Design and discovery of nonpeptide endothelin antagonists

Annette M. Doherty

The involvement of the endothelin family of peptide vasoconstrictors has been implicated in a variety of human disease states. Several classes of endothelin antagonists are in early clinical development, and the therapeutic applications of this new mechanistic class of agents are beginning to emerge. This article reviews the different compound classes, their receptor selectivity for the endothelin receptor subtypes ET_A and ET_B and some important studies of selected compounds in animal models of different disease states.

The potent vasoconstrictor endothelin (ET), which was first isolated and characterized from endothelial cells in 1988, belongs to a family of peptides with diverse biological actions[1–5]. These peptides have potent effects on hemodynamic parameters and on renal, cardiovascular, neurological and endocrine function[6,7]. Endothelin is important in fetal development and may play a physiological role in cardiovascular homeostasis[8]. It has been implicated as a potential causative factor in several human disease states, including hypertension, congestive heart failure, atherosclerosis, renal failure, pulmonary hypertension, ischemia and cerebral vasospasm[9–19].

Endothelin receptor subtypes

Two subtypes of ET receptors classified as ET_A and ET_B have been cloned and characterized in animal and mammalian systems[20–23]. A third endothelin receptor subtype has been cloned from *Xenopus* dermal melanophores and heart[24,25], but this subtype has not been described in mammalian tissues.

Both subtypes are widely distributed in animal and human tissues[26–36]. In many animal tissues, vasoconstriction occurs via activation of ET_A and/or ET_B receptors, depending upon the species and vascular bed[27–36]. The ET_B receptor on endothelial cells mediates vasodilation[27], and there is some functional evidence to suggest that ET_B receptor subtypes may exist on smooth muscle cell and endothelial cell receptors[35]. The importance of ET_B receptors in mediating vasoconstrictor responses in mammalian tissues is a subject of current study[30–33]. It has been reported that ET_A-mediated vasoconstriction plays a major role in some human vessels, such as coronary artery, but ET_B-receptor-mediated contractions in human tissues using ET_B-selective agonists such as [Ala 1,3,11,15]ET-1 and BQ 3020 appear considerably less potent[30]. However, Luscher and coworkers have reported that ET_B receptor mRNA was detected by northern blot analysis in human internal mammary artery and aortic smooth muscle cells[33]. Several groups have shown that the selective ET_B receptor agonist sarafotoxin-6C (SRTX-6C) can elicit vasoconstriction in human vessels, albeit with lower potency than is seen with ET-1 itself[34–36]. Downregulation of ET_B receptors in isolated tissues may be responsible for these observations[37].

Several peptide ET antagonists have been reported since the discovery of endothelin; these have been identified both through compound library screening, with subsequent optimization, and through rational design techniques from the

Annette M. Doherty, Department of Chemistry, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48105, USA. tel: +1 313 996 7710, fax: +1 313 996 3107

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agonist itself[38–49]. Many of these have proved to be useful tools in exploration of the pharmacology and physiology of the endothelin system, but this article focuses on recently identified nonpeptide receptor antagonists. The development of nonpeptide, low molecular weight antagonists with high potency, selectivity and oral activity is an important objective to enable exploration of the potential role of ET and its isopeptides in both acute and chronic human diseases[3,9–19].

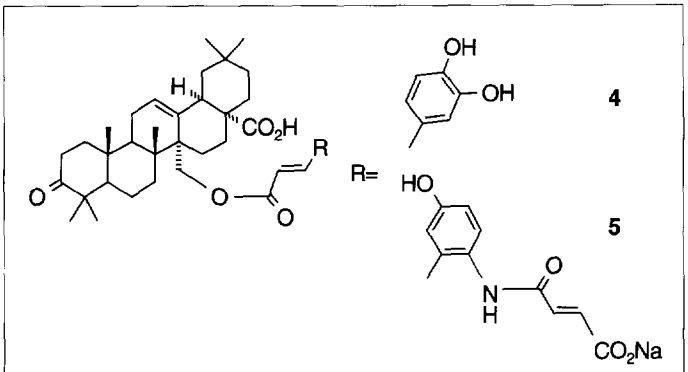


Figure 1. *ET_A-selective antagonist compound 50-235 (**4**), isolated from bayberry* Myrica cerifera. *Optimization led to the steroid analog 97-139 (**5**).*

