Eur J Med Chem (1997) 32, 515–522

Selective endothelin A receptor antagonists. 2. Discovery and structure–activity relationships of 5-ketopentanoic acid derivatives

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(Received 15 July 1996; accepted 16 December 1996)

Summary — The second in this series of papers describes the further progress made in the discovery of a potent and selective endothelin ET_A receptor antagonist for the potential treatment of diseases in which endothelin has been shown to have a pathophysiological role including hypertension, ischaemic diseases and atherosclerosis. We describe herein the synthesis and structure–activity relationships of a novel series of 5-ketopentanoic acid derivatives exemplified by the lead compound **1** (IC_{50} 0.72 μM, rat aortic ET_AR). Optimisation of the in vitro binding of **1** led to the identification of a more potent compound (**37**) which exhibited an $IC_{50} < 0.1$ μM with > 300-fold selectivity for the ET_A receptor over the ET_B receptor. This compound demonstrated functional antagonism of endothelin-induced vasoconstriction in vitro.

endothelin receptor antagonist / ketopentanoic acid / structure–activity relationship / vasodilator activity

Introduction

The endothelins (ET), a family of 21 amino acid peptides, have been implicated in the pathophysiology of several disease states including hypertension, pulmonary hypertension, cerebral and myocardial ischaemia, renal failure and atherosclerosis [1, 2]. The three human isopeptides (ET-1, ET-2 and ET-3) exert their biological effects by selectively binding to specific membrane bound G protein coupled receptors. Two distinct types of endothelin receptors have been cloned and expressed from human and other mammalian tissue. The ET_A receptor subtype is highly selective for ET-1 and is located principally on vascular smooth muscle where it mediates vasoconstriction and smooth muscle cell proliferation. The ET_B subtype has equal affinity for all three isopeptides and is widely distributed in a variety of vascular and non vascular tissue. Endothelial ET_B receptors mediate vasodilation, whereas ET_B receptors on smooth muscle can induce contraction.

The therapeutic potential for endothelin receptor antagonists has led to numerous reports in the literature of structurally diverse antagonists with varying potency and receptor subtype selectivity. Many of the compounds initially described were peptidic in nature [3] and have been valuable in demonstrating the involvement of endothelin receptors in animal in vivo models of hypertension and renal failure. However, it is the recent disclosure of non peptidic orally active antagonists that has allowed a more extensive exploration of the pathophysiological role of endothelin in animal models of disease and consequently the potential uses of an orally bioavailable ET antagonist [4]. These non peptide antagonists have been recently reviewed [3, 5]. There is debate about the merits of a selective ET_A receptor antagonist compared with those of a mixed ET_A/ET_B antagonist [6]. Potential advantages for an ET_A selective antagonist have been outlined in part 1 of this series [7].

Our initial goal in the search for a selective antagonist was to develop a pharmacophore model for ET_A ligand binding and use the technique of 3-D-database searching to rationally select a set of compounds from the RPR corporate database [8]. These compounds would then be screened for their ability to inhibit the binding of [^{125}I]ET-1 to rat aortic ET_A receptors. This process has been described in detail in part 1 of this series [7]. A number of moderately active non peptide antagonists were identified by this approach, including 2,4-dibenzyloxy benzoic acid [7] (IC_{50} 9.0 μM, rat ET_AR) and the 5-ketopentanoic acid derivative **1** (0.72 μM). Neither of these compounds significantly

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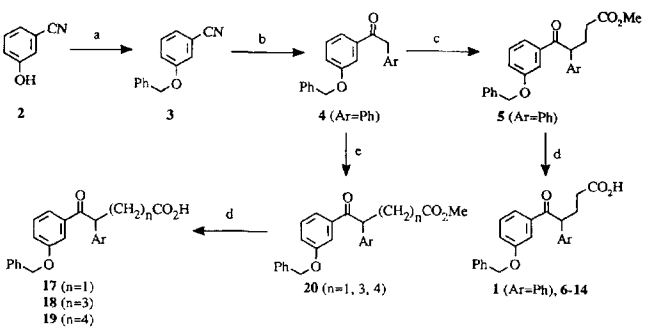
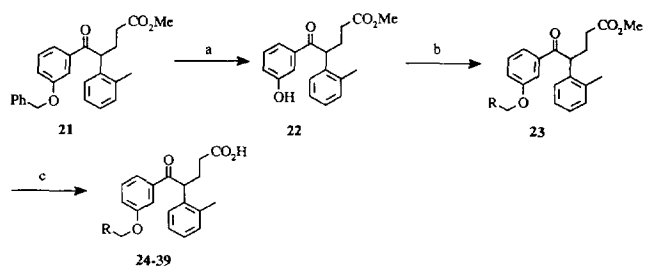
inhibited the binding of [^{125}I]ET-1 to rat cerebellum ET_B receptors at a concentration of 30 μM. This paper outlines the syntheses and structure activity relationships of analogues the 5-ketopentanoic acid lead **1.**

