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Selective endothelin A receptor ligands. 1. Discovery and structure–activity of 2,4-disubstituted benzoic acid derivatives

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Summary — This paper describes the discovery of a new non-peptide endothelin A (ET_A) selective ligand, 2,4-dibenzyloxybenzoic acid **3**, which inhibits the binding of [^{125}I]ET-1 to ET_A receptors with an IC_{50} of 9 μM (ET-1 = endothelin-1). Optimisation of **3** resulted in compound **52** which had an IC_{50} of 1 μM. One of the analogues of **3**, compound **15**, was examined in a functional assay and shown to antagonise ET-1-induced contraction of rat aorta. The identification of **3** was made through the application of ChemDBS-3D searching of our corporate database. The 3D query, using an aromatic ring to a carboxylic acid group separated by 10.2 ± 1.1 Å, was derived from an examination of common pharmacophoric distances found in the low energy conformations of two known ET_A antagonists, the cyclic pentapeptide BQ 123 **1** and myriceron caffeoyl ester **2**.

endothelin receptor ligand / benzoic acid

Introduction

The endothelins are a family of closely related peptides whose biological actions are mediated, in mammals, by at least two subtypes of receptor designated ET_A and ET_B [1]. Endothelin-1 (ET-1) is a 21 amino-acid peptide with potent vasoconstrictor properties that was originally identified in conditioned medium from cultured bovine endothelial cells [2]. However its distribution has been found to extend beyond the endothelium to many cell types of animals and humans including smooth muscle, macrophages, glial cells and mesangial cells [3]. Similarly both ET_A and ET_B receptor subtypes are widely distributed in tissues [4]. In the vasculature ET_A receptors were first identified on smooth muscle and were recognised to mediate the ET-1-induced vasoconstrictor actions whereas ET_B receptors were found on endothelial cells and were responsible for the ET-1-induced vasodilatation. Subsequently, it was recognised that ET_B receptors were also distributed to smooth muscle and were able to mediate contractions [5]. The relative importance of the ET_A and ET_B vasoconstrictor receptors varies between tissue and species [6].

Evidence of a causal role for endothelin in disease states was provided with the availability of peptide antagonists of the endothelin receptors including the ETA receptor antagonists BQ 123 and FR 139317 [7], the ETB antagonist BQ 788 [8] and the ET_A/ET_B mixed antagonists PD 142893 and PD 145065 [9]. Since the start of this work, a number of non-peptide, ET_A-selective (including BMS 182874 [10] and PD 156707 [11]) and mixed ET_A/ET_B antagonists (including Ro 47-0203 [12] and SB 209670 [13]) have been described. The availability of these compounds has further reinforced both the potential role endothelin plays in a number of disease models, especially congestive heart failure, ischaemic myocardial injury, subarrachnoid haemorrhage-induced vasospasm, renal ischaemia, and the consequent therapeutic potential of endothelin receptor antagonists in man [9].

Herein we describe the discovery of 2,4-dibenzyloxy benzoic acid as a selective ET_A receptor ligand and structure–activity relationships leading to some small improvements in binding to the ET_A receptor.

Lead generation

Our lead compound **3** was discovered using 3D searching of our UK corporate databases (60 000 compounds). In order to apply this technique, a pharmacophore query suitable for the ET_A receptor has to be generated. Such a query is best derived from small rigid ligands. At the start of our work, the only

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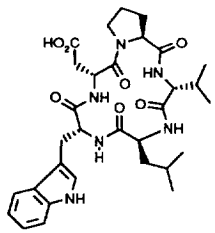
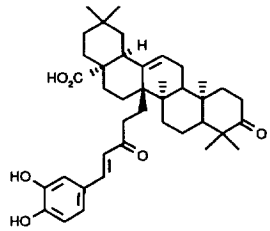
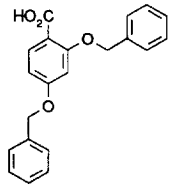


BQ 123 **1**



Myriceron caffeoyl ester **2**



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conformationally restricted ligand available was the cyclic pentapeptide BQ 123 **1** [15], which shows rigidity in the backbone but demonstrates considerable conformational freedom about the side chains [16]. Notwithstanding, we initiated our modelling work by recreating the aqueous solution backbone conformation previously described for BQ 123 following NMR and CD studies [16]. However, the pharmacophore of BQ 123 is almost certainly formed by the flexible side chains hanging from this backbone and not the backbone itself, and the positions of these side chains had not been determined. At about this time, Shionogi reported the discovery of a potent ET_A-selective antagonist: myriceron caffeoyl ester **2** [17]. We noted that **2** and BQ 123 **1** possess an acid group and an aromatic ring, and it was proposed that the combination of these groups represent at least a portion of a common pharmacophore. To use this hypothesis for 3D searching, the pharmacophoric distance range between these two groups had to be defined. The equivalent calculation on BQ 123 alone gave a range of 3–13 Å. Therefore, conformational analysis was carried out on the two compounds within the Chem-X modelling package [18] and the two sets of conformations were compared to identify common aromatic to acid group distances. This was carried out using logical combination of conformational plots, a facility within Chem-X. The range 9.1–11.3 Å was identified as the only common distance thus enabling a two-point pharmacophore, acid group to aromatic ring separated by 10.2 ± 1.1 Å, to be defined. In addition, pairs of conformers (BQ 123 and **2**), showing the common aromatic to acid distances, were considered in superimposition studies. It was found that an excellent superimposition could be achieved, from which a possible third pharmacophore point was identified: a hydrophobic region formed by either the valine residue of BQ 123 or one of the *gem* dimethyl groups of the Shionogi terpenoid (fig 1). Unfortunately, at that time 3D searching tools were not capable of identifying hydrophobic groups and searches were therefore carried out using the simple two-point pharmacophore only. All searches were carried out using the ChemDBS-3D [18] system which, as well as considering 3D geometry, also allows full searching of conformational space. A set of 383 compounds, from the corporate database of 60 000, were identified as fitting the two-point query and were screened for activity in the ET_A binding assay. The lead structure **3** (IC_{50} 9 μM) was identified within the first 50 compounds tested. A superimposition of this lead with the BQ 123 conformation used to generate the query is shown in figure 2. The aromatic ring centroid to acid group oxygen atom for this conformation is 10.6 Å.

Chemistry

The preparation of 2,4-dibenzyloxybenzoic acid **3** and its analogues described in this paper is illustrated in schemes 1–5. The physical properties of all compounds for which biological data is available are pre-

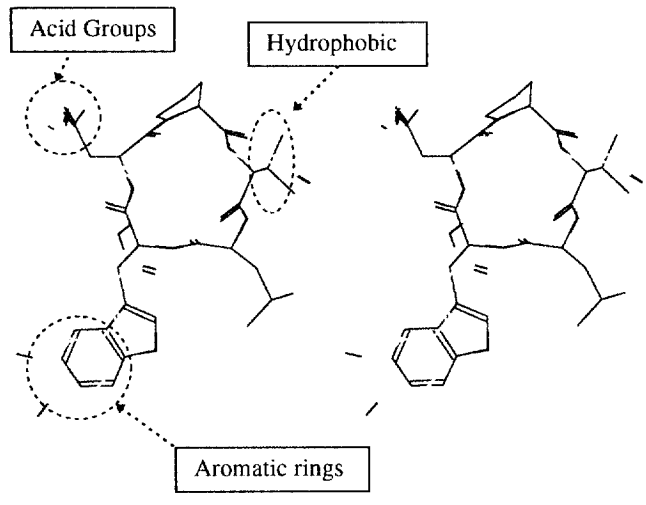


Fig 1. Stereoview of the superimposition of myriceron caffeoyl ester **2** (in yellow) on BQ 123 **1** (in green).

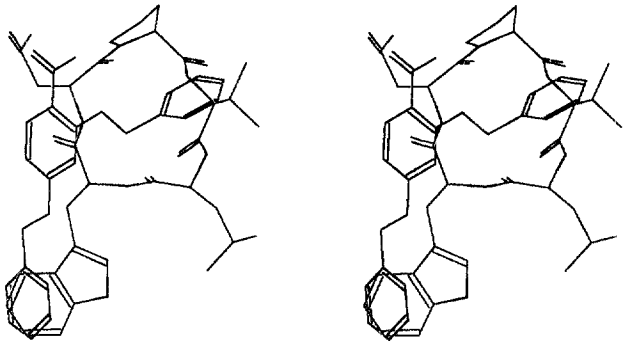
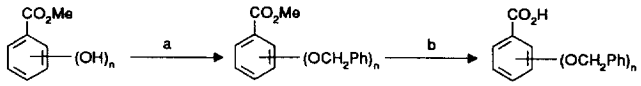
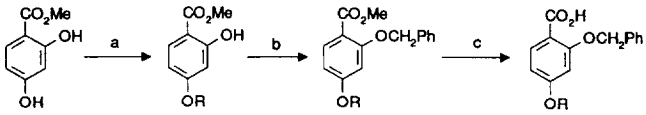


Fig 2. Stereoview of the superimposition of the lead structure **3** (in purple) on BQ 123 **1** (in green).



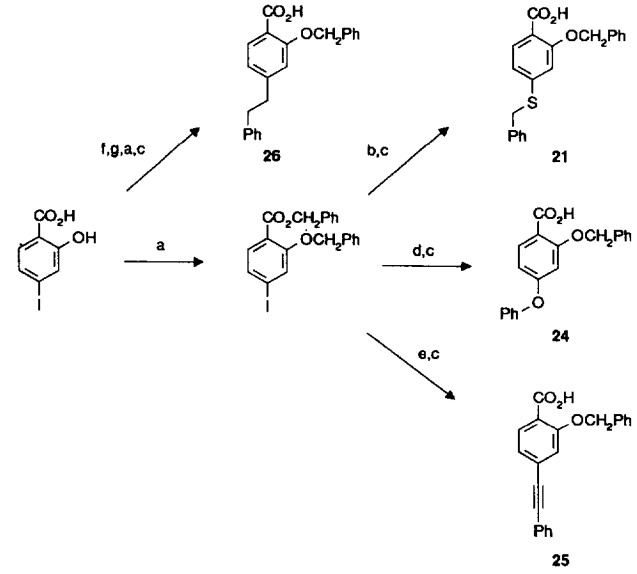
Scheme 1. Preparation of benzyloxy and dibenzyloxybenzoic acids. (Method A). Reagents: (a) $PhCH_2Br$, NaH, DMF, 100 °C; (b) 1 M NaOH, dioxan, reflux.



