 204 (100%). ^{1}H NMR (DMSO-d$_6$): δ 11·15 (br d, 1H, NH), 7·49 (d, 2H, H-o), 7·40 (t, 2H, H-m), 7·32 (t, 1H, H-p), 7·31 (d, 1H, H-7), 7·27 (d, 1H, H-2), 7·22 (d, 1H, H-4), 6·86 (dd, 1H, H-6), 5·11 (s, 2H, CH$_2$O), 4·33 (q, 1H, CH), 3·62 (s, 3H, CH$_3$), 3·45 and 3·13 (AB, 2H, CH$_2$N). ^{13}C NMR (DMSO-d$_6$): δ 172·0 (CO), 152·3 (C-5), 137·6 (C-i), 131·6 (C-7a), 128·3 (C-m), 127·6 (C-o, -p), 126·1 (C-3a), 124·6 (C-2), 112·5 (C-7), 112·2 (C-6), 107·7 (C-3), 102·0 (C-4), 69·9 (CH$_2$O), 52·1 (OCH$_3$), 40·4 (CH$_2$N), 40·3 (CH). HRFABMS m/z: 325·1575 (calcd for C$_{19}$H$_{21}$O$_3$N$_2$ (M$^+$ + H): 325·1552).

Preparation of 3-amino-2-(5-benzyloxy-1H-indol-3-yl)propa-nol oxalate (2c)

Aqueous K$_2$CO$_3$ (6%; 40 mL) was added to a suspension of **1c** (500 mg; 1·38 mmol) in CH$_2$Cl$_2$ (85 mL) and the mixture was stirred vigorously at room temp for 30 min. The organic layer was separated and washed with brine, dried over Na$_2$SO$_4$, fil-tered and concentrated in-vacuo to a syrup. The syrup was

dissolved in dry THF (20 mL) and added dropwise to a stirred suspension (15–20°C) of LiAlH$_4$ (200 mg) in dry THF (10 mL). Stirring was continued for 3 h at 40°C and then overnight at room temp. After cooling to 5°C the reaction mixture was treated cautiously with H$_2$O (8 mL), the pH of the solution was adjusted to 8 with 10% aqueous AcOH and more H$_2$O (10 mL) was added. The mixture was filtered, the THF was evaporated in-vacuo below 40°C, and the remaining aqueous layer was washed with cold CH$_2$Cl$_2$ and lyophilized. The solid residue was dissolved in dry MeOH (4 mL), a solution of oxalic acid (0·15 g; 1·66 mmol) in dry MeOH (1 mL) was added and the mixture was diluted with dry Et$_2$O. The solid formed was removed by filtration and washed with cold Et$_2$O, to afford the oxalate salt 2c (251 mg; 46·9%); mp 164–165°C. UV (MeOH): λ (log ε) 202 (4·62), 275 (3·80), 296 (3·67), 308 (3·50). IR (KBr, cm^{-1}): 3025 (NH$_3^+$). EIMS m/z: 296 (M$^+$, 21%), 249 (100%). ^{1}H NMR (DMSO-d$_6$): δ 10·98 (br d, 1H, NH), 7·49 (d, 2H, H-o), 7·40 (t, 2H, H-m), 7·32 (t, 1H, H-p), 7·27 (d, 1H, H-7), 7·22 (d, 1H, H-2), 7·20 (d, 1H, H-4), 5·10 (CH$_2$O), 3·72 (m, 2H, CH$_2$OH), 3·34 (m, CH$_2$N), 3·14 (CH). ^{13}C NMR (DMSO-d$_6$): δ 152·2 (C-5), 137·8 (C-i), 131·7 (C-7a), 128·4 (C-m), 127·7 (C-o), 127·6 (C-p), 126·9 (C-3a), 123·7 (C-2), 112·3 (C-7), 111·9 (C-6), 111·3 (C-3), 102·1 (C-4), 70·0 (CH$_2$O), 63·4 (CH$_2$OH), 41·7 (CH$_2$N), 37·2 (CH). HRFABMS m/z 296·1527 (calcd for C$_{18}$H$_{20}$O$_2$N$_2$ (M$^+$): 296·1525).