Nonpeptide ET antagonists from screening and lead optimization approaches

Anthraquinone ET_A-selective antagonists

Several anthraquinones (Box 1) were reported to be ET_A-selective antagonists produced by a strain of *Streptomyces*[50,51]. The two acids **1** and **3** have micromolar affinity for the ET_A receptor (porcine aorta) and no activity against a variety of ET_B tissues. Esterification of the acid group eliminated all ET_A receptor affinity (WS009A ester, **2**). Both acids are antagonists that block the ET-1-induced increase of inositol phosphates in rat aortic rings (ET_A). When administered intravenously at a dose of 10 mg kg^{-1}, WS009A (**1**) antagonizes the pressor effect of an intravenous bolus injection of ET-1 (3.3 μg kg^{-1}) in spontaneously hypertensive rats without any effect on the depressor response.

Another anthraquinone series of weakly active ET receptor antagonists of unknown specificity, discovered through screening from a *Streptomyces* strain, have been disclosed[52,53]. Optimization of the activity of these compounds has not been reported.

Steroid ET_A-selective antagonists

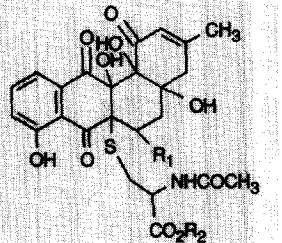
Another nonpeptide ET_A receptor antagonist of natural origin has been isolated from bayberry, *Myrica cerifera* (Figure 1)[54]. This compound, 50-235 (**4**), selectively antagonized specific binding of [125I]ET-1, but not of [125I]ET-3, to rat cardiac membranes and antagonized the ET-1-induced increase in intracellular free calcium concentration in Swiss 3T3 fibroblasts and contraction of rat aortic strips[54]. This compound inhibited [125I]ET-1 binding to rat aortic smooth muscle cells A7r5 (ET_A) with a K_i of 51±12 nM, and had no effect on [125I]ET-1 or [125I]ET-3 binding to Girardi heart cells (ET_B). This antagonist also inhibited the contractile response elicited by ET-1 in isolated rat thoracic aorta (ET_A) with a pA_2 value of 6.65 (Ref. 54). It exhibits 500-fold selectivity for human ET_A receptors compared with ET_B receptors[55]. Consistent with some other studies that indicate mitogenesis to be mediated primarily via the ET_A receptor in certain tissues, 50-235 inhibited ET-1 promoted mitogenesis of A7r5 cells[56]. Structure–activity studies have revealed that the 3-keto, 17-carboxyl and 27-caffeoyl groups in 50-235 are important for ET_A blocking activity[57].

Antagonist 50-235 has been optimized further to lead to the steroid analog 97-139 (**5**; Figure 1)[58]. In rat aortic smooth muscle cells that express ET_A receptors and human Girardi heart cells that express ET_B receptors, 97-139 displaced specifically bound [125I]ET-1 with the K_i values of 1 and 1,000 nM respectively. It also inhibited ET-1-induced thymidine incorporation in A7r5 cells with an IC_{50} of 0.92 nM. Administration of this antagonist to pithed rats (0.03–1.0 mg kg^{-1}) resulted in a dose-dependent inhibition of the pressor response to ET-1, although the compound was not as potent *in vivo* as might have been expected from the *in vitro* activity.

Box 1. Structure and binding affinities of some anthraquinone ET_A- selective antagonists

Compound	R_1	R_2	Binding IC_{50} (μM) ET_A	ET_B
WS009A **(1)**	H	H	5.8	ia[b]
WS009A ester **(2)**	H	CH_3	nr[a]	ia
WS009B **(3)**	OH	H	0.67	ia

[a]nr, not reported
[b]ia, inactive



Diphenyl ethers from screening of fungal broths

A series of substituted diphenyl ethers (Box 2), including asterric acid (**6**), discovered by screening of fungal broths, has been reported to possess ET antagonist activity in the micromolar range[59,60]. Analogs with increased activity have not been reported. Asterric acid (R_1 = COOH, R_2 = OH, R_3 = CO_2CH_3, R_4 = OH) exhibits ET_A receptor binding in A10 cells with an IC_{50} of 10 μM. Conversion of the acidic groups of asterric acid to either an ester or a hydrazide, gave inactive derivatives. Hydrolysis of the ester group of asterric acid to a second acid group also abolished activity.