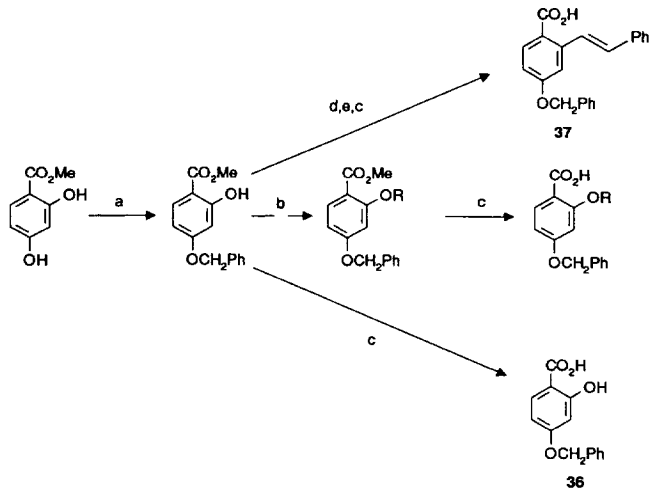
Scheme 2. Preparation of 4-heteroarylalkoxy- and 4-arylalkoxy-2-benzyloxybenzoic acid derivatives. (Method B). Reagents: (a) RX, $Bu_4N^+Cl^-$, K_2CO_3, KI, acetone, reflux; (b) NaH/DMF, then $PhCH_2Cl$, 60 °C; (c) 1 M NaOH, dioxan, reflux.

sented in tables I–IV. The monobenzyloxybenzoic acids **4** and **5** and the dibenzyloxybenzoic acids **3** and **6–9** in table I were prepared by benzylation of the appropriate methyl hydroxybenzoates followed by alkaline hydrolysis of the intermediate ester (scheme 1).

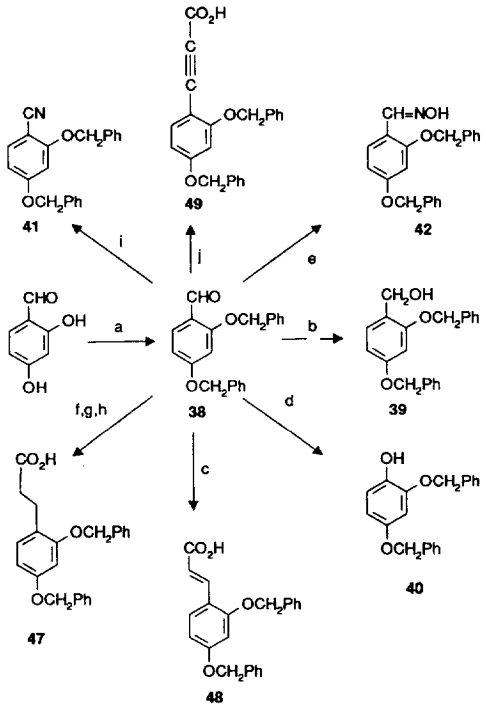
The synthesis of analogues of 2,4-dibenzyloxybenzoic acid **3** modified at the 4-position are summarised in schemes 2 and 3 and the compounds prepared are listed in table II. 4-Arylalkoxy-2-benzyloxybenzoic acid derivatives **10** to **18** and 4-heteroarylalkoxy-2-benzyloxybenzoic acid derivatives **19** and **20** were prepared by selective alkylation of methyl 2,4-dihydroxybenzoate to introduce the 4-substituent followed by benzylation to introduce the 2-benzyloxy group and subsequent alkaline hydrolysis of the benzoate ester to the corresponding benzoic acid.



Scheme 3. Preparation of 4-substituted 2-benzyloxybenzoic acid derivatives. Reagents: (a) $PhCH_2Br$, NaH, DMF; (b) $PhCH_2SSnMe_3$, $(Ph_3P)_4Pd$; (c) aqueous NaOH, dioxan; (d) PhONa, $CuBr{\cdot}Me_2S$, pyridine; (e) $PhC{\equiv}CH$, $(Ph_3P)_4Pd$, CuBr, Et_3N; (f) $PhCH{=}CH_2$, $(Ph_3P)_2PdCl_2$, Et_3N; (g) H_2, Pd/C.



Scheme 4. Preparation of 2-substituted 4-benzyloxybenzoic acid derivatives. (Method C). Reagents: (a) $PhCH_2Cl$, $Bu_4N^+Br^-$, K_2CO_3, KI, acetone, reflux; (b) NaH/DMF, then RX, 60 °C; (c) 1 M NaOH, dioxan, reflux; (d) $(CF_3SO_2)_2O$, pyridine; (e) *E*-$PhCH{=}CHSnBu_3$, $(Ph_3P)_4Pd$.



Scheme 5. Synthesis of analogues of **3** modified at the carboxylic acid position. Reagents: (a) $PhCH_2Br$, K_2CO_3, DMF, RT; (b) $NaBH_4$, CH_3OH, RT; (c) BuLi, THF, –70 °C, $(EtO)_2P(=O)CH_2CO_2H$; (d) H_2SO_4, H_2O_2, RT; (e) NH_2OH•HCl, pyridine, reflux; (f) $(EtO)_2P(=O)CH_2CO_2Et$, NaH; (g) Et_3SiH, $(Ph_3P)_3RhCl$; (h) KOH, MeOH; (i) nitropropane, $(NH_4)_2HPO_4$, CH_3CO_2H, reflux; (j) (i) Ph_3P, CBr_4, 0 °C; (ii) BuLi, –70 °C; (iii) CO_2.

Alkylation of 4-iodosalicylic acid with benzyl bromide using sodium hydride in DMF gave benzyl 2-benzyloxy-4-iodobenzoate. This iodo compound was used to carry out a number of transformations. (i) Treatment with benzylthiotrimethylstannane using tetrakis triphenylphosphinepalladium, according to the procedure of Widdowson [19], gave 4-benzylthio-2-benzyloxybenzoic acid **21**. The corresponding sulphone **22** was prepared by oxidation of the thioether **21** with peracetic acid. (ii) An Ullmann reaction between the aryl iodide and sodium phenoxide in the presence of CuBr•Me_2S provided 4-phenoxy-2-benzyloxybenzoic acid **24**. (iii) The aryl iodide was reacted with phenylacetylene under Heck conditions using $(Ph_3P)_4Pd$ to give 4-phenylethynyl-2-benzyloxybenzoic acid **25**. A similar Heck reaction between 4-iodosalicylic acid and styrene provided 2-hydroxy-4-phenylethenylbenzoic acid. Hydrogenation of the alkene followed by alkylation of the 2-hydroxy provided 4-phenylethyl-2-benzyloxybenzoic acid **26**.

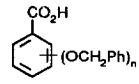
4-Benzylamino-2-benzyloxybenzoic acid **23** was prepared by reductive alkylation of 4-amino-2-benzyloxybenzoic acid with benzaldehyde and sodium cyanoborohydride.

The syntheses of analogues of 2,4-dibenzyloxybenzoic acid **3** modified at the 2-position are summarised in scheme 4 and the compounds prepared are listed in table III. 2-Alkoxy-, 2-arylalkoxyoxy- and 2-heteroarylalkoxyoxy-4-benzyloxybenzoic acid derivatives **27–35** were prepared by a similar approach to that used for the preparation of 4-arylalkoxy-2-benzyloxybenzoic acid derivatives and involved selective benzylation of methyl 2,4-dihydroxybenzoate to introduce the 4-benzyloxy group, followed by alkylation to introduce the 2-alkoxy or arylalkoxyoxy group and then alkaline hydrolysis of the appropriate benzoate ester to the corresponding benzoic acid derivative. Methyl 2-hydroxy-4-benzyloxybenzoate was converted to its triflate and then treated with (*E*)-tributyl phenylethenylstannane in the presence of $(Ph_3P)_4Pd$ to give, after saponification, (*E*)-2-phenylethenyl-4-benzyloxybenzoic acid **37**. The *trans* configuration of the alkene was confirmed by ^{1}H-NMR (J = 16 Hz for the alkene protons).

Analogues of **3** modified at the carboxylic acid position are summarised in table IV. The synthetic methods are summarised in scheme 5.

2,4-Dibenzyloxybenzaldehyde **38** was prepared by benzylation of 2,4-dihydroxybenzaldehyde and this compound was used as a common intermediate to prepare: (i) 2,4-dibenzyloxybenzyl alcohol **39** following reduction with sodium borohydride; (ii) (*E*)-3-(2,4-dibenzyloxyphenyl)prop-2-enoic acid **48** following a Wittig reaction with the ylide generated from diethyl phosphonoacetic acid; (iii) 2,4-dibenzyloxyphenol **40** following Baeyer–Villiger oxidation with hydrogen peroxide in concentrated sulphuric acid; (iv) 2,4-dibenzyloxybenzonitrile **41** following reaction with nitropropane in acetic acid in the presence of diammonium hydrogen phosphate, according to the method of Blatter [20]; (v) 2,4-dibenzyloxybenzaldehyde oxime **42** following reaction with hydroxylamine; (vi) 3-(2,4-dibenzyloxyphenyl)prop-2-ynoic acid **49** following reaction with carbon tetrabromide and triphenylphosphine and subsequent treatment of the resulting dibromoethylene derivative with butyllithium then carbon dioxide; (vii) 2,4-dibenzyloxyphenylpropanoic acid **47** following a Wittig reaction with the ylide generated from triethyl phosphonoacetate, reduction of the cinnamic ester with triethylsilane in the presence of Wilkinson's catalyst, then alkaline hydrolysis of the propanoic ester; (viii) (*E*)-2-benzyl-3-(2,4-dibenzyloxyphenyl)propenoic acid **51** following Wittig reaction with the appropriate ylide then alkaline hydrolysis of the substituted propenoate ester; and (ix) 2,4-dibenzyloxyphenylacetic acid **46**

Table I. Physical properties and in vitro activity of benzyloxy and dibenzyloxybenzoic acids.