Chemistry

The preparation of **1** and the majority of the analogues described in this paper is illustrated in schemes 1 and 2. The physical properties of all compounds for which biological data is presented are summarised in tables I–IV. 3-Cyanophenol **2** was converted to 3-benzyloxybenzonitrile **3** under standard conditions as shown in scheme 1. Reaction of **3** with an arylmethyl magnesium chloride followed by acid hydrolysis of the intermediate imine *(Method A)* gave the ketomethylene compounds exemplified by **4**. All the Grignard reagents were prepared in ethereal solution from the appropriate commercially available benzyl chlorides. The acidic methylene position of **4** could be deprotonated with potassium *tert*-butoxide in an inert solvent such as tetrahydrofuran *(Method B)* and quenched with methyl acrylate to construct the racemic pentanoate skeleton **5**. This Michael addition generally proceeded smoothly at room temperature, with the exception of analogues where the Ar group contained an *ortho* substituent; in these cases refluxing temperatures were required. Basic hydrolysis *(Method C)* then afforded the target 5-ketopentanoic acids. This procedure was used to prepare analogues **1** and **6–13** (table II). The cyclopentyl compound **14** was synthesised by a slightly different

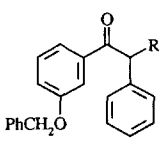


Scheme 1. Reagents: (a) NaH, DMF, benzyl bromide; (b) *Method A:* i) $ArCH_2Cl$, Mg, I_2, ether, reflux, ii) aq HCl; (c) *Method B: tert*-BuOK, THF, methyl acrylate; (d) *Method C:* 1.0 N NaOH, 1,4-dioxane; (e) *Method D:* $Br(CH_2)_nCO_2Me$, *tert*-BuOK, THF.



Scheme 2. Reagents: (a) H_2, 5% Pd/C, MeOAc, MeOH, conc HCl; (b) *Method E:* RCH_2Cl or RCH_2Br, NaH, DMF; (c) *Method C.*

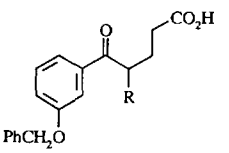
Table I. Physical properties and in vitro activity of side chain analogues.



Compound	*R*	*Formula*[a]	*Mp (°C)*	*ET_A IC_{50} (μM)*[b]
1	$CH_2CH_2CO_2H$	$C_{24}H_{22}O_4$	127–129	0.72, 0.78
5	$CH_2CH_2CO_2Me$	$C_{25}H_{24}O_4$	58–60	>10, > 10
15	$CH_2CH_2SO_2Me$	$C_{24}H_{24}O_4S$[d]	119–121	10.0, 10.0
16	CH_2CH_2CN	$C_{24}H_{21}NO_4$	[c]	>10, > 30
17	CH_2CO_2H	$C_{23}H_{20}O_4$	150–152	2.0, 3.0
18	$(CH_2)_3CO_2H$	$C_{25}H_{24}O_{24}$	107–109	3.0, 4.0
19	$(CH_2)_4CO_2H$	$C_{26}H_{26}O_4$	[c]	8.0, 10.0

[a]Satisfactory microanalyses were obtained (± 0.4%) for C, H, N unless otherwise stated; [b]inhibition of [^{125}I] ET-1 binding in vitro to rat A10 cell ET_A receptors (results of two experiments are shown); [c]oil; [d]C, H, N, S.

Table II. Physical properties and in vitro activity of compounds **6–14**.



Compound	*R*	*Formula*[a]	*Mp (°C)*	ET_A IC_{50} (μM)[b]
1	Ph	$C_{24}H_{22}O_4$	127–129	0.72, 0.78
6	4-(MeO)Ph	$C_{25}H_{24}O_5$	74	20.0, 22.0
7	4-(Cl)Ph	$C_{24}H_{21}ClO_4$	91	7.0, 8.0
8	4-(Me)Ph	$C_{25}H_{24}O_4$	105–106	7.0, 7.0
9	3,4-(Cl_2)Ph	$C_{24}H_{20}Cl_2O_4$:0.2 H_2O	87–88	6.0, 7.0
10	2-(Cl)Ph	$C_{24}H_{21}ClO_4$	131–132	0.20, 0.22
11	2-(Me)Ph	$C_{25}H_{24}O_4$	121–122	0.21, 0.26
12	2-(MeO)Ph	$C_{25}H_{24}O_5$	143–144	0.5, 0.65
13	2,6-(Cl_2)Ph	$C_{24}H_{20}Cl_2O_4$	113–114	0.39, 0.40
14	Cyclopentyl	$C_{23}H_{26}O_4$	65–67	15.0, 15.0

[a,b]See footnotes in table I.

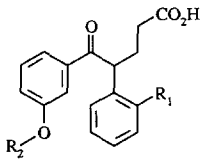
route from cyclopentylmethyl magnesium bromide. Tetra-*n*-butylammonium fluoride was used to effect the Michael addition to methyl acrylate, potassium *tert*-butoxide and other bases having failed to induce any reaction [9].

The Michael reaction *(Method B)* could be carried out with other acceptors such as methyl vinyl sulphone and acrylonitrile to provide the non-acidic analogues **15** and **16** (table I) respectively. The homologous ketoalkanoic acid derivatives **17–19** (table I) were obtained by alkylating the ketomethylene compound **4** with the appropriate bromoalkyl ester *(Method D)* followed by hydrolysis of the resultant intermediate **20**.