Preparation of 3-amino-2-(5-fluoro-1H-indol-3-yl)propanol oxalate (2b)

In a manner similar to that described above, 1b was converted to the oxalate salt, 2b. Treatment of 1b (400 mg; 1·46 mmol) with 10% aqueous K$_2$CO$_3$ and subsequent work-up afforded the free amine as a syrup. The syrup was dissolved in THF and reduced with LiAlH$_4$. The reaction mixture was treated with 10% aqueous AcOH to pH 8, filtered and evaporated in-vacuo to remove THF, and the product was extracted into AcOEt (3 × 15 mL). The extract was dried over Na$_2$SO$_4$ and the solution was concentrated to 10 mL. The concentrate was treated with a solution of oxalic acid (0·15 g, 1·66 mmol) in MeOH (3 mL) and the solid formed was removed by filtration to afford the oxalate salt 2b (107 mg; 24·5%); mp 164–166°C. UV (MeOH): λ (log ε) 200 (4·53), 219 (4·52), 280 (3·96), 286 (3·96), 296 (3·83). IR (KBr, cm^{-1}): 3050 (NH$_3^+$). EIMS m/z: 208 (M$^+$, 7%), 161 (100%). ^{1}H NMR (DMSO-d$_6$): δ 11·23 (br d, 1H, NH), 7·40–7·33 (overlapped m, 3H, H-2, H-4, H-7), 6·93 (td, 1H, H-6), 3·70 (m, 2H, CH$_2$O), 3·30 (m, 2H, CH$_2$N), 3·11 (m, 1H, CH). ^{13}C NMR (DMSO-d$_6$): δ 156·7 (C-5), 133·0 (C-7a), 126·8 (C-3a), 125·1 (C-2), 112·5 (C-7), 111·9 (C-3), 109·2 (C-6), 103·1 (C-4), 63·3 (CH$_2$OH), 41·4 (CH$_2$N), 37·2 (CH). HRFABMS m/z: 209·1090 (calcd for C$_{11}$H$_{14}$ON$_2$F (M$^+$ + H): 209·1090).

Pharmacology

Antihypertensive effects of 1a-c and 2a-c in spontaneously hypertensive rats (SHR). Male SHR, 250–300 g, were anaesthetized with sodium pentobarbital (30 mg kg^{-1}). The trachea, a carotid artery and a femoral vein were cannulated for adequate ventilation, to record blood pressure and for drug administration, respectively. Mean arterial blood pressure (MAP) and heart rate (HR) were recorded with a Statham P23 ID pressure transducer and a 7P4F tachograph, respec-

tively (both from Grass Instrument Co., Quincy, MA). Both haemodynamic parameters were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co.). The body temperature of the animals was maintained between 37–38°C. After 15 min stabilization, a single dose of the tryptamine derivative was injected and the MAP and HR were monitored for 30 min. Doses (given intravenously) were 1·0 and 3·1 mg kg^{-1} for 1a and 3·1 and 10 mg kg^{-1} for 1b, c and 2a-c.

Direct effect of 1a-c and 2a-c on the blood pressure of pithed rats. Male Wistar normotensive rats, 250–300 g, were pithed under ether anaesthesia (Shipley & Tilden 1947). The tracheas were cannulated for artificial respiration by mean of a BioScience pump (Sheerness, Kent, UK; 56 cycles min^{-1}; volume 2 mL (100 g body weight)$^{-1}$ and both vagus nerves were sectioned. A carotid artery and the right femoral vein were cannulated for blood pressure monitoring and bolus administration of the compounds (3·1 and 10 mg kg^{-1}), respectively. After the bolus injections, the corresponding cannula was flushed with physiological saline solution (0·1 mL). The haemodynamic parameters were recorded as described above.