Aryl sulfonamides from compound library screening

A nonpeptide ET_A-selective antagonist, BMS 182874 (**17**), has recently been designed through optimization of a compound library screening hit, sulfathiazole (**10**)[61–63]. These aryl sulfonamides (**10–17**; Box 3), have IC_{50} values ranging from 150 nM to 69 μM at the ET_A receptor (A10 cells), with reportedly no binding affinity to ET_B receptors. The sulfonamide hydrogen appears critical to receptor binding affinity because the *N*-methyl derivative was devoid of activity. The two methyl groups of the isoxazole are also important for binding, the 4-methyl group being critical for binding. Modification of the 3-methyl group to larger alkyls and aryls led to a loss of binding affinity. Substitution on the phenyl group yielded several potent compounds, but only those containing an alkyl- or arylalkyl-amino group exhibited functional activity (**17**, K_b = 100 μM; **16**, K_b >> 100 μM).

As lipophilicity on the phenyl group was increased, activity was found to improve, and thus a series of naphthalenesulfonamides was synthesized (Box 3). The 1,5-disubstitution pattern was preferred for optimal receptor affinity, **14–17**. BMS 182874 (**17**) exhibits ET_A binding affinity IC_{50} of 150 nM (A10 cells) and displays functional antagonism by inhibition of ET-1-induced increase in intracellular Ca^{2+} in A10 cells with an IC_{50} of 570 nM and a K_b value of 520 nM in rabbit coronary artery ring. Oral activity was also demonstrated for BMS 182874 in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. A dose of 100 μmol kg^{-1}, either intravenous or oral, provided a maximal drop in blood pressure of 32–45% with a sustained drop of 25–12%, 12–24 h after administration. A similar series of aryl sulfonamides has been reported by the Immunopharmaceutics group[64].

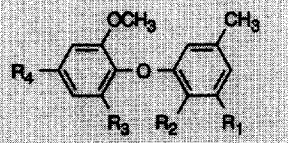
Butenolides as ET_A-selective and ET_A/ET_B antagonists

An initial lead structure identified from library screening, PD 012527, was modified to discover more potent orally-active, ET_A-selective antagonists, exemplified by PD 155080 and PD 156707 (**18**, **19**, **20**; Figure 2)[65,66]. Preliminary enhancement of the receptor binding affinity of the initial lead compound was achieved through application of the Topliss 'Decision Tree' approach for lead optimization, which is based upon QSAR principles[67,68]. This is a nonmathematical, nonstatistical and noncomputerized guide to the use of basic Hansch principles, including electronic, lipophilic and steric considerations, for the optimization of activity of a lead structure containing benzene rings. This approach to optimization of the substituents on each of the phenyl rings in the butenolide structure led to PD 155080 and, with further SAR development, to PD 156707 (Ref. 65).

PD 155080 is a potent competitive inhibitor of [^{125}I]ET-1 and [^{125}I]ET-3 binding to human cloned ET_A and ET_B receptors, with IC_{50} values of 7.8 nM and 3.5 μM, respectively. The compound also antagonizes ET-1-induced arachidonic acid release in rabbit renal artery vascular smooth muscle cells (VSMC) with an IC_{50} of 0.15 μM. PD 156707 is approximately tenfold more potent than PD 155080 in binding to human cloned ET_A and ET_B receptors, with IC_{50} values of 0.3 nM and

Box 2. Series of diphenyl ether ET antagonists isolated from fungal broths

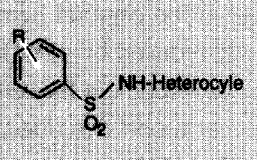
Compound	R_1	R_2	R_3	R_4	Binding IC_{50} (μM) ET_A	ET_B
6	COOH	OH	$COOCH_3$	OH	10[a]	>10
7	$COOCH_3$	OCH_3	$COOCH_3$	OCH_3	>10	nr[b]
8	COOH	OH	COOH	OH	>10	nr
9	$CONHNH_2$	OH	$COOCH_3$	OH	>10	nr



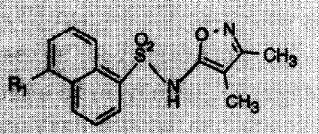
[a]A10 cells
[b]nr, not reported

Box 3. Aryl sulfonamide ET_A antagonists

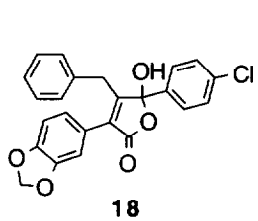
Compound	Heterocycle	R	Binding IC_{50} (μM) ET_A[a]	ET_B[b]
10	2-thiazolyl	4-NH_2	69	ia[c]
11	3,4-dimethyl-5-isoxazolyl	4-NH_2	0.78	ia
12	3,4-dimethyl-5-isoxazolyl	4-OH	9.2	ia
13	3,4-dimethyl-5-isoxazolyl	4-$NHCH_2C_6H_5$	9.8	ia