The synthetic procedure depicted in scheme 2 was used to routinely vary the nature of the 3-benzyloxy substituent. Catalytic hydrogenolysis of **21** in acidic medium effected debenzylation and gave rise to the phenol **22**. This intermediate could be deprotonated with sodium hydride and quenched with a variety of alkyl bromides or chloromethyl substituted aromatic and heterocyclic rings *(Method E)*. These alkylating agents were either obtained from commercial sources or prepared from available hydroxymethyl derivatives by literature procedures. Hydrolysis of the resulting esters **23** afforded the compounds **24–39** (table III). The 3-phenoxy analogue **40** was prepared from 3-phenoxybenzonitrile using the chemistry described in scheme 1.

Scheme 3 outlines some modifications to the carbonyl group in this series (table IV). The ketone in **21** could be reduced to a methylene group using the Huang–Minlon modification of the Wolff–Kishner reaction. This conversion proceeded with concomitant ester hydrolysis to give the target compound **41**. Standard Wittig chemistry could not be carried out on these 4,5-diaryl ketones owing to their ready enolisation. Conversion of the carbonyl oxygen of **5** to a methylene group was carried out, however, with the milder Tebbe reagent [10]; this allowed us to obtain the alkenyl analogue **42**. Borohydride reduction of **1** proceeded stereoselectively to give the expected *rel*-4*R*, 5*R* isomer **43** as the sole isolated product; the other possible stereoisomer was not detected. Conversion to the lactone **44** comfirmed the stereochemistry of reduction; the vicinal coupling constant of the two ring methine protons was 4 Hz, as predicted for a *cis* orientation. The amide **45** was prepared in a straightforward manner via condensation of the acid chloride of 3-benzyloxybenzoic acid and ethyl (2-phenylamino)-propanoate and alkaline hydrolysis of the resultant ester.

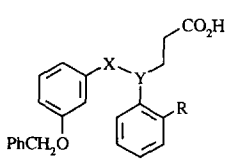
Table III. Physical properties and in vitro activity of compounds **24–40**.



Compound	R_1	R_2	*Formula*[a]	*Mp (°C)*	ET_A IC_{50} (μM)[b]
11	CH_3	$PhCH_2$	$C_{25}H_{24}O_4$	121–122	0.21, 0.26
24	CH_3	$PhCH_2CH_2$	$C_{26}H_{26}O_4$	74–76	7.0, 8.0
25	CH_3	(Cyclopent)CH_2	$C_{24}H_{28}O_4$	104–105	4.0, 5.0
26	CH_3	4-(MeO)$PhCH_2$	$C_{26}H_{26}O_5$	132–133	10.0, 10.0
27	CH_3	4-(Cl)$PhCH_2$	$C_{25}H_{23}ClO_4$	131–133	6.0, 8.0
28	CH_3	3-(MeO)$PhCH_2$	$C_{26}H_{26}O_5$	72–74	0.9, 1.0
29	CH_3	3-(Cl)$PhCH_2$	$C_{25}H_{23}ClO_4$	103–105	0.45, 0.52
30	CH_3	3-(F)$PhCH_2$	$C_{25}H_{23}FO_4$	106–108	0.37, 0.38
31	H	2-(MeO)$PhCH_2$	$C_{25}CH_{24}O_5$	111–113	> 30, > 30
32	CH_3	(2-Pyridyl)CH_2	$C_{24}H_{23}NO_4$	152–154	6.0, 8.0
33	CH_3	(3-Pyridyl)CH_2	$C_{24}H_{23}NO_4$:0.3 H_2O	[d]	0.12, 0.14
34	CH_3	(4-Pyridyl)CH_2	$C_{24}H_{23}NO_4$	124–125	0.67, 0.72
35	CH_3	(4-Pyridazinyl)CH_2	$C_{23}H_{22}N_2O_4$:0.2 H_2O[e]	72–74	1.0, 1.0
36	CH_3	(2-Thienyl)CH_2	$C_{23}H_{22}O_4S$	96–98	0.23, 0.25
37	CH_3	(3-Thienyl)CH_2	$C_{23}H_{22}O_4S$:H_2O	94–98	0.09, 0.10
38	CH_3	(2-Furanyl)CH_2	$C_{23}H_{22}O_5$	92–94	0.65, 0.79
39	CH_3	(3-Furany)CH_2	$C_{23}H_{22}O_5$	83–85	0.56, 0.60
40	H	Ph	$C_{23}H_{20}O_4$	72–74	12.0, 15.0

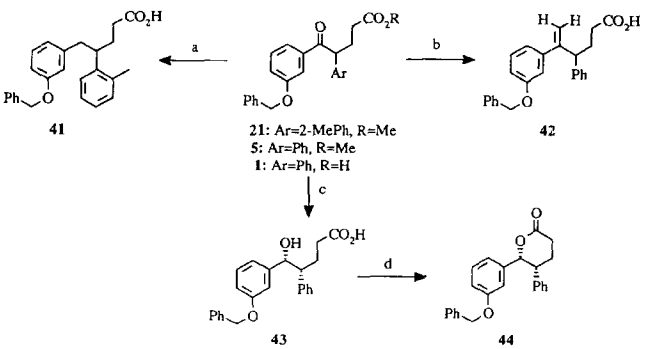
[a,b]See footnotes in table I; [c]C: calc 74.2%; found 73.1%; [d]oil; [e]N: calc 7.17%; found 6.56%.

Table IV. Physical properties and in vitro activity of compounds **41–43** and **45**.