Effect of 1a-c on canine basilar artery rings. Mongrel dogs of both sexes were anaesthetized with sodium pentobarbital (30 mg kg^{-1}, i.v.) and killed by rapid exsanguination. The brain was removed and the corresponding basilar arteries were dissected free at room temperature and placed in modified Krebs solution of composition (mM): NaCl 118, KCl 4·7, KH$_2$PO$_4$ 1·2, MgSO$_4$·7H$_2$O 1·2, CaCl$_2$·2H$_2$O 2·5, NaHCO$_3$, 25·0, dextrose 11·7, and calcium disodium EDTA 0·026 (Furchgott & Bhadrakom 1953). Ring segments (3–4 mm) of the canine basilar artery were mounted in separate 10-mL organ baths containing modified Krebs solution at 37°C and bubbled with a mixture of 95% O$_2$ and 5% CO$_2$. The segments were carefully suspended between two L-shaped nikrom hooks (diameter 0·2 mm) and inserted into the lumen to record changes in isometric tension. No attempt was made to remove the endothelium.

Ring segments were placed under an optimum baseline tension of 3000 mg; this amount of resting tension has been shown to maximize the C$_{max}$ value obtained with 5-HT (Allen et al 1974) and is essentially equivalent to the contractions induced by 15 mM KCl or 0·5 mM CaCl$_2$. The preparations were left to equilibrate for 120 min; within this period three contractile responses to 5-HT (10^{-7} M) were elicited. The resting tension was adjusted continuously throughout the experiments. Isometric contractions were recorded as changes in force (g) on a Grass model 7D polygraph with Statham FTO3C transducer. Concentration–response curves to 5-HT (10^{-10} to 10^{-6} M) and to 1a-c (10^{-7} to 10^{-4} M) were obtained using a cumulative concentration schedule (spaced by a factor of 10$^{1/2}$). For this purpose, 100-μL volumes of drug dilutions were added to the fluid bathing the basilar artery preparations and successive additions were made only after a stable or maximum response to the previous concentration had been attained. After washing arterial rings were left for at least 45 min (changing the Krebs solution every 15 min) before the next concentration–response curve was plotted. One or two

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Table 1. Cardiovascular effect of the tryptamine analogues **1a-c** and **2a-c** in anaesthetized spontaneously hypertensive rats.

Compound	Dose 3·1 mg kg^{-1} (i.v.)		Dose 10 mg kg^{-1} (i.v.)	
	Mean arterial blood pressure (mm Hg)	Heart rate (beats min^{-1})	Mean arterial blood pressure (mm Hg)	Heart rate (beats min^{-1})
1a*	$-75\cdot83 \pm 15\cdot5†$	$-78\cdot48 \pm 20\cdot00†$	$-$	$-$
2a	$-53\cdot12 \pm 6\cdot32†$	$-72\cdot70 \pm 12\cdot56†$	$-77\cdot38 \pm 3\cdot82†$	$-68\cdot00 \pm 12\cdot60†$
1b	$-8\cdot02 \pm 3\cdot57$	$-24\cdot40 \pm 7\cdot11†$	$-8\cdot82 \pm 4\cdot09$	$-47\cdot40 \pm 4\cdot13†$
2b	$-2\cdot50 \pm 2\cdot50$	$-45\cdot00 \pm 15\cdot00†$	$-5\cdot00 \pm 0\cdot65$	$-50\cdot00 \pm 6\cdot89†$
1c	$-35\cdot50 \pm 8\cdot59†$	$-47\cdot00 \pm 5\cdot83†$	$-61\cdot30 \pm 8\cdot87†$	$-68\cdot20 \pm 13\cdot08†$
2c	$-45\cdot00 \pm 7\cdot58†$	$-23\cdot00 \pm 3\cdot74†$	$-97\cdot80 \pm 4\cdot46†$	$-33\cdot00 \pm 3\cdot75†$