Compound[a]	*$(OCH_2Ph)_n$*[b]	*Mp (°C)*	*Formula*[c]	*Inhibition of ET-1 binding IC_{50} (μM)*[d] ET_A[e]	ET_B[f]
BQ 123				0.022	23
BQ 788					0.06
3	2,4-$(OCH_2Ph)_2$	220–222	$C_{21}H_{18}O_4$	9 ± 0.5 (2)	<20% at 30 μM (2)[g]
4	2-OCH_2Ph	68–70	$C_{14}H_{12}O_3$	<20% at 30 μM (1)	
5	4-OCH_2Ph	188–190	$C_{14}H_{12}O_3$	<20% at 30 μM (1)	
6	3,4-$(OCH_2Ph)_2$	184–186	$C_{21}H_{18}O_4$	28 ± 2.3 (2)	
7	2,3-$(OCH_2Ph)_2$	118–120	$C_{21}H_{18}O_4$	<20% at 30 μM (1)	
8	2,5-$(OCH_2Ph)_2$	109–111	$C_{21}H_{18}O_4$	<20% at 30 μM (1)	
9	3,5-$(OCH_2Ph)_2$	207–208	$C_{21}H_{18}O_4$	<20% at 30 μM (1)	

[a]All compounds prepared according to method A; [b] ^{1}H-NMR spectra were consistent with the assigned structures; [c]±0.4% unless otherwise stated; [d]mean IC_{50} ± standard error of the mean (SEM) with the number of experiments given in parentheses; [e]rat A10 cell receptors; [f]rat cerebellum receptors; [g]% inhibition of [^{125}I]ET-1 binding at given concentration.

following condensation with *N*-benzoylglycine, alkaline hydrolysis of the resulting benzylideneoxazolone, and Baeyer Villiger oxidation of the resulting phenylpyruvic acid.

2,4-Dibenzyloxyphenoxyacetic acid **50** was prepared by alkylation of 2,4-dibenzyloxyphenol **40** with ethyl bromoacetate and subsequent alkaline hydrolysis of the phenoxyacetate.

The tetrazole **45** was prepared by the reaction of the benzonitrile with trimethyltin azide, prepared in situ from sodium azide and trimethyltin chloride.

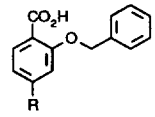
The pyrazole-5-carboxylic acid **52** was prepared by reaction of the corresponding acetophenone **44** with diethyl oxalate in the presence of potassium ethoxide, subsequent reaction of the intermediate 2,4-dioxobutanoate with hydrazine hydrate in EtOH in the presence of acetic acid, then alkaline hydrolysis of the pyrazole carboxylate moiety. The 3-(2,4-dibenzyloxyphenyl)isoxazole-5-carboxylic acid **53** was prepared in a similar manner from reaction of the intermediate 2,4-dioxobutanoate with hydroxylamine.

Substituted amino-acid amides **54** and **55** were prepared by reaction of 2,4-dibenzyloxybenzoic acid **3** with the appropriate amino-acid ester (glycine methyl ester or β-alanine ethyl ester) in the presence of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide and 1-hydroxybenzotriazole.

Results and discussion

Initial attempts to improve the potency of the lead compound **3** were based on the superimposition of that compound with BQ 123 (fig 2). This superimposition shows the benzoic acid moiety overlaying the carboxylic acid of the aspartate residue in BQ 123, the phenyl ring associated with the 4-benzyloxy group of **3** overlaying the indole ring of the tryptophan residue, and the 2-benzyloxy group is seen projecting into the region occupied by the valine residue of BQ 123. Thus, receptor binding may be improved by more closely mimicking the BQ 123 structure by replacement of the phenyl ring of the 4-benzyloxy residue with an indole ring. One such compound **20** was prepared, but failed to show an increase in activity compared with **3**. Another obvious modification suggested by the superimposition was the replacement of the phenyl ring of the 2-benzyloxy group with an aliphatic group in order to better mimic the valine residue of BQ 123. Whilst the cyclohexylmethoxy derivative **29** was approximately equipotent with **3**, the 2-methylpropyl derivative **28** was threefold less active, and the methoxy derivative **27** was inactive, suggesting that an aliphatic group of the correct size could replace the phenyl ring of the 2-benzyloxy group.

Table II. Physical properties and in vitro activity of 4-substituted 2-benzyloxybenzoic acid derivatives



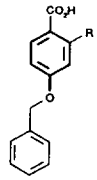
Compound	*R*[a]	*Method*	*Mp (°C)*	*Formula*[c]	*Inhibition of ET-1 binding IC_{50} (μM)*[d]	
					ET_A[e]	ET_B[f]
3	$PhCH_2O$–	A	220–222	$C_{21}H_{18}O_4$	9 ± 0.5 (2)	<20% at 30 μM (2)[g]
10	$PhCH_2CH_2O$–	B	116–118	$C_{22}H_{20}O_4$	25 ± 0.4 (2)	
11	4-$ClC_6H_4CH_2O$–	B	150–152	$C_{21}H_{17}ClO_4$	7 ± 1.5 (2)	
12	4-$MeOC_6H_4CH_2O$–	B	127–129	$C_{22}H_{22}O_5$	38%, 52% at 30 μM (2)	
13	4-*i*-$PrC_6H_4CH_2O$–	B	162–164	$C_{24}H_{24}O_4$	26%, 35% at 30 μM (2)	
14	3-$MeOC_6H_4CH_2O$–	B	104–105	$C_{22}H_{20}O_5$	16 ± 1.6 (2)	
15	3,4-$(OCH_2O)C_6H_3CH_2O$–	B	112–114	$C_{22}H_{18}O_6$	4 ± 0.4 (2)	<20% at 30 μM (1)
16	2,3-$(OCH_2O)C_6H_3CH_2O$–	B	110–112	$C_{22}H_{18}O_6$	<20% at 3 μM (1)	
17	3,4-$(MeO)_2C_6H_3CH_2O$–	B	118–120	$C_{23}H_{22}O_6$	<20% at 30 μM (1)	
18	1-NaphthylCH_2O–	B	145–147	$C_{25}H_{20}O_4$	26%, 30% at 30 μM (2)	
19	3-PyridylCH_2O–	B	156–158	$C_{20}H_{17}NO_4$	4 ± 0.4 (2)	
20	3-IndolylCH_2CH_2O–	B	68–69	$C_{24}H_{21}NO_4 \cdot 0.3H_2O$	41%, 51% at 30 μM (2)	
21	$PhCH_2S$–	[b]	110–112	$C_{21}H_{18}O_3S$	30 ± 2.4 (2)	
22	$PhCH_2SO_2$–	[b]	187–189	$C_{21}H_{18}O_5S$	<20% at 30 μM (1)	
23	$PhCH_2NH$–	[b]	126–128	$C_{21}H_{19}NO_3$	26 ± 3.0 (2)	
24	PhO–	[b]	125–127	$C_{20}H_{16}O_4$	<20% at 30 μM (1)	
25	$PhC{\equiv}C$–	[b]	125–127	$C_{22}H_{16}O_3$	15 ± 1.7 (2)	
26	$PhCH_2CH_2$–	[b]	83–85	$C_{22}H_{20}O_3$	15 ± 0.3 (2)	

[a] ^{1}H-NMR spectra were consistent with the assigned structures; [b]see *Experimental protocols*; [c]±0.4% unless otherwise stated; [d]mean IC_{50} ± standard error of the mean (SEM) with the number of experiments given in parentheses; [e]rat A10 cell receptors; [f]rat cerebellum receptors; [g]% inhibition of [^{125}I]ET-1 binding at given concentration.

As well as modifications inspired by the superimposition of our lead compound with BQ 123 **1**, we turned our attention to empirical modification of the individual components of 2,4-dibenzyloxybenzoic acid.

Our initial modifications concentrated on examining the receptor binding properties of the positional isomers of monobenzyloxy- and dibenzyloxybenzoic acid (table I). The monobenzyloxybenzoic acids **4** and **5** were inactive in the receptor binding assay, demonstrating that both benzyloxy groups contributed towards the binding of **3** to the receptor. As both benzyloxy groups were necessary for activity we examined the effects of varying the substitution pattern of these groups on the central aromatic ring to determine the optimal pattern for receptor binding. Of the four

Table III. Physical properties and in vitro activity of 2-substituted 4-benzyloxybenzoic acid derivatives.



Compound	*R*[a]	*Method*	*Mp (°C)*	*Formula*[c]	*Inhibition of ET-1 binding IC_{50} (μM)*[d]	
					ET_A[e]	ET_B[f]
3	$PhCH_2O$–	A	220–222	$C_{21}H_{18}O_4$	9 ± 0.5 (2)	<20% at 30 μM (2)[g]
27	MeO–	C	119–124	$C_{15}H_{14}O_4$	<20% at 30 μM (1)	
28	Me_2CHCH_2O–	C	96	$C_{18}H_{20}O_4$	42%, 54% at 30 μM (2)	
29	$CyclohexylCH_2O$–	C	99–100	$C_{21}H_{24}O_4$	19 ± 1.8 (2)	
30	$PhCH_2CH_2O$–	C	100–101	$C_{22}H_{20}O_4$	31%, 37% at 30 μM (2)	
31	$PhCH_2CH_2CH_2O$–	C	101–102	$C_{23}H_{22}O_4$	17%, 23% at 30 μM (2)	
32	4-$ClC_6H_4CH_2O$–	C	145–146	$C_{21}H_{17}ClO_4$	21 ± 2.4 (2)	
33	4-$MeOC_6H_4CH_2O$–	C	104–105	$C_{22}H_{20}O_5$	<20% at 30 μM (1)	
34	4-*i*-$PrC_6H_4CH_2O$–	C	88–89	$C_{24}H_{24}O_4$	<20% at 30 μM (1)	
35	2-$PyridylCH_2O$–	C	147–148	$C_{20}H_{17}NO_4$	12 ± 1.0 (2)	<20% at 30 μM (1)
36	HO–	C	182	$C_{14}H_{12}O_4$	25 ± 2.5 (2)	
37	(*E*)-PhCH=CH–	C	147–149	$C_{22}H_{18}O_3$	25 ± 1.5 (2)	

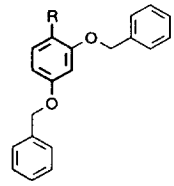
[a] ^{1}H-NMR spectra were consistent with the assigned structures; [b]see *Experimental protocols*; [c]±0.4% unless otherwise stated; [d]mean IC_{50} ± standard error of the mean (SEM) with the number of experiments given in parentheses; [e]rat A10 cell receptors; [f]rat cerebellum receptors; [g]% inhibition of [^{125}I]ET-1 binding at given concentration.

positional isomers synthesised only 3,4-dibenzyloxybenzoic acid showed greater than 20% inhibition of the binding of ET-1 to the ET_A receptor from rat A10 cells (see compounds **6–9** in table I) and this compound was threefold less active than the lead compound **3**. It was concluded that the 2,4-substitution pattern of the central aromatic ring leads to optimal binding at the ET_A receptor.