Compound	*R*	*X–Y*	*Formula*[a]	*Mp (°C)*	ET_A IC_{50} (μM)[b]
41	CH_3	CH_2–CH	$C_{25}H_{26}O_3$	108–110	6.0, 6.0
42	H	C(=CH_2)–CH	$C_{25}H_{26}O_3$:0.3 H_2O	93–96	6.0, 8.0
43	H	(*R*, *R*)–CH(OH)–CH	$C_{24}H_{24}O_4$	[c]	> 30, > 30
45	H	CO–NH	$C_{23}H_{21}NO_4$	96–98	8.0, 10.0

[a,b,c]See footnotes in table I.



Scheme 3. Reagents: (a) **21** $[HO(CH_2)_2]_2O$, hydrazine hydrate, KOH, 180 °C; (b) **5** (i) Tebbe reagent, toluene, THF, 0 °C (ii) *Method C*; (c) **1**, $NaBH_4$, EtOH, 5 °C; (d) AcCl, Et_3N, CH_2Cl_2.

Results and discussion

All test compounds were assayed in vitro for their ability to antagonise the binding of [^{125}I]ET-1 to rat aortic A10 cell membrane ET_A receptors. The results are shown in tables I–IV. The 5-ketopentanoic acid analogues described in this paper were screened against a rat cerebellum ET_B preparation, and in all cases caused < 50% inhibition of the binding of [^{125}I]ET-1 at a concentration of 30 μM (data not shown).

The length of the alkyl chain bearing the carboxylic acid was systematically varied (compounds **17–19**; table I) and there appeared to be some tolerance; shortening and lengthening the chain by one to two atoms produced compounds of similar potency. We were interested to see whether a carboxylic acid was important for binding, or if this group could be replaced with a non acidic H-bond acceptor. The methyl ester **5**, the sulphone **15** and the nitrile **16** were prepared, and all were found to be approximately 10-fold less potent than **9** (table I). This is perhaps not surprising, as an anionic centre appears to be a critical pharmacophoric element in all reported endothelin antagonists [4, 5].

In an attempt to improve the binding affinity of **1**, a Topliss 'batchwise' analysis [11] was initially applied to the 4-phenyl ring. In this QSAR method, aromatic substituents are grouped according to π, σ and π^2 parameters and an initial set of five derivatives are synthesised: 4H, 4-OMe, 4-Cl, 4-CH_3, and 3,4-dichloro. These are then ranked in order of decreasing potency in a bioassay. The results of this approach are shown in table II (compounds **1**, **6–9**) and indicate that no QSAR parameter is dominant, but that there is clearly an unfavourable steric effect from 4-substitution. A moderate *ortho* effect was observed, however, when small substituents were introduced into the 2-position. Thus the 2-chloro and 2-methyl derivatives, **10** and **11** respectively were approximately three-fold more potent than **1** with a 2-methoxy analogue **12** exhibiting an IC_{50} of 0.65 μM, a level equipotent with **1**. We hypothesised that the *ortho* substituent was exerting its beneficial effect by restricting the rotation of the 4-phenyl ring and locking it in a bioactive conformation. Introduction of a second *ortho* atom as in the 2,6-dichloro derivative **13** did not lead to a further improvement in binding potency. The IC_{50} for the cyclopentyl analogue **14** was greater than 10 μM, implying that the interaction of the 4-phenyl ring with the receptor is aromatic in nature and not merely hydrophobic. A cyclopentyl group was felt to most closely resemble a phenyl ring in shape. We were disappointed that a simple aromatic substitution strategy did not furnish the dramatic increases in receptor binding activity observed by other groups with their series of antagonists.

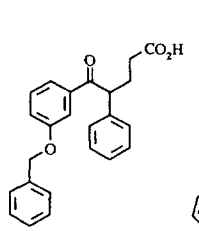
The compounds **24–40** shown in table III were synthesised to explore and optimise the interaction of the 3-benzyloxy group with the receptor. Again this approach was unsuccessful, with the original benzyloxy substituent giving rise to the best receptor binding. A methyloxy linker was optimum, as demonstrated by the weak binding of the phenethyl and phenoxy analogues **24** and **40** respectively. The poor receptor binding potency of the cyclopentyl analogue **25** indicated that the interaction at this position was aromatic in nature rather than just lipophilic. The testing of analogues **26** and **27** revealed that substitution at the 4-position of the phenyl ring was deleterious to binding, leading to greater than 20-fold drops in potency when compared to **11**. Halo groups appeared to be tolerated at the 3-position (compounds **29** and **30**) but noteworthy is the dramatic loss of binding potency on introduction of a 2-substituent as in **31**.

Since the 3-benzyloxy position appeared to be sterically demanding, we looked at replacement of the phenyl ring with a variety of 5- and 6-ring heterocycles (compounds **32–39**). Some modest improvements in receptor binding were achieved as a result of this strategy, with IC_{50} values of 0.14 and 0.09 μM being measured for the 3-pyridyl **33** and 3-thienyl **37** analogues respectively. The fact that polar heterocyclic rings were tolerated at this position meant that the aqueous solubility of this series of antagonists could be improved. For example, the solubilities of **11** and the 3-pyridyl analogue **33** in pH 7.4 buffer were found to be 0.16 g/L and 2.25 g/L respectively.