Data, shown as maximum changes in mean resting arterial blood pressure and heart rate, are means ± s.e.m. of results from at least six animals. *The changes produced with 1 mg kg^{-1} were $-48\cdot28 \pm 11\cdot82†$ mmHg and $-38\cdot50 \pm 10\cdot84$ (beats min^{-1}) respectively. $†P < 0\cdot05$ compared with the mean resting blood pressure or heart rate.

control curves to 5-HT were first obtained in each ring to ensure consistency in the response. Two or three consecutive concentration–response curves to 5-HT and to **1a-c** were obtained; the rings challenged with 5-HT acted as a control to monitor any spontaneous change in sensitivity to 5-HT. Agonist potency (−log EC50) was estimated as the concentration (M) of the drug producing half the maximum response.

Effect of 1a-c and 2a-c on rat aortic rings. Male Wistar normotensive rats, 250–300 g, were stunned and the thoracic aorta was immediately excized, placed in cold buffer (Furchgott & Bhadrakom 1953), cleaned and freed from surrounding connective tissue. The isolated arteries were cut into rings (3–4 mm long) and placed in 10-mL tissue chambers filled with Krebs solution. Tissue baths were maintained at 37°C, pH 7·4 and bubbled with a mixture of 95% O_2 and 5% CO_2. Rings were mounted on two nikrom hooks in order to fix them to the bottom of the chamber, and to a Grass FTO3 force-displacement transducer connected with a 7D Grass Polygraph to record the isometric tension developed by the aortic rings. The rings were given 4 g initial tension and left to equilibrate for 2 h with changes of buffer at 30-min intervals after contracting them with phenylephrine (10^{-7} M). Optimum tension was selected from preliminary experiments in which the rings were stretched to obtain the greatest response to phenylephrine (10^{-7} M). When a 120-min equilibration period had elapsed, one or two concentration–response curves were obtained for 5-HT (10^{-7} to 10^{-4} M; spaced by a factor of $10^{1/2}$). After wash-out, arterial rings were left at least 45 min (changing Krebs solution every 15 min) before concentrations of **1a-c** and **2a-c** (10^{-7} to 10^{-4} M) were added using a cumulative concentration schedule. To determine whether the compounds antagonize the 5-HT contraction in the aorta, concentration–response curves to 5-HT (10^{-7} to 10^{-4} M; spaced by a factor of $10^{1/2}$) were obtained before and after aortic rings had been incubated with **1a-c** and **2a-c** (10^{-5} M) for 20 min. Concentration–response curves elicited by the compounds are expressed as a percentage of maximum contraction to 5-HT. Agonist potency ($-$log EC50) was estimated as the concentration of drug (M) producing half of the maximum response.

Statistical analysis
The results are expressed as mean ± s.e.m. The difference between several dependent means (MAP and HR) and their own control were evaluated by analysis of variance with repeated measures using the method O'Brien & Kaiser (1985) and Dunnett's method was used to compare mean values to control. Student's *t*-test for paired comparisons was used to evaluate differences between $-$log EC50 values to 5-HT in aortic rings before and after incubation with **1a-c** and **2a-c**.

Results and Discussion

The effects of intravenous injection of compounds **1a** (1·0 and 3·1 mg kg^{-1}), **1b**, **1c** and **2a-c** (3·1 and 10 mg kg^{-1}) on mean resting arterial blood pressure (MAP) and on heart rate (HR) studied in anaesthetized SHR are shown in Table 1. Treatment with indorenate (**1a**) induced a significant long-lasting and dose-dependent reduction in blood pressure and in heart rate, whereas 5-alkoxytryptamine derivatives **1c** and **2a**, **2c** caused a short-lasting and dose-dependent immediate antihypertensive and bradycardic response after which blood pressure and heart rate gradually returned to baseline. In contrast, the 5-fluorinated tryptamine derivatives **1b** and **2b** did not modify systemic blood pressure, even when the dose was increased to 10 mg kg^{-1}, but induced a significant reduction in heart rate at a dose of 3·1 mg kg^{-1}. Of the four 5-alkoxylated compounds tested (**1a**, **1c** and **2a**, **2c**), compound **1a** showed the most potent antihypertensive effect.