The results of modification of the 4-benzyloxy group of 2,4-dibenzyloxybenzoic acid **3** are summarised in table II.

Substitution in the phenyl ring associated with the 4-benzyloxy residue had variable effects on receptor binding. Introduction of a chlorine atom in the *para*-position of the phenyl ring had no effect on receptor binding. A small reduction in receptor binding was observed on introducing a 4-OMe group, whilst the 4-*i*-Pr derivative had a further reduction in binding potency (compare the receptor binding properties of **11–13** with **3** in table II). The 3-OMe analogue **14** had similar receptor binding potency to the 4-OMe compound **12**. Whilst introduction of a 3,4-methylenedioxy group (compound **15**) gave a twofold improvement in potency, introduction of a 2,3-methylenedioxy group (compound **16**) led to loss of activity, indicating a regiospecific requirement for this functional group. The 3,4-dimethoxybenzyloxy analogue **17** was inactive, presumably due to the different orientation of the oxygen lone pairs compared with **15**.

Table IV. Physical properties and in vitro activity of analogues of **3** modified at the carboxylic acid position.



Compound	*R*[a]	*Method*	*Mp (°C)*	*Formula*[c]	*Inhibition of ET-1 binding IC_{50} (μM)*[d]	
					ET_A[e]	ET_B[f]
3	$-CO_2H$	A	220–222		9 ± 0.5 (2)	<20% at 30 μM (2)[g]
38	$-CHO$	[b]	80–81	$C_{21}H_{18}O_3$	8 ± 0.6 (2)	
39	$-CH_2OH$	[b]	87	$C_{21}H_{20}O_3$	13 ± 1.5 (2)	
40	$-OH$	[b]	82–83	$C_{20}H_{18}O_3$	18 ± 1.0 (2)	
41	$-CN$	[b]	100	$C_{21}H_{17}NO_2$	6 ± 0.6 (2)	<20% at 30 μM (1)
42	$-CH=NOH$	[b]	130	$C_{21}H_{19}NO_3$	13 ± 1.1 (2)	
43	$-NO_2$	[b]	101–103	$C_{20}H_{17}NO_4$	10 ± 1.2 (2)	
44	$-COCH_3$	[b]	64–65	$C_{22}H_{20}O_3$	11 ± 0.2 (2)	
45	–5-Tetrazole	[b]	178–179	$C_{21}H_{18}N_4O_2 \cdot 0.2H_2O$	6 ± 0.1 (2)	
46	$-CH_2CO_2H$	[b]	137–139	$C_{22}H_{20}O_4$	15 ± 1.4 (2)	
47	$-CH_2CH_2CO_2H$	[b]	124–126	$C_{23}H_{22}O_4$	5 ± 0.1 (2)	
48	(*E*)-$CH=CHCO_2H$	[b]	153–155	$C_{23}H_{20}O_4$	5 ± 0.3 (2)	<20% at 30 μM (1)
49	$-C\equiv CCO_2H$	[b]	125 (dec)	$C_{23}H_{18}O_4$	3 ± 0.05 (2)	<20% at 10 μM (1)
50	$-OCH_2CO_2H$	[b]	72	$C_{22}H_{20}O_5$	10 ± 0.2 (2)	
51	(*E*)-$C=C(CH_2Ph)CO_2H$	[b]	175–176	$C_{30}H_{26}O_4$	5 ± 0.3 (2)	
52	–3-Pyrazole-5-CO_2H	[b]	218–219	$C_{24}H_{20}N_2O_4$	1 ± 0.01 (2)	<20% at 10 μM (1)
53	–3-Isoxazole-5-CO_2H	[b]	192–193	$C_{24}H_{19}NO_5$	5 ± 1.1 (2)	
54	$-CONHCH_2CO_2H$	[b]	183	$C_{23}H_{21}NO_5$	2 ± 0.03 (2)	<20% at 10 μM (1)
55	$-CONHCH_2CH_2CO_2H$	[b]	145	$C_{24}H_{23}NO_5$	3 ± 0.3 (2)	<20% at 10 μM (1)

[a] ^{1}H-NMR spectra were consistent with the assigned structures; [b]see *Experimental protocols*; [c]±0.4% unless otherwise stated; [d]mean IC_{50} ± standard error of the mean (SEM) with the number of experiments given in parentheses; [e]rat A10 cell receptors; [f]rat cerebellum receptors; [g]% inhibition of [^{125}I]ET-1 binding at given concentration.

The presence of a basic nitrogen atom in the 3-position of the phenyl ring gave a twofold increase in potency, compare the receptor binding properties of the 3-pyridylmethoxy derivative **19** with **3** in table II. The increases in activity observed with the 3-pyridyl compound **19** and the 3,4-methylenedioxyphenyl compound **15** are consistent with interaction with a H-bond donor group at this position.

Replacement of the oxygen atom in the linking group by sulphur (compound **21**) or nitrogen (compound **23**) resulted in a small loss (up to threefold) of activity. The sulphone derivative **22** was inactive in the binding assay. Carbon analogues, irrespective of the degree of unsaturation, were twofold less active than the parent benzyloxy compound **3** (compare the phenylacetylene **25** and phenethyl **26** with **3** in table II). It was concluded that there was no apparent advantage in changing the oxygen atom of the 4-benzyloxy substituent for carbon, sulphur or nitrogen.

Comparison of the potency of the phenoxy **24** and phenethyloxy **10** derivatives with the parent benzyloxy derivative **3** showed that the optimal number of atoms in the linker between the central aromatic ring and the phenyl ring of the 4-benzyloxy substituent is two.

The results of modification of the 2-benzyloxy group of 2,4-dibenzyloxybenzoic acid **3** are summarised in table III.

Introduction of a *para*-chlorine atom into the phenyl ring resulted in a twofold decrease in activity. The introduction of methoxy or isopropyl resulted in a complete loss of activity (compare the activities of **32–34** with **3**). The presence of a basic nitrogen atom in the 2-position of the phenyl ring was tolerated, compare the receptor binding properties of the 2-pyridylmethoxy derivative **35** with **3**.

Increasing the length of the bridging group decreased activity, compare the receptor binding properties of the phenethyloxy **30** and the 3-phenylpropoxy derivative **31** with the parent benzyloxy derivative **3** in table III.

The results of modification of the carboxylic acid group of 2,4-dibenzyloxybenzoic acid **3** are summarised in table IV.

It was found that other H-bond acceptor groups could replace the carboxylic acid moiety in **3** and the receptor binding potency could be maintained. For example, replacement of the carboxylic acid group in **3** with $COCH_3$ **44**, CN **41**, CHO **38**, NO_2 **43** and tetrazole **45** gave compounds with similar receptor binding potency. Even replacement of the carboxylic acid group with weak H-bond acceptors such as CH_2OH **39** and OH **40** resulted in compounds active in the receptor binding assay, albeit weaker in potency than **3**.

Introducing a methylene spacer between the central aromatic ring and the carboxylic acid residue, as in 2,4-dibenzyloxyphenylacetic acid **46**, resulted in a twofold loss of activity compared to **3**. The corresponding phenylpropionic acid **47** was twice as active as **3**, and the conformationally restricted phenylpropenoic **48** and phenylpropynoic acids **49** were 2–3-fold more active than **3**. The phenoxyacetic acid derivative **50** was equipotent with the benzoic acid derivative **3**.

In view of the initial results from modifying the carboxylic acid moiety, incorporation of a H-bond acceptor group into the spacer between the central aromatic ring and the carboxylic acid group was examined. Compounds with a rigidified chain, or heterocyclic ring, containing both acidic and H-bond acceptor groups were found to be beneficial, for example the benzamidoacetic acid **54**, the benzamidopropionic acid **55** and the pyrazole carboxylic acid **52** had IC_{50} values in the range 1 to 3 μM. The oxazole acid **53** was fivefold less active than the corresponding pyrazole acid **52**.

The distances between the aromatic centroid of the 4-benzyloxy residue and the carboxylic acid group are 9.9 Å for the benzoic acid **3**, 12.6 Å for the propynoic acid **49**, 12.1 Å for the benzamidoacetic acid **54**, and 12.9 Å for the pyrazolecarboxylic acid **52**. The binding activities observed for the analogues of **3** modified in the carboxylic acid position may be explained by a model of the receptor containing a H-bond donor site close to the 1-position of **3** and a cationic site somewhat further removed. The nitrile **41** is postulated to bind to the H-bond donor site. The propynoic acid **49** binds to the cationic site. The benzamido acetic acid **54** and pyrazolecarboxylic acid **52** bind at both the H-bond donor site and the cationic site, resulting in increased potency. The benzoic acid **3** could bind to either the H-bond donor site or the cationic site (modelling studies showed that it was possible to construct a cationic site which interacts with all four carboxylic acids **3**, **49**, **52** and **54**). This model has been successfully used to design more potent compounds which will be the subject of further communications from this laboratory.

In order to determine whether these compounds were acting as antagonists or agonists, one of the compounds in this series **15** was examined in a functional assay based on the ET-1 induced contraction of isolated rat aortic rings. Compound **15** antagonised the effects of ET-1 with a pK_B of 4.6 ± 0.5 ($n = 6$). By comparison, BQ 123, the peptidic ET_A-selective antagonist had a pK_B of 7.2 ± 0.6 ($n = 9$).