The final SAR study on the lead molecule **1** involved examining the role of the carbonyl group in

receptor binding. It is clear from the compounds described in table IV that the carbonyl group, in addition to any binding contribution, has an important effect in controlling the bioactive conformation of the molecule. Thus reduction of the carbonyl group and replacement of the carbonyl oxygen atom with the larger CH_2, as in compounds **41–43**, leads to a significant drop in binding activity. Analogue **45** in which the ketomethine group has been changed to an amide bond still retains some weak activity. The conformational consequence of this modification is to place the 4- and 5-phenyl rings in a *cis* geometry (the expected *cis*oid conformation of the two phenyl rings in **45** was confirmed by X-ray crystallography). A likely receptor bound conformation of this series of antagonists then requires the 5-keto group to be in conjugation to the phenyl ring and the 4- and 5-phenyl rings to be in a close to *cis*oid arrangement.

It was essential to determine whether this series of compounds could antagonize a biological effect of ET-1 in an ET_A receptor mediated process. As the



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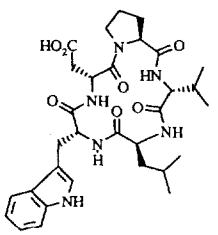

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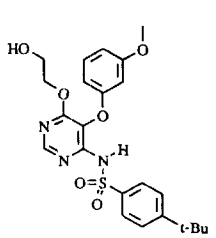

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ET-1 induced contraction of isolated endothelium denuded rat aortic rings is mediated by ET_A receptors [12], the in vitro functional activity of the most potent analogue **37** was determined in this system. For purposes of comparison, the ET_A selective antagonist BQ 123 (**46**) [13] and the mixed antagonist Ro 46-2005 (**47**) [12] were also examined. The results are summarised in table V. Compound **37** showed no agonist activity, but antagonized the ET-1 induced contractions in a concentration-dependent manner with a pK_B of 5.7. The concentration–response curves of ET-1 were shifted to the right in a parallel fashion by increasing concentrations of **37** with no significant reduction in the maximal response. BQ 123 possessed a pK_B of 6.8 and Ro 46-2005 was equipotent with **37**, having a pK_B of 5.7.

Table V. In vitro functional activity of compounds **37**, **46** and **47**.

Compound	*IC_{50} (nM)*[a]		*pK_B against ET-1*
	ET_A[b]	*ET_B*[c]	*Rat aorta*[d]
46 BQ 123	8.0, 12	> 30000	6.8
47 Ro 46-2005	300, 330	30	5.7
37	90, 100	> 30000	5.7

[a]Inhibition of [^{125}I] ET-1 binding in vitro; [b]rat A10 aortic cell receptors (two experiments); [c]rat cerebellum receptors (single experiment); [d]isolated deendothelialised rat aortic rings (single experiment).

All the ketopentanoic acid analogues described in this paper were prepared and assayed as racemates. We decided to look at the separation and chiral stability of the enantiomers of the thiophene **37**, our most potent analogue. The use of HPLC on a chiral stationary phase allowed both enantiomers of **37** to be obtained in greater than 98% ee (the enantiomers of **37** were separated on a Chiralcel OD column eluting with methanol / isopropyl alcohol / acetic acid / hexane (4:1:1:100). Typical retention times under these conditions were $t(1)$ = 26.5 min and $t(2)$ = 33.5 min). Stability studies were carried out at 50 °C under a range of pH values, the racemisation being followed by analytical chiral HPLC. At a pH of greater than 8.3, racemisation of the enantiomers was complete and rapid within 4 h. In a medium of aqueous phosphate buffer (pH 7.4), 50% racemisation occurred after 0.5 h. The enantiomers appeared to be stable to acidic conditions. It appeared unlikely then that a homochiral form of these compounds would exhibit significant chiral stability under physiological conditions.

Conclusion

Optimisation of the screening lead **1** on ET_A receptor binding allowed us to obtain more potent antagonists such as **37** (IC_{50} 90 nM). This compound showed modest functional activity (pK_B 5.7) and > 300-fold selectivity for the ET_A receptor over the ET_B receptor. The ready racemisation of the α-keto chiral centre meant that we did not attempt to extend this particular series of compounds further. However, the extensive SAR studies described above and in the previous paper [7] have helped us to design a further novel series of potent ET_A selective antagonists which demonstrate oral bioavailability and in vivo activity. Such compounds, which will be the subject of future communications from this laboratory, will be of use in understanding the biological role of the ET_A receptor and the therapeutic uses of a selective antagonist.

Experimental protocols

Pharmacology

In vitro radioligand binding assays

These were carried out as previously described [7].

In vitro functional assay
This was carried out as previously described [7].

Chemistry

Reagents, starting materials and solvents were purchased from commercial suppliers and used as received, or distilled from the appropriate drying agent. All organic solutions were dried over magnesium sulphate and concentrated at reduced pressure under aspirator vacuum using a Buchi rotary evaporator. Reaction products were purified when necessary by flash chromatography on silica gel (40–63 μm) eluting with the solvent system indicated. Yields were not optimised. Melting points were determined on a Gallenkamp 595 apparatus and were uncorrected. ^{1}H-NMR spectra were acquired on a Varian VXR-400 spectrometer; peak positions were reported in parts per million (ppm) relative to internal tetramethylsilane on the δ scale. Elemental analyses were performed by the analytical department, Rhône-Poulenc Rorer. The structure and purity of all compounds were confirmed by microanalytical and/or spectroscopic methods. Satisfactory microanalyses (± 0.4%) were obtained for C, H, N unless otherwise stated.