As shown in Table 2, the administration of alcohols **2a-2c** at a dose up to 10 mg kg^{-1} to pithed rats did not reduce MAP significantly, as had previously been observed in anaesthetized SHR. The lack of the hypotensive effect in this model for **2a-c** suggests that these compounds might reduce systemic blood pressure in whole animals by activation of 5-HT$_1$ central receptors. Indeed, compounds **1b** and **1c** are not only devoid of hypotensive capacity but significantly increased the MAP values in pithed rats. The pressor effect of compound **1b** had a potency comparable with that observed for the 5-HT$_{1A}$ agonist indorenate (Castillo et al 1994) in pithed rats (Table 2). The observed increase in blood pressure caused by indorenate has been attributed to the activation of 5-HT$_2$ peripheral receptors

Table 2. Cardiovascular effect of the tryptamine analogues 1a-c and 2a-c in pithed rats.

Compound	Dose 3·1 mg kg^{-1} (i.v.)		Dose 10 mg kg^{-1} (i.v.)	
	Mean arterial blood pressure (mm Hg)	Heart rate (beats min^{-1})	Mean arterial blood pressure (mm Hg)	Heart rate (beats min^{-1})
1a	35·16 ± 4·78*	11·67 ± 5·27	75·83 ± 3·96*	18·33 ± 2·79
2a	− 9·16 ± 3·05	− 13·33 ± 6·67	− 16·00 ± 4·00	− 43·33 ± 10·13
1b	44·17 ± 3·00*	− 14·17 ± 7·35	101·33 ± 4·67*	8·33 ± 6·14
2b	− 5·23 ± 1·12	50·13 ± 18·54	10·09 ± 2·89	49·00 ± 16·43
1c	12·50 ± 2·50*	− 25·00 ± 3·54	35·00 ± 2·04*	− 18·75 ± 4·27
2c	− 1·67 ± 1·67	− 26·67 ± 3·33	− 18·33 ± 3·33	− 38·33 ± 7·26

Data, shown as maximum changes in mean resting arterial blood pressure and heart rate, are means ± s.e.m. of results from at least six animals. *$P < 0.05$ compared with the mean resting blood pressure or heart rate.

(Castillo et al 1995). The striking difference in biological activity between 2a-c and 1a-c indicates that the carbomethoxy group on the tryptamine side-chain is significant in the structure–activity profile.

The study on receptor activity of compounds 1a-c and of 5-hydroxytryptamine (5-HT) used as reference standard for 5-HT-ergic 5-HT$_{1D}$-like and 5-HT$_{2A}$ receptors was assessed by measuring canine basilar contractions (Martin 1994) and rat aortic ring contractions (Cohen et al 1981), respectively.

Measurement of the canine basilar artery contractions after treatment of the segments with increasing concentrations of 5-HT and tryptamine derivatives 1a-c (Fig. 2) showed that agonist potency values (−log EC50) estimated as the concentration of drug producing half of the maximum effect were 7·24 ± 0·03, 5·87 ± 0·07, 5·70 ± 0·08 and 5·68 ± 0·03, respectively. The rank order of potency for vasoconstriction in this vessel was, therefore: 5-HT > 1a > 1b, 1c. The E$_{max}$ for 1a-c were equivalent to 72·5 ± 3·5, 76·7 ± 5·48 and 51·9 ± 1·39%, respectively, of the 5-HT value. These findings suggest that 1a-c behave as partial agonists of the 5-HT$_{1D}$ receptor in the canine basilar artery. Treatment of rat aortic rings carrying intact endothelium with increasing concentrations of 5-HT (10^{-7} to 10^{-4} M), used as reference to characterize 5-HT$_{2A}$-binding sites, also elicited dose-dependent contractions, but with lower potency (−log EC50 = 4·72 ± 0·08) than those previously determined on the canine basilar artery (−log EC50 = 7·24 ± 0·03). Unlike the results obtained with the canine basilar artery, no changes in resting tension of the rat aortic artery were observed after the addition of 1a-c at increasing concentrations from 10^{-7} to 10^{-4} M.