Conclusion

A 3D search of a compound database, based on a model derived from the superimposition of BQ 123 **1** and myriceron caffeoyl ester **2**, revealed a non-peptide lead compound 2,4-dibenzyloxybenzoic acid **3** which selectively bound to ET_A receptors with an IC_{50} of 9 μM. Empirical modification at the carboxylic acid position of this lead compound gave the 3-phenylpyrazole-5-carboxylic acid **52** with a modest improvement in IC_{50} to 1 μM. The SAR at this position has been rationalised using a model of the receptor containing both a

cationic site and an H-bond donor site. Rational modification of the benzyloxy groups at either the 2 or 4 positions did not result in significant increases in potency. One of the compounds in this series, **15**, antagonised the ET-1-induced contraction of rat aorta with a pK_B of 4.6.

Experimental protocols

Melting points were determined using an Electrothermal apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian VXR 400 instrument. Analyses indicated by the symbols of the elements or functions were within ±0.4% of theoretical values. Flash chromatography was performed using 'Sorbsil' (Crosfield) silica gel, mesh size 40–60 μm supplied by Rhône Poulenc.

Molecular modelling

Molecule construction
BQ 123 was constructed within the Builder module of Insight [21] and minimised using the Insight Optimise routine. This optimised structure was used as an input for DGEOM [22], which was used to produce 50 randomly generated conformers. These 50 structures were individually relaxed and energy minimised using the CVFF force field within the Discover programme. A short low temperature dynamics run (0.2 ps, 300 K) was followed by minimisation until the convergence criteria were reached (maximum derivative less than 0.1). The analysis tools of Insight were used to cluster these structures according to position of backbone heavy atoms. Four clusters were selected as representative. One of these was found by visual inspection to be very similar to the reported solution structure [16] and was used for all further dynamics studies that used the published nOe constraints [16]. The backbone conformation of BQ 123 was further investigated using a simulated annealing protocol: high temperature dynamics were performed (100 ps, 900 K), archiving the current structure every 1 ps. Each archived structure was energy minimised without constraints using quasi Newton–Raphson (maximum derivative less than 0.1). The structure closest to the required torsional constraints was selected as a possible bioactive conformation for the backbone atoms.

Myriceron caffeoyl ester **2** was constructed using the CONCORD [23] builder (v2.93).

Conformational analysis
Conformational analyses of BQ 123 and **2** were carried out within Chem-X using the Chem-X force field, applying Gasteiger charges and considering VDW and electrostatic non-bonded interactions. Only bonds causing movement of either the acid or aryl group centroid were rotated systematically using 12 points for unconjugated bonds and two points for conjugated. Other non-conjugated bonds not involved in the direct movement of these groups were rotated systematically, but only with a three-point rotation. Conformations within 10 kcal were considered as acceptable if they were within 10 kcal of the global minimum found. The acid-aromatic ring distances for the two sets of conformations were then compared on a molecule by molecule basis and using a tolerance of 0.5 Å. This comparison was carried out using the combination of conformational plots facilities in Chem-X. It was found that common conformations existed between the two molecules in the distance range 9.1–11.3 Å. Two paired conformations were selected from the centres of this range and superimposed (fig 1).

3D searching
ChemDBS-3D was used for database building and searching. The search query was generated from the structure of BQ 123 shown in figure 1, by deletion of all atoms except the aromatic ring centroid and the acid function; the aromatic centre-acid group oxygen was 10.2 Å. Searching was carried out using a tolerance of ±1.1 Å. The first search was on a database of 60 000 compounds, 383 hits were produced, 98 of these resulted from a search of the stored conformation (CONCORD [23] generated) and 285 from a complete rules based conformational search of the remaining compounds. The lead structure **3** was discovered in the set of stored conformer matches. Other leads were discovered using these search criteria. These will be the subject of a later publication.

Chemistry

*3,4-Dibenzyloxybenzoic acid **6**. Method A*
A stirred solution of methyl 3,4-dihydroxybenzoate (7.0 g) in DMF (200 mL) under N_2 was treated with NaH (60%, 2.78 g) and maintained at 25 °C for 1 h. The mixture was treated with benzyl bromide (9.66 mL) and stirred at 100 °C for 8 h. The mixture was evaporated (45 °C/0.1 mm Hg). The residue was partitioned between EtOAc and water. The organic layer was dried and evaporated to give methyl 3,4-dibenzyloxybenzoate (6.9 g, 48%). This ester (4.5 g) was saponified with 1 M NaOH (15 mL) in dioxan (100 mL) at reflux for 6 h. The dioxan was evaporated, the residue diluted with water (10 mL), washed with EtOAc (2 x 20 mL), brought to pH 1 giving a solid precipitate. This was separated by filtration and recrystallised from MeOH giving **6**, colourless crystals (2.0 g, 46%), mp 184–186 °C. ^{1}H-NMR (DMSO) 5.18 (2H, s), 5.23 (2H, s), 7.16 (1H, d), 7.3–7.5 (10H, m), 7.55 (1H, d), 7.57 (1H, s). Anal $C_{21}H_{18}O_4$ (C, H).

*2-Benzyloxy-4-(4-chlorobenzyloxy)benzoic acid **11**. Method B*
A solution of methyl 2,4-dihydroxybenzoate (6.73 g) in acetone (250 mL) was treated with potassium iodide (2.66 g), tetrabutylammonium chloride (0.05 g), potassium carbonate (5.53 g), and 4-chlorobenzyl chloride (7.08 g), and stirred at reflux for 24 h. The mixture was filtered, and the filtrate evaporated. The residue was taken up in EtOAc, washed with water, dried, and evaporated. The residue was recrystallised from EtOAc to yield methyl 2-hydroxy-4-(4-chlorobenzyloxy)benzoate as colourless crystals (5.3 g, 45%), mp 119–121 °C. ^{1}H-NMR (DMSO) 3.9 (3H, s), 5.2 (2H, s), 6.6 (2H, m), 7.5 (4H, m), 7.7 (1H, d).

A solution of methyl 2-hydroxy-4-(4-chlorobenzyloxy)-benzoate (5.2 g) in dry dimethylformamide (DMF) (50 mL) was treated with NaH (60% dispersion, 0.71 g) and stirred at 25 °C for 20 min. Benzyl bromide (2.12 mL) was added and the solution heated at 60 °C for 2 h. The solution was evaporated. The residue was dissolved in EtOAc, washed with water, dried, and evaporated. The residue was recrystallised from EtOAc to yield methyl 2-benzyloxy-4-(4-chlorobenzyloxy)benzoate as colourless crystals (5.1 g, 75%), mp 99–101 °C. ^{1}H-NMR (DMSO) 3.8 (3H, s), 5.18 (2H, s), 5.21 (2H, s), 6.7 (1H, d), 6.85 (1H, s), 7.3–7.5 (9H, m), 7.75 (1H, d).

A solution of methyl 2-benzyloxy-4-(4-chlorobenzyloxy)-benzoate (1.88 g) in dioxan (37 mL) and 1 M NaOH (12.5 mL) was refluxed for 4 h. The solution was evaporated, the residue

diluted with water, and brought to pH 1 with 2 M HCl. The precipitate was extracted with EtOAc, washed with water, dried and evaporated. The residue was recrystallised from EtOAc to yield **11** (0.8 g, 44%) as colourless crystals, mp 150–152 °C. ^{1}H-NMR (DMSO) 5.15 (2H, s), 5.2 (2H, s), 6.7 (1H, d), 6.8 (1H, s), 7.3–7.5 (9H, m), 7.7 (1H, d). Anal $C_{21}H_{17}NO_4$ (C, H).

*2-Benzyloxy-4-benzylthiobenzoic acid **21***
A stirred solution of 4-iodosalicylic acid (4.4 g) in DMF (44 mL) was treated with NaH (60%, 1.34 g). After 10 min the suspension was treated with benzyl bromide (5.71 g) and heated at 60 °C for 0.5 h. The solution was partitioned between water and ether. The ether layer was separated, washed with 1 M NaOH, water dried and evaporated. The residual solid was washed with MeOH to give benzyl 2-benzyloxy-4-iodobenzoate, yellow solid (5.83 g, 79%), mp 61–63 °C.

A stirred solution of benzyl 2-benzyloxy-4-iodobenzoate (2.9 g), benzylthio trimethylstannane (1.87 g) and $(Ph_3P)_4Pd$ (380 mg) in toluene (60 mL) was refluxed for 0.5 h. The solution was washed with 10% aqueous KF, water, dried and evaporated. The residual oil was purified by flash chromatography on silica, eluting with pentane/EtOAc 5:1 to give benzyl 2-benzyloxy-4-benzylthiobenzoate, a colourless oil (2.34 g, 81%).

This ester (1.1 g) was saponified in 1 M NaOH (4.5 mL) and dioxan (10 mL) at reflux for 1 h. The solution was evaporated, the residue diluted with water, brought to pH 1. The precipitate was extracted into EtOAc, dried and evaporated. The residual solid was recrystallised from EtOH to give **21**, colourless crystals (740 mg, 85%), mp 110–112 °C. Anal $C_{21}H_{18}O_3S$ (C, H).

*2-Benzyloxy-4-benzylsulphonylbenzoic acid **22***
A solution of 2-benzyloxy-4-benzylthiobenzoic acid **21** (1.0 g) in AcOH (10 mL) and CH_2Cl_2 (5 mL) was treated with aqueous peracetic acid (38%, 1.06 mL) and stirred at 25 °C for 2 h, during which a solid crystallised. The mixture was filtered to give **22**, colourless crystals (0.80 g, 73%), mp 187–189 °C. Anal $C_{21}H_{18}O_5S$ (C, H).

*4-Benzylamino-2-benzyloxybenzoic acid **23***
A stirred suspension of 2-benzyloxy-4-nitrobenzoic acid (8.5 g) in EtOH (26 mL) and conc HCl (52 mL) was treated with $SnCl_2$ (21.8 g) in EtOH (77 mL) and stirred at 25 °C for 24 h. The resulting solution was evaporated to remove EtOH and diluted with water (120 mL). The resulting precipitate was filtered off and partitioned between EtOAc and water. The organic layer was separated, dried and evaporated to ethyl 4-amino-2-benzyloxybenzoate, a light-brown solid (5.0 g, 60%).