3-Benzyloxybenzonitrile ***3***. A stirred solution of 3-cyanophenol (**2**) (20.0 g, 168 mmol) in dry dimethylformamide (200 mL) was treated with sodium hydride (5.6 g of a 60% suspension w/w, 140 mmol) portionwise over 40 min. Benzyl bromide (20.0 mL, 168 mmol) was added dropwise and the reaction mixture heated to 90 °C for 4 h. After this time, the reaction mixture was concentrated to dryness and the residue partitioned between ethyl acetate (400 mL) and water (400 mL). The organic layer was washed with 100 mL portions of 1.0 M aqueous sodium hydroxide solution and water before being dried and concentrated. The residue was recrystallised from methanol to leave **3** as a cream-coloured solid (21.0 g, 68%); mp = 38–40 °C (lit mp = 39–40 °C).

Method A: 1-(3-benzyloxyphenyl)-2-phenyl-ethanone ***4***. A stirred suspension of magnesium turnings (9.0 g, 321 mmol) in dry diethyl ether (100 mL) was treated with iodine (0.6 g) followed by dropwise addition of benzyl chloride (22.8 g, 180 mmol) in ether (150 mL) at such a rate as to maintain a gentle reflux. When the addition was complete, 3-benzyloxybenzonitrile (**3**) (12.7 g, 61 mmol) was added in one portion and the reaction mixture heated to reflux for 5 h. The reaction mixture was cooled, acidified to pH 1.0 with 10% aqueous hydrochloric acid and stirred vigorously for 4 h at room temperature. The ethereal layer was separated and the aqueous phase extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with water (3 x 100 mL), dried and concentrated. The residue was recrystallised from methanol to leave **4** in the form of a white solid (14.8 g, 82%); mp = 70–72 °C. ^{1}H-NMR ($CDCl_3$) 4.26 (s, 2H), 5.20 (s, 2H), 7.15–7.62 (m, 14H). Anal $C_{21}H_{18}O_2$ (C, H).

Method B: (±)-methyl 5-(3-benzyloxyphenyl)-5-oxo-4-phenylpentanoate ***5***. A solution of the ketone **4** (10.0 g, 33 mmol) in dry tetrahydrofuran (100 mL) was cooled in an ice bath before being treated with potassium *tert*-butoxide (370 mg, 3.3 mmol) and methyl acrylate (2.9 g, 26.0 mmol). The reaction mixture was stirred at room temperature for 18 h and then concentrated to dryness. The residue was partitioned between ethyl acetate (200 mL) and water (200 mL). The organic layer was then washed with water (100 mL), dried and concentrated to leave **5** as a colourless solid (11.6 g, 91%); mp = 58–60 °C. ^{1}H-NMR ($CDCl_3$) 2.16 (m, 1H), 2.30 (t, J = 7 Hz, 2H), 2.45 (m, 1H), 3.62 (s, 3H), 4.61 (t, J = 7 Hz, 1H), 5.03 (s, 2H), 7.08–7.55 (m, 14H). Anal $C_{25}H_{24}O_4$ (C, H).

Method C: (±)-5-(3-benzyloxyphenyl)-5-oxo-4-phenylpentanoic acid ***1***. A mixture of the ester **5** (11.0 g, 28 mmol), 1.0 N sodium hydroxide (60 mL) and 1,4-dioxane (200 mL) was stirred at room temperature for 18 h and then concentrated to low volume. The residue was taken up in water (50 mL), cooled in an ice bath and acidified to pH 1.0 with concentrated hydrochloric acid. The resulting mixture was extracted with dichloromethane (200 mL) and the organic layer dried and concentrated to leave an oil. This was dissolved in a minimum of diethyl ether, and acid **1** was precipitated as a white solid (5.1 g, 48%) by addition of pentane; mp = 127–129 °C. ^{1}H-NMR ($CDCl_3$) 2.18 (m, 1H), 2.34 (m, 2H), 2.45 (m, 1H), 4.62 (t, J = 8 Hz, 1H), 5.10 (s, 2H), 7.10–7.55 (m, 14H). Anal $C_{24}H_{22}O_4$ (C, H).

Method D: Methyl (±)-4-(3-benzyloxyphenyl)-4-oxo-3-phenylbutanoate ***20*** *(n = 1).* A solution of the ketone **4** (3.02 g, 10 mmol) in dry tetrahydrofuran (80 mL) was cooled in an ice bath and treated portionwise with potassium *tert*-butoxide (1.12 g, 10 mmol) over 10 min. A solution of methyl bromoacetate (1.04 g, 11 mmol) in tetrahydrofuran (10 mL) was added dropwise and the reaction mixture stirred with warming to room temperature over 18 h. The reaction was concentrated to dryness and partitioned between ethyl acetate (60 mL) and water (60 mL). The organic layer was dried, concentrated and the residue recrystallised from ethyl acetate/pentane to afford **20** (n = 1) as a white solid (2.1 g, 56%); mp = 88–89 °C. ^{1}H-NMR (DMSO) 2.71 (dd, J = 16.4 Hz, 1H), 3.21 (dd, J = 16.8 Hz, 1H), 3.56 (s, 3H), 5.12 (m, 2H), 5.24 (dd, J = 8.4 Hz, 1H), 7.20–7.65 (m, 14H).