Compounds 1a-c and 2a-c were also tested to assess their capacity to antagonize 5-HT-induced contractions in rat aortic rings mediated by 5-HT$_{2A}$-receptor stimulation (Cohen et al 1981). Concentration–response curves for 5-HT obtained before and after aortic ring incubation with 1a-c and 2a-c (10^{-5} M) for 20 min, showed no significant change in −log EC50 value for the 5-HT-induced contractions, nor change in E$_{max}$ (Table 3). The lack of inhibitory activity of 1a-c and 2a-c demonstrates that these compounds have no significant interaction with 5-HT$_{2A}$ receptors.

There is a relationship between the chemical structure of some newly synthesized tryptamine derivatives and their activity on 5-HT-ergic receptors. Several findings have emerged from this work: compound 1b, which has 5-fluorine
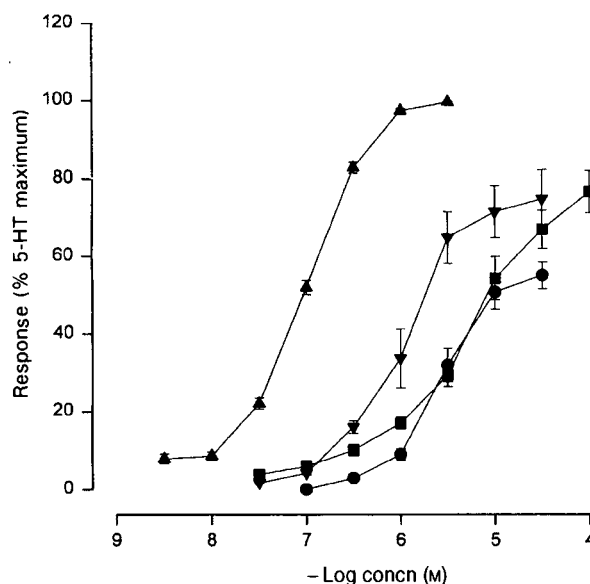

FIG. 2. Contractile effects of 5-HT (▲), 1a (▼), 1b (■), and 1c (●) in the canine basilar artery. Maximum response of each agonist is compared with that achieved by 5-HT. Each value represents the mean ± s.e.m. (vertical bars) of at least six experiments.

Table 3. Effect of the tryptamine analogues 1a-c and 2a-c (10^{-5} M) on −log EC50 values for 5-HT-induced contractions in rat aortic rings.

Compound	Before compound −log EC50	After compound −log EC50
Control	4·72 ± 0·08	4·54 ± 0·15
1a	4·97 ± 0·19	4·75 ± 0·14
2a	4·98 ± 0·21	4·90 ± 0·14
1b	5·06 ± 0·13	4·82 ± 0·09
2b	4·92 ± 0·14	4·92 ± 0·16
1c	4·89 ± 0·14	4·54 ± 0·22
2c	5·01 ± 0·17	4·97 ± 0·14

Data shown are means ± s.e.m. from at least six experiments. *$P < 0.05$ compared with −log EC50 before incubation with the

substitution, was shown to be a good vasoconstrictor in canine basilar artery, but was practically inactive as agonist or antagonist in rat aorta, showing a preferential affinity for 5-

HT$_1$ receptors over 5-HT$_2$ sites. Although the 5-fluorinated compound **1b** was able to modify systemic blood pressure in pithed rats, it showed an action profile different from that of **1a**, which appeared to be a more potent antihypertensive agent than the 5-alkoxylated compounds **1c**, **2a** and **2c**. The results suggest that the presence of the 5-alkoxy group is an important requirement for the antihypertensive effect. A carbomethoxy group at the side-chain might increase their potency.

Acknowledgements

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