The ester (3.7 g) was saponified by refluxing with 1 M NaOH (42 mL) in dioxan (37 mL) for 6 h. The solution was evaporated to remove dioxan, diluted with water, brought to pH 5 with 2 M HCl. The precipitate was extracted into EtOAc, dried and evaporated. The residue was purified by flash chromatography on silica, eluting with pentane/EtOAc/AcOH 50:50:0.8, followed by trituration with pentane to give 4-amino-2-benzyloxybenzoic acid, light-brown solid (1.66 g, 50%).

A solution of 4-amino-2-benzyloxybenzoic acid (500 mg), benzaldehyde (320 mg), and $NaBH_3CN$ (190 mg) in EtOH was stirred at 25 °C for 24 h. The solution was evaporated, the residue partitioned between EtOAc and water, the organic layer separated and dried. The residual oil was purified by flash chromatography on silica, eluting with pentane/EtOAc/AcOH 50:50:0.8, followed by trituration with ether to give **23**, colourless crystals (230 mg, 34%), mp 126–128 °C. Anal $C_{21}H_{19}NO_3$ (C, H, N).

*2-Benzyloxy-4-phenoxybenzoic acid **24***
A solution of phenol (1.1 g) in pyridine (40 mL) was treated with NaH (60%, 0.47 g). After 10 min benzyl 2-benzyloxy-4-iodobenzoate (4.44 g), (intermediate in the preparation of **21**), and $CuBr{\cdot}Me_2S$ (4.1 g) were added. The solution was refluxed for 5 h. The solution was evaporated, the residue partitioned between EtOAc and water. The EtOAc layer was washed with 2 M AcOH, water, dried and evaporated. The residue was purified by flash chromatography, eluting with pentane/EtOAc 5:1 to give benzyl 2-benzyloxy-4-phenoxybenzoate, colourless oil (2.02 g, 51%).

This oil was saponified as in compound **21**. Recrystallisation from cyclohexane/toluene gave **24**, colourless crystals (1.3 g, 83%), mp 125–127 °C. Anal $C_{20}H_{16}O_4$ (C, H).

*2-Benzyloxy-4-phenylethynylbenzoic acid **25***
Benzyl 2-benzyloxy-4-iodobenzoate (7.6 g) was added to triethylamine (120 mL) followed by phenylacetylene (2.94 g) then tetrakis(triphenylphosphine)palladium(0) (0.664 g) and copper(I) bromide (0.248 g). The resulting mixture was stirred at room temperature for 3 h. The triethylamine was distilled off and the residue partitioned between ether (600 mL) and saturated aqueous ammonium chloride solution. The organic phase was dried over magnesium sulphate and evaporated. The residue was purified by flash chromatography on silica eluting with a mixture of pentane and EtOAc (5:1 v/v). Fractions homogeneous in the required product were combined and evaporated to give benzyl 2-benzyloxy-4-phenylethynylbenzoate (2 g, 28%) as a cream solid, mp 98–100 °C.

A stirred solution of benzyl 2-benzyloxy-4-phenylethynylbenzoate (2 g) and 1 N sodium hydroxide (5 mL) in dioxan (80 mL) was heated at reflux for 1 h. The reaction mixture was evaporated, the residue dissolved in water (6 mL) and the solution washed twice with EtOAc (20 mL). The pH of the aqueous phase was adjusted to pH 1 by addition of 2 N HCl and resulting solid was filtered and washed well with water to give **25** (0.6 g, 38%), mp 125–127 °C. Anal $C_{22}H_{16}O_3$ (C, H).

*2-Benzyloxy-4-phenylethylbenzoic acid **26***
A solution of 4-iodosalicylic acid (20 g), styrene (10.7 mL), and $(Ph_3P)_2PdCl_2$ (530 mg) in triethylamine (45 mL) was heated at 100 °C for 2 h. The mixture was partitioned between 2M HCl and EtOAc. The organic layer was separated, dried, evaporated, and the residue recrystallised from MeOH to give 2-hydroxy-4-phenylethenylbenzoic acid, colourless crystals (8.0 g, 44%).

A solution of 2-hydroxy-4-phenylethenylbenzoic acid (8.0 g) in EtOH (200 mL) was treated with 5% Pd/C and shaken under hydrogen at 25 °C and atmospheric pressure. When hydrogen uptake was complete, the mixture was filtered and the filtrate evaporated to give 2-hydroxy-4-phenylethylbenzoic acid (6.0 g, 74%).

A stirred solution of 2-hydroxy-4-phenylethylbenzoic acid (4.0 g) in DMF (100 mL) was treated with NaH (60%, 1.12 g). After 10 min the suspension was treated with benzyl bromide (5.0 mL) and heated at 60 °C for 0.5 h. The solution was partitioned between water and ether. The ether layer was separated, washed with 1 M NaOH, water, dried and evaporated. The residue was purified by flash chromatography on silica, eluting with pentane/EtOAc 8:1, to give benzyl 2-benzyloxy-4-phenylethylbenzoate, a colourless solid (6.0 g, 86%), mp 68–70 °C.

The ester (2.0 g) was saponified as in compound **6**. Recrystallisation from pentane/EtOAc gave **26**, colourless crystals (0.46 g, 29%), mp 83–85 °C. Anal $C_{22}H_{20}O_3$ (C, H).

4-Benzyloxy-2-cyclohexylmethoxybenzoic acid ***29****. Method C*
A solution of methyl 2,4-dihydroxybenzoate (51 g) in acetone (600 mL) was treated with KI (18.7 g), tetrabutylammonium chloride (0.25 g), K_2CO_3 (42 g), and benzyl chloride (38 mL) and refluxed with stirring for 7 h. The mixture was filtered, the filtrate evaporated. The residue was dissolved in EtOAc, washed with water, dried, and evaporated. The residue was triturated with MeOH to give methyl 2-hydroxy-4-benzyloxybenzoate, colourless solid (66 g, 84%), mp 101–102 °C.

A solution of methyl 2-hydroxy-4-benzyloxybenzoate (2.58 g) in DMF (30 mL) was treated with NaH (60%, 0.4 g). After 10 min, the mixture was treated with cyclohexylmethyl bromide (2.7 g) and stirred at 100 °C for 24 h. The mixture was evaporated (45 °C/0.1 mm Hg), the residue was partitioned between EtOAc and water. The organic layer was separated, dried and evaporated. The residue was purified by flash chromatography, eluting with pentane/ether 4:1, to give methyl 2-cyclohexylmethoxy-4-benzyloxybenzoate, colourless solid (1.75 g, 49%).

The ester (1.75 g) was saponified using 15% KOH in MeOH (50 mL) at reflux for 2 h. The solution was evaporated, the residue was diluted with water, washed with EtOAc, brought to pH 1 with conc HCl. The precipitate was extracted into EtOAc, dried and evaporated. The residue was recrystallised from EtOAc/pentane to give **29**, colourless solid (1.1 g, 65%), mp 99–100 °C. Anal $C_{21}H_{24}O_4$ (C, H).

(E)-4-Benzyloxy-2-phenylethenylbenzoic acid ***37***
A solution of methyl 2-hydroxy-4-benzyloxybenzoate (6.45 g) in pyridine (30 mL) was treated with trifluoromethanesulphonic anhydride (6.65 mL) at 0 °C. The solution was stirred at 25 °C for 24 h and then partitioned between EtOAc/water. The organic layer was washed with 2 M HCl, water, dried and evaporated. The residue was triturated with pentane to give methyl 2-trifluoromethylsulphonyloxy-4-benzyloxybenzoate, light-brown solid (6.7 g, 69%), mp 53–55 °C.

A solution of the triflate (1.95 g) in dioxan (25 mL) was treated with (*E*)-tributyl phenylethenylstannane (2.06 g), LiCl (0.64 g), $(Ph_3P)_4Pd$ (0.12 g), and 2,6-di-*tert*-butyl-4-methylphenol (5 mg) and refluxed for 4 h. The solution was cooled and treated with pyridine (2.52 mL) and pyridine hydrofluoride (0.7 mL) and stirred at 25 °C for 24 h. The solution was diluted with ether, filtered, the filtrate washed with 1 M HCl, water, dried and evaporated. The residue was purified by flash chromatography, eluting with pentane/EtOAc 19:1, to give crude methyl 2-phenyethenyl-4-benzyloxybenzoate (0.2 g, 12%).

The ester (100 mg) was saponified with 1 M NaOH (0.73 mL) and dioxan (1.5 mL) at 60 °C for 2 h. The solution was diluted with water and brought to pH 1. The product was extracted into EtOAc, dried and evaporated. The residue was recrystallised from cyclohexane/EtOAc to give **37**, colourless crystals (30 mg, 31%), mp 147–149 °C. ^{1}H-NMR (DMSO) 5.25 (2H, s), 7.0 (1H, d), 7.2 (1H, d, J = 16 Hz), 7.3–7.6 (11H, m), 7.9 (1H, d), 8.05 (1H, d, J = 16 Hz). Anal $C_{22}H_{18}O_3$ (C, H).

2,4-Dibenzyloxybenzaldehyde ***38***
A mixture of 2,4-dihydroxybenzaldehyde (17.5 g), potassium carbonate (42 g) and benzyl bromide (35 mL) in DMF (100 mL) was stirred at room temperature for 18 h. The reaction mixture was poured into vigorously stirred ice-water (300 mL) and stirring was continued for 30 min. The resulting brown solid was filtered and dried to give **38** (28.5 g, 71%), mp 80–81 °C. Anal $C_{21}H_{18}O_3$ (C, H).

2,4-Dibenzyloxybenzyl alcohol ***39***
A solution of 2,4-dibenzyloxybenzaldehyde (2.26 g) in MeOH (20 mL) was treated portionwise with sodium borohydride (0.268 g) over 5 min. Water (5 mL) was added dropwise to the reaction mixture. The resulting white solid was filtered, dried and crystallised from a mixture of ether and petroleum ether to give **39** (0.6 g, 26%) as a white solid, mp 87 °C. Anal. $C_{21}H_{20}O_3$ (C, H).