(±)-Methyl 5-(3-hydroxyphenyl)-4-(2-methylphenyl)-5-oxopentanoate ***22***. A mixture of **21** (11.4 g, 28.3 mmol), 5% palladium on carbon (0.55 g), concentrated hydrochloric acid (11 mL), methyl acetate (110 mL) and methanol (110 mL) was stirred under an atmosphere of hydrogen for 6 h. After this time, the catalyst was removed by filtration and the filtrate concentrated. The residue was boiled in hexane, cooled and filtered to leave **22** as a white solid (6.3 g, 71%); mp = 102–104 °C. ^{1}H-NMR ($CDCl_3$) 2.02 (m, 1H), 2.40 (m, 3H), 2.50 (s, 3H), 3.66 (s, 3H), 4.81 (m, 1H), 5.82 (br s, 1H), 6.95–7.40 (m, 13H).

Method E: (±)-methyl 4-(2-methylphenyl)-5-oxo-5-[3-(2-phenethyloxy)phenyl]pentanoate ***23****; R = 2-phenethyl.* A solution of **22** (1.0 g, 3.2 mmol) in dry dimethylformamide (10 mL) was treated in one portion with sodium hydride (0.14 g of a 60% suspension w/w, 3.52 mmol) and the reaction mixture stirred for 30 min at room temperature. 2-Bromoethylbenzene (0.6 g, 3.3 mmol) was added and the reaction mixture stirred for a further 18 h. The reaction mixture was concentrated to dryness and partitioned between ethyl acetate (50 mL) and 1.0 M sodium hydroxide (50 mL). The organic layer was dried, concentrated and the residue chromatographed on silica gel (pentane/ethyl acetate, 9:1) to give **23** (R = 2-phenethyl) as a colourless oil (0.2 g, 31%). ^{1}H-NMR ($CDCl_3$) 2.02 (m, 1H), 2.40 (m, 3H), 2.50 (s, 3H), 3.06 (t, J = 7 Hz, 2H), 4.16 (m, 2H), 4.78 (m, 1H), 6.98–7.38 (m, 13H).

(±)-5-(3-Benzyloxyphenyl)-5-oxo-4-cyclopentylpentanoic acid ***14***. A mixture of magnesium turnings (0.6 g, 24.6 mmol), 10 mL diethyl ether and a crystal of iodine was stirred at room temperature and treated with a few drops of neat bromomethylcyclopentane. When the reaction mixture had begun to reflux, a solution of bromomethylcyclopentane (2.0 g, 12.3 mmol) in 30 mL ether was added dropwise at a rate sufficient to maintain a gentle reflux. When the reflux had subsided, the 3-benzyloxy-

benzonitrile (**3**) (1.28 g, 6.1 mmol) was introduced in one portion and the reaction mixture heated at reflux for 5 h. The reaction was then poured onto a stirred slurry of 50 g ice and 10 mL concentrated hydrochloric acid, and stirring continued for 2 h. The mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organics dried and concentrated. The residue was chromatographed on silica gel (pentane/ethyl acetate, 9:1) to leave 1-(3-benzyloxyphenyl)-2-cyclopentyl-ethanone as a yellow oil (1.0 g, 56%). ^{1}H-NMR ($CDCl_3$) 1.0–1.90 (m, 8H), 2.38 (m, 1H), 2.97 (d, J = 7 Hz, 2H), 5.11 (s, 2H), 7.16–7.55 (m, 9H).

A mixture of 1-(3-benzyloxyphenyl)-2-cyclopentyl-ethanone (1.0 g, 3.4 mmol), tetra-*n*-butyl ammonium fluoride (1.0 mL of a 1.0 M solution in THF, 1.0 mmol) methyl acrylate (0.37 mL, 4.2 mmol) and 30 mL of tetrahydrofuran was stirred for 18 h at room temperature before being concentrated to dryness and partitioned between ethyl acetate (100 mL) and water (50 mL). The organic layer was dried and concentrated, and the residue purified by chromatography on silica gel (pentane/ethyl acetate, 19:1) to leave a yellow oil (0.5 g, 39%). This material was hydrolysed according to method C to leave **14** as a cream-coloured solid (0.28 g, 58%) after trituration with pentane; mp = 65–67 °C. Anal $C_{23}H_{26}O_4$ (C, H).

(±)-5-(3-Benzyloxyphenyl)-4-(2-methylphenyl)-pentanoic acid ***41***. A mixture of **21** (3.5 g, 8.9 mmol) hydrazine hydrate (2.0 mL), diethylene glycol (75 mL) and potassium hydroxide (4.1 g, 73 mmol) was heated at 185–190 °C for 3 h. The reaction mixture was cooled, poured into 400 mL water, acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate (150 mL). The organic extract was washed with water (100 mL), dried and concentrated. The residue was recrystallised from acetonitrile to leave **41** (0.75 g, 23%) as a white solid; mp = 108–110 °C. ^{1}H-NMR ($CDCl_3$) 1.90–2.12 (m, 4H), 2.08 (s, 3H), 2.80 (m, 2H), 3.15 (m, 1H), 4.90 (m, 2H), 6.60–7.38 (m, 13H). Anal $C_{25}H_{26}O_3$ (C, H).