2,4-Dibenzyloxyphenol ***40***
Concentrated sulphuric acid (two to three drops) was added to a stirred solution of dibenzyloxybenzaldehyde **38** (1.5 g) in MeOH (50 mL), resulting in a thick precipitate. The mixture was stirred for 5 min, then hydrogen peroxide (1 g, 27.5% in water) was added in one portion and stirring was continued for 2 h. The resulting brown solution was treated with 10% aqueous sodium sulphite solution (10 mL) then evaporated to low bulk. The residue was treated with water (50 mL), the solid washed with water, dried and crystallised from cyclohexane, with charcoal treatment, to give **40** (0.3 g, 21%) as a white powder, mp 82–83 °C. Anal $C_{20}H_{18}O_3$ (C, H).

2,4-Dibenzyloxybenzonitrile ***41***
A mixture of 2,4-dibenzyloxybenzaldehyde **38** (1.59 g), diammonium hydrogen phosphate (3.5 g) and nitropropane (15 mL) in glacial acetic acid (5 mL) was refluxed for 15 h. The reaction mixture was evaporated to dryness in vacuo and the residue was stirred with water (100 mL). The insoluble material was filtered and dried at 50 °C to yield **41** as a tan solid (0.6 g, 40%), mp 100 °C. Anal $C_{21}H_{17}NO_2$ (C, H, N).

2,4-Dibenzyloxybenzaldehyde oxime ***42***
A mixture of 2,4-dibenzyloxybenzaldehyde **38** (2 g), hydroxylamine hydrochloride (0.45 g) and pyridine (0.53 mL) in EtOH (20 mL) was heated at reflux for 1 h. The solvent was then removed in vacuo, the residue dissolved in EtOAc and the solution washed with 1 N HCl then with brine. Removal of the solvent in vacuo gives a beige solid which crystallises from a mixture of ether and petroleum ether to give **42** (0.75 g, 36%) in the form of a white solid, mp 130 °C. Anal $C_{21}H_{19}NO_3$ (C, H, N).

2,4-Dibenzyloxynitrobenzene ***43***
Benzyl alcohol (5.4 g) was added slowly to a stirred suspension of sodium hydride (2 g, 60% dispersion in mineral oil) in dry DMF (100 mL). The mixture was stirred for 30 min then 2,4-difluoronitrobenzene (3.2 g) was added in one portion. The resulting black solution was stirred at room temperature for 18 h, evaporated and the residue partitioned between ether (200 mL) and water (100 mL). The organic phase was washed thoroughly with water, dried and evaporated. The residue was triturated with hot EtOH (50 mL) and the mixture cooled in an ice-bath. The insoluble material was purified by filtration through a bed of silica eluting with a mixture of CH_2Cl_2 and petroleum ether, bp 40–60 °C (1:1 v/v). The filtrate was evaporated and the solid crystallised from EtOH to give **43** (2.3 g, 34%) as a cream solid, mp 101–103 °C. Anal $C_{20}H_{17}NO_4$ (C, H, N).

2,4-Dibenzyloxyacetophenone ***44***
2,4-Dihydroxyacetophenone (15.2 g) was alkylated according to the conditions used for compound **38**. Recrystallisation from cyclohexane gave **44**, pale pink solid (28.4 g, 86%), mp 64–65 °C. Anal $C_{22}H_{20}O_3$ (C, H).

5-(2,4-Dibenzyloxyphenyl)-2H-tetrazole hydrate ***45***
A suspension of 2,4-dibenzyloxybenzonitrile **41** (1.89 g) in dry toluene (200 mL) was treated with trimethyltin chloride (6 g) and sodium azide (2 g). The mixture was stirred at reflux for 3 d, evaporated and the residue partitioned between CH_2Cl_2

(250 mL) and water (165 mL). The organic phase was washed with brine (165 mL), dried over magnesium sulphate and evaporated. The resulting semi-solid was triturated with ether (30 mL) and the solid washed with pentane and crystallised from a mixture of EtOAc and pentane to give **45** (1.55 g, 71%) as a white solid, mp 178–179 °C. Anal $C_{21}H_{18}N_4O_2$, 0.2 H_2O (C, H, N).

2,4-Dibenzyloxyphenylacetic acid ***46***

A mixture of 2,4-dibenzyloxybenzaldehyde **38** (10 g), *N*-benzoylglycine (6.32 g), NaOAc (2.65 g), and Ac_2O (10 mL) was heated at 100 °C for 4 h. Water (40 mL) was added and the resulting precipitate filtered, washed with EtOH, water to give 4-(2,4-dibenzyloxybenzylidene)-2-phenyl-4*H*-oxazol-5-one (12.2 g, 84%).

A solution of 4-(2,4-dibenzyloxybenzylidene)-2-phenyl-4*H*-oxazol-5-one (10 g) in 10% aqueous NaOH (300 mL) was refluxed for 24 h. The solution was treated with 40% aqueous NaOH (5 mL), 35% H_2O_2 (5 mL) followed by water (5 mL) at <15 °C, then stirred at 25 °C for 24 h. The solution was acidified, extracted with EtOAc, the extract dried and evaporated. The residue was purified by flash chromatography, eluting with CH_2Cl_2/MeOH 19:1, followed by recrystallisation from EtOAc/pentane to give **46**, yellow solid (0.45 g, 6%), mp 137–139 °C. Anal $C_{22}H_{20}O_4$ (C, H).

3-(2,4-Dibenzyloxyphenyl)propionic acid ***47***

A solution of triethyl phosphonoacetate (7.4 g) in THF (150 mL) was treated with NaH (60%, 1.32 g) at –10 °C and maintained at this temperature for 15 min. A solution of 2,4-dibenzyloxybenzaldehyde **38** (10.0 g) in THF (75 mL) was added dropwise giving an oily precipitate. The THF was decanted and the residue partitioned between aqueous NH_4Cl and EtOAc. The organic layer was dried and evaporated giving ethyl 3-(2,4-dibenzyloxyphenyl)propenoate.

A solution of the unsaturated ester (1.52 g) in toluene (10 mL) was treated with $(Ph_3P)_3RhCl$ (18 mg) and triethylsilane (1.6 mL) and stirred at 25 °C under N_2 for 24 h. The solution was concentrated and the residue purified by flash chromatography, eluting with pentane/EtOAc 5:1 to give ethyl 3-(2,4-dibenzyloxyphenyl)propionate, a colourless oil (1.48 g, 97%).

The ester (1.40 g) was saponified with 15% KOH in MeOH according to the conditions used in compound **29**. Recrystallisation from EtOH gave **47**, colourless solid (0.52 g, 40%), mp 124–126 °C. Anal $C_{23}H_{22}O_4$ (C, H).

(E)-3-(2,4-Dibenzyloxyphenyl)prop-2-enoic acid ***48***

To a solution of 2.5 M *n*-butyllithium (3.64 mL) in dry THF (THF) (20 mL) that was cooled to –70 °C under N_2 was added a solution of diethyl phosphonoacetic acid (0.89 g) in THF (5 mL). After 30 min a solution of 2,4-dibenzyloxybenzaldehyde **38** (1.46 g) in THF (5 mL) was slowly added to the cooled solution so as to maintain the temperature. The resulting mixture was stirred at –70 °C for 3 h and then at 25 °C for 18 h. The resulting suspension was treated with water, the THF was removed in vacuo, the residue was extracted with EtOAc, the extract was dried and evaporated. The residual solid was recrystallised from isopropanol to yield **48** as colourless crystals (0.6 g, 36%), mp 153–155 °C. ^{1}H-NMR (DMSO) 5.15 (2H, s), 5.21 (2H, s), 6.4 (1H, d, J = 16 Hz), 6.7 (1H, d), 6.85 (1H, s), 7.3–7.5 (10H, m), 7.6 (1H, d), 7.8 (1H, d, J = 16 Hz). Anal $C_{23}H_{20}O_4$ (C, H).

3-(2,4-Dibenzoyloxyphenyl)prop-2-ynoic acid ***49***

A mixture of triphenylphosphine (15.7 g) and carbon tetrabromide (9.93 g) in dry CH_2Cl_2 (100 mL) was cooled to 0 °C and stirred for 30 min. A solution of 2,4-dibenzyloxybenzaldehyde (4.76 g) in CH_2Cl_2 (30 mL) was added and the solution stirred at 0 °C for 2 h. The solution was treated with pentane to precipitate triphenylphosphine oxide. The filtrate was evaporated to give 1-(2,4-dibenzyloxyphenyl)-2,2-dibromoethylene as a yellow oil (4.85 g, 67%).

A solution of 1-(2,4-dibenzyloxyphenyl)-2,2-dibromoethylene (4.85 g) in dry THF (100 mL) was treated with 2.5 M *n*-butyllithium (9 mL) at –70 °C under nitrogen. The mixture was stirred for 45 min at –70 °C and then at 25 °C for 45 min. The mixture was then cooled to –40 °C, treated with solid CO_2 (10 g) and then stirred at 25 °C for 24 h. The solution was evaporated, the residue taken up in water, and the pH was adjusted to pH 14 with 1 M NaOH. The aqueous layer was washed with pentane, the pH adjusted to pH 1 and extracted with CH_2Cl_2. The extract was washed with brine, dried and evaporated. The residue was recrystallised from EtOAc/pentane to yield **49** as yellow crystals (1.5 g, 42%), mp 125 °C (dec). ^{1}H-NMR (DMSO) 5.15 (2H, s), 5.25 (2H, s), 6.7 (1H, d), 6.85 (1H, s), 7.3–7.5 (11H, m). Anal $C_{23}H_{18}O_4$ (C, H).

2,4-Dibenzyloxyphenoxyacetic acid ***50***

2,4-Dibenzyloxyphenol **40** (3.1 g) was added to a stirred suspension of sodium hydride (0.44 g, 60% dispersion in mineral oil) and the mixture was heated at reflux for 30 min. The mixture was cooled, treated with ethyl bromoacetate (1.8 g), and then refluxed for 1 h. The reaction mixture was evaporated and the residue partitioned between EtOAc and water. The organic phase was washed with water, dried over magnesium sulphate and evaporated to give a light-brown oil which was purified by column chromatography on silica eluting with CH_2Cl_2. Fractions homogeneous in the required product were combined and evaporated. The residue was triturated with pentane to give ethyl (2,4-dibenzyloxyphenoxy)acetate (3.2 g, 81%) as a white powder, mp 62–64 °C.

This ester (2.0 g) was saponified using 10% aqueous KOH (10 mL) in MeOH (50 mL) at 25 °C for 1 h. The solution was evaporated, brought to pH 1 with aqueous HCl. The precipitate was extracted into EtOAc, dried and evaporated. The residue was recrystallised from cyclohexane/EtOAc to give **50** (0.56 g, 30%), colourless crystals, mp 72 °C. Anal $C_{22}H_{20}O_5$ (C, H).

(E)-2-Benzyl-3-(2,4-dibenzyloxyphenyl)propenoic acid ***51***

A stirred solution of triethyl 2-phosphono-3-phenylpropionate (5.17 g) in dry THF (50 mL) was treated with a solution of *n*-butyllithium in hexane (8 mL, 2.5 M) at –78 °C. After stirring for 15 min a solution of 2,4-dibenzyloxybenzaldehyde **38** (5 g) in dry THF (50 mL) was added dropwise over 5 min at –78 °C. The reaction mixture was stirred at –78 °C for 1 h and allowed to warm to room temperature. After standing at room temperature for 18 h, the reaction mixture was evaporated and the residue partitioned between EtOAc (120 mL) and water (75 mL). The organic phase was washed with brine (50 mL), dried over magnesium sulphate and evaporated. The residue was purified by flash chromatography eluting with CH_2Cl_2. Fractions homogeneous in the required product were evaporated to give ethyl (*E*)-2-benzyl-3-(2,4-dibenzyloxyphenyl) propenoate (1.5 g, 20%) as a yellow oil.

A mixture of this oil (1.5 g) and aqueous KOH (15%) in MeOH (50 mL) was stirred and heated at reflux for 3 h. The reaction mixture was diluted with water (30 mL) plus 3 N KOH solution (5 mL), acidified to pH 1 by addition of concentrated HCl and the resulting solid filtered. Recrystallisation from a mixture of EtOAc and pentane gives **51** (1.13 g, 79%) as a white solid, mp 175–176 °C. Anal $C_{30}H_{26}O_4$ (C, H).

3-(2,4-Dibenzyloxyphenyl)-pyrazole-5-carboxylic acid **52**
A suspension of potassium ethoxide (0.63 g) in ether (50 mL) was treated with diethyl oxalate (1.2 g), cooled to 0 °C, further treated with 2,4-dibenzyloxyacetophenone **44** (2.5 g) and stirred at 25 °C for 24 h. The mixture was partitioned between ether and 0.1 M HCl. The ether layer was washed with water, dried, and evaporated to yield ethyl 4-(2,4-dibenzyloxyphenyl)-2,4-dioxobutanoate as an oil (3.23 g, 99%).

A solution of ethyl 4-(2,4-dibenzyloxyphenyl)-2,4-dioxobutanoate (3.23 g) in EtOH (10 mL) was treated with hydrazine hydrate (0.4 mL), refluxed for 2 h and then cooled to room temperature. The mixture was filtered to yield ethyl 3-(2,4-dibenzyloxyphenyl)pyrazole-5-carboxylate as a solid (1.5 g, 47%).

A solution of ethyl 3-(2,4-dibenzyloxyphenyl)pyrazole-5-carboxylate (1.5 g) in MeOH (20 mL) and THF (10 mL) was treated with aqueous 10% KOH (7.5 mL) and refluxed for 2 h. The solution was evaporated, brought to pH 1 with 1M HCl, and the precipitate filtered. The solid was purified by flash chromatography on silica, eluting with CH_2Cl_2/MeOH 98:2. Fractions homogeneous in the required product were combined and evaporated. The residue was recrystallised from EtOAc to give **52** as colourless crystals (0.13 g, 9%), mp 218–219 °C. ^{1}H-NMR ($CDCl_3$) 5.1 (2H, s), 5.2 (2H, s), 6.68 (1H, d), 6.72 (1H, s), 7.3–7.5 (11H, m), 7.7 (1H, d). Anal $C_{24}H_{20}N_2O_4$ (C, H, N).

3-(2,4-Dibenzyloxy-phenyl)isoxazole-5-carboxylic acid **53**
A mixture of 2,4-dibenzyloxyacetophenone **44** (33.2 g) and diethyl oxalate (21.9 g) in EtOH (250 mL) was treated with sodium (2.53 g) added in small portions over 2 h, and the resulting mixture was stirred at room temperature for 18 h and filtered. The solid was washed with a little EtOH and then washed thoroughly with ether. A portion of this solid (5 g) and hydroxylamine hydrochloride (0.84 g) were suspended in EtOH (100 mL) and 1 N HCl was added until the pH of the mixture reached pH 1. The stirred mixture was heated at reflux for 3 h, during which time all the solids dissolve, then left at room temperature for 18 h. The resulting solid was filtered and washed with ether to give ethyl 3-(2,4-dibenzyloxyphenyl)-isoxazole-5-carboxylate (2.2 g).

A solution of ethyl 3-(2,4-dibenzyloxyphenyl)isoxazole-5-carboxylate (2.2 g) in hot MeOH (100 mL) was treated with 10% aqueous KOH solution (10 mL w/v) and the mixture heated to reflux for 2 h. The reaction mixture was evaporated to low bulk, stirred with 1 N HCl (50 mL) for 15 min and filtered. The resulting solid was washed with water, dried and crystallised from EtOAc to give **53** (1 g, 49%), in the form of a white solid, mp 192–193 °C. Anal $C_{24}H_{19}NO_5$ (C, H, N).

(2,4-Dibenzyloxy-benzamido)acetic acid **54**
A solution of 2,4-dibenzyloxybenzoic acid (3.34 g) and glycine methyl ester hydrochloride (1.25 g) in DMF (40 mL) was treated with triethylamine (2.23 g) and 1-hydroxybenzotriazole (1.53 g) at 0 °C. The mixture was stirred for 10 min, treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride (2.05 g) and allowed to warm to 25 °C. The solution was evaporated. The residue was partitioned between EtOAc and water. The organic layer was washed with 1 M HCl, 1 M NaOH, water, dried and evaporated. The residue was recrystallised from MeOH to yield methyl (2,4-dibenzyloxybenzamido)acetate as a solid (900 mg, 22%). ^{1}H-NMR ($CDCl_3$) 3.7 (3H, s), 4.2 (2H, d), 5.1 (2H, s), 5.2 (2H, s), 6.6 (1H, s), 6.7 (1H, d), 7.3–7.5 (10H, m), 8.2 (1H, d), 8.4 (1H, t).

A solution of methyl (2,4-dibenzyloxybenzamido)acetate (900 mg) in MeOH (20 mL) was treated with 1 M NaOH (20 mL) and stirred at 25 °C for 2 h. The solution was evaporated, the residue diluted with water, brought to pH 1 and extracted with EtOAc. The extract was dried and evaporated. The residue was recrystallised from MeOH to yield **54** as a colourless solid (300 mg, 35%), mp 183 °C. ^{1}H-NMR ($CDCl_3$) 4.15 (2H, d), 5.05 (2H, s), 5.2 (2H, s), 6.6 (1H, s), 6.7 (1H, d), 7.3–7.5 (10H, m), 8.2 (1H, d), 8.4 (1H, t). Anal $C_{23}H_{21}NO_5$ (C, H, N).

3-(2,4-Dibenzyloxybenzamido)propionic acid **55**
The title compound was prepared according to the procedure used for compound **54**, using β-alanine, ethyl ester. The product was recrystallised from MeOH to give **55**, a colourless solid, mp 145 °C. Anal $C_{24}H_{23}NO_5$ (C, H, N).

Pharmacology

Preparation of ET_A receptors
A10 cells were grown to confluence in Dulbecco's modified essential medium containing 10% foetal calf serum. Two days after the final medium change cells were harvested by scraping from the base of the flask and centrifuged at 1500 rpm for 10 min at 4 °C in a bench centrifuge. The resulting pellets were washed in 50 mM Hepes buffer pH 7.3 containing calcium chloride (1 mM) and magnesium chloride (5 mM) and resuspended at a density of 140 000 cells/mL in the same. Cell suspensions were then frozen using a mixture of MeOH and solid carbon dioxide and stored at –20 °C until required. For use in the assay cells were diluted to the required density with Hepes buffer pH 7.3.

Preparation of ET_B receptors
Rats were killed by cervical dislocation and the cerebellum tissue was removed into ice-cold Tris buffer pH 7.4 containing sucrose (0.25 M), ethylenediaminetetracetic acid (3 mM), and a cocktail of protease inhibitors. After homogenising using a glass/Teflon manual homogenise, the samples were centrifuged at 4 °C for 17 min at 1000 *g* and the resulting supernatants were retained. This material was centrifuged at 4000 *g* for 35 min at 4 °C and the pellets were resuspended in 50 mM Tris buffer pH 7.4, and the protein concentration was measured. Aliquots of 100 mL were frozen in a mixture of MeOH and solid carbon dioxide and stored at –20 °C until required. For use in the assay samples were diluted to the required concentration with Tris buffer pH 7.4 containing 0.1% bovine serum albumin.

Binding assay
Assays were performed using Millipore 96-well filtration plates with 0.22 μm filters in a final volume of 250 mL. Mixtures consisting of test compound and [^{125}I]-ET-1 (20 pM) in Tris buffer pH 7.4 containing 0.1% bovine serum albumin were treated with either A10 cells or cerebellum protein. Total and non-specific binding were measured in the absence and presence of unlabelled ET-1 (100 nM). Approximately 60 000 A10 cells were used per well or 5 μg of cerebellum protein. Plates are incubated for 2 h at 37 °C before the reaction is terminated by vacuum filtration. Plates are washed twice with assay buffer at 4 °C and the filters are punched out for gamma counting.

Functional assay
Male Sprague–Dawley rats were sacrificed, the aortae removed, de-endothelialised and placed in organ baths containing oxygenated Krebs at 37 °C. The tissues were equilibrated under 2 g tension and exposed to phenylephrine (30 nM). A maxi-

mum contractile response to 3 μM phenylephrine was then measured. Tissues were incubated for 30 min with vehicle (DMSO) or ligand in the presence of protease inhibitors leupeptin (1 μM), thiorphan (1 μM), bestatin (1 μM), bacitracin (750 units l-1) and captopril (1 μM). A cumulative concentration response curve to ET-1 (0.01–1000 nM) in 0.1% BSA was generated. All results are expressed as percentage of maximal PE response and pK_B values calculated.

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