(±)-5-(3-Benzyloxyphenyl)-4-phenyl-5-hexenoic acid ***42***. A solution of **5** (1.0 g, 2.5 mmol) in tetrahydrofuran (5 mL) was cooled to 0 °C under nitrogen, and treated with dropwise with a solution of Tebbe reagent in toluene (0.5 M, 6.0 mL, 3 mmol). After 30 min at 0 °C, the reaction mixture was warmed to room temperature and stirred a further 1 h before being quenched by the addition of 15% potassium hydroxide in methanol (2 mL). The reaction mixture was poured into ethyl acetate (100 mL), filtered through a short pad of diatomaceous earth and concentrated. The residue was chromatographed on silica gel (dichloromethane) to leave a pale yellow oil (0.7 g, 72%). This material was hydrolysed according to *Method C* to leave **42** as pale yellow solid (0.33 g, 49%) after chromatography on silica gel (dichloromethane/methanol, 19:1); mp = 93–96 °C. ^{1}H-NMR ($CDCl_3$) 2.10 (m, 1H), 2.25 (m, 1H), 2.32 (m, 2H), 3.78 (m, 1H), 4.98 (s, 2H), 5.20 (s, 1H), 5.41 (s, 1H), 6.80–7.40 (m, 14H). Anal $C_{25}H_{24}O_3$:0.3 H_2O (C, H).

(±)-(4R, 5R)-5-(3-Benzyloxyphenyl)-5-hydroxy-4-phenyl-pentanoic acid ***43***. A solution of **1** (1.0 g, 2.67 mmol) in ethanol (30 mL) was treated portionwise with sodium borohydride (0.22 g, 5.8 mmol) and the reaction mixture stirred at room temperature for 4 h. The reaction mixture was concentrated, taken up in ethyl acetate (200 mL) and washed with 50 mL portions of 2.0 M hydrochloric acid and water. The organic layer was dried and concentrated. The residue was chromatographed on silica gel (ethyl acetate/pentane, 3:1) to leave **43** as a colourless oil (0.4 g, 40%). ^{1}H-NMR ($CDCl_3$) 1.75 (m, 1H), 1.85 (m, 1H), 2.03 (m, 2H), 2.85 (m, 1H), 4.70 (d, J = 8 Hz, 1H), 5.02 (m, 2H), 6.90–7.42 (m, 14H). Anal $C_{24}H_{24}O_4$ (C, H).

(±)-(rel-5R, 6R)-6-(3-Benzyloxyphenyl)-5-phenyl-tetrahydropyran-2-one ***44***. A mixture of **43** (1.0 g, 2.7 mmol), triethylamine (0.4 mL, 2.9 mmol) and dichloromethane (40 mL) was cooled to 5 °C and treated dropwise with acetyl chloride (0.2 mL). The reaction mixture was stirred at 5 °C for 45 min and washed with water (4 x 50 mL). The organic layer was dried, concentrated and the residue recrystallised from methanol to leave **44** as white solid (0.43 g, 43%); mp = 96–98 °C. ^{1}H-NMR (DMSO) 2.00 (m, 1H), 2.28 (m, 1H), 2.68 (m, 1H), 3.42 (m, 1H), 3.68 (m, 1H), 4.89 (s, 2H), 5.83 (d, J = 4 Hz, 1H), 6.48–7.35 (m, 14H). Anal $C_{24}H_{22}O_3$ (C, H).

N-(3-Benzyloxybenzoyl)-N-phenyl-3-aminopropanoic acid ***45***. A solution of 3-benzyloxybenzoic acid (5.0 g, 22 mmol) in dichloromethane (50 mL) was treated with a solution of oxalyl chloride in dichloromethane (2.0 M, 16 mL, 32 mmol) and the reaction mixture stirred at room temperature for 2 h. The reaction mixture was concentrated and the residue taken up in dichloromethane (20 mL). This solution was added to a stirred mixture of ethyl 3-phenylaminopropanoate (6.3 g, 33 mmol), triethylamine (15 mL, 108 mmol) and dichloromethane (100 mL). The reaction mixture was stirred at room temperature for 6 h and washed with 2.0 M hydrochloric acid (100 mL). The organic layer was dried and concentrated and the residue chromatographed on silica gel (pentane/ethyl acetate, 2:1) to leave a colourless oil (6.0 g, 68%); ^{1}H-NMR (DMSO) 1.12 (t, J = 8 Hz, 3H), 2.58 (t, J = 7 Hz, 2H), 3.98 (q, J = 8 Hz, 2H), 4.08 (t, J = 7 Hz, 2H), 4.95 (s, 2H), 6.80–7.38 (m, 14H).

A mixture of the above ester (3.0 g, 7.4 mmol) and 15% methanolic potassium hydroxide (50 mL) was heated at reflux for 1 h before being cooled and concentrated. The residue was taken up in water (50 mL) and acidified to pH 1.0 with concentrated hydrochloric acid. The mixture was extracted with ethyl acetate (100 mL) and the organic layer dried and concentrated. The residue was dissolved in diethyl ether (10 mL), from which **45** crystallised as colourless solid (1.08 g, 38%); mp = 96–98 °C. Anal $C_{23}H_{23}NO_4$ (C, H, N).

Acknowledgments

We thank I Ahmed, A Al-Shaar, J Archer, D Chatterjee, B Pedgrift, R Petheram, C Handscombe, P Lockey, A Stoppard, and M Wong for expert technical assistance; M Podmore and M Vine for spectroscopy, A Stevens for microanalysis, B Slater for solubility measurements and R Mackenzie for chiral HPLC studies.

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