Compound	R	Binding IC_{50} (μM) ET_A	ET_B
14	H	20	ia
15	OH	7.8	ia
16	NH_2	4.0	ia
17	$N(CH_3)_2$	0.15	>200



[a]A10 cells
[b]rat cerebellum
[c]ia, inactive



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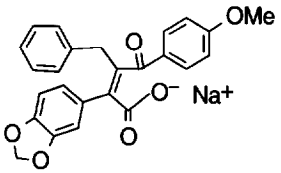

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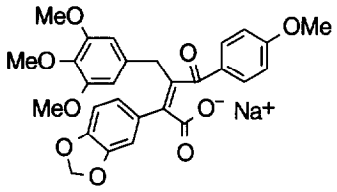

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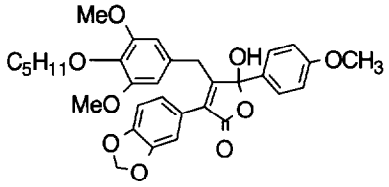

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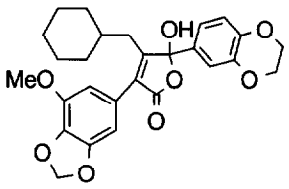

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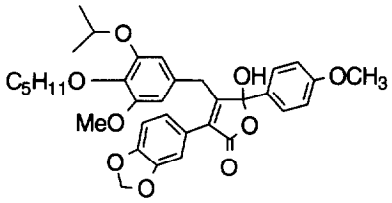

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Figure 2. *Butenolide ET antagonists. PD 012527 (**18**) has been modified to yield more potent compounds exemplified by PD 155080 (**19**) and PD 156707 (**20**). Modification of substituents around the butenolide ring in PD 155080 and PD 156707 has led to compounds with differing selectivities for human ET_A and ET_B receptor subtypes: PD 160672 (**21**), PD 160874 (**22**) and PD 162073 (**23**).*

0.42 μM, respectively. PD 156707 antagonizes ET-1-induced arachidonic acid release in rabbit renal artery VSMC with an IC_{50} of 1.1 nM (Ref. 65).

The pharmacokinetics of PD 155080 and PD 156707 have been compared in male Wistar rats following a 15 mg kg^{-1} intravenous or oral gavage dose (three animals per dose). Oral bioavailabilities are 87% for PD 155080 and 41% for PD 156707 (Ref. 69). Both compounds are also highly potent and selective in the human vasculature[70].

The cerebrovascular effects of the endothelin antagonists PD 155080 and PD 156707 have been described in addition to efficacy in a cat model of acute stroke with PD 156707.

Intravenous administration of PD 156707 (5 μMol kg^{-1} bolus followed by 3 μMol kg^{-1} infusion), 30 min after occlusion of the middle cerebral artery, restored cerebral blood flow to pre-occlusion baseline levels at 6 h. The volume of ischemic damage in the cerebral hemisphere after occlusion was significantly reduced by 45% (Refs 71,72). PD 156707 is in early clinical development for cerebral ischemia.

A recent report described the SAR and ET_A/ET_B selectivity of the PD 155080 and PD 156707 series of orally-active nonpeptide ET antagonists[69]. Modification of the substituents around the butenolide ring has led to compounds with differing selectivity for human ET_A and ET_B receptors (Figure 2). Thus, compounds with increased lipophilicity at R_2 show increased ET_B affinity and a more balanced ET_A/ET_B profile. For example, PD 160672 (**21**), the 4-*O*-*n*-pentyl analog of PD 156707, is a potent competitive inhibitor of [^{125}I]ET-1 and [^{125}I]ET-3 binding to human cloned ET_A and ET_B receptors, with IC_{50} values of 0.8 nM and 44 nM respectively[69].

PD 160874 (**22**) is a competitive inhibitor of [^{125}I]ET-1 and [^{125}I]ET-3 binding to human cloned ET_A and ET_B receptors, with IC_{50} values of 3.5 nM (ET_A) and 8.9 nM (ET_B) respectively. Another compound of interest in this series is PD 162073 (**23**) with IC_{50} values of 2.4 nM (ET_A) and 50 nM (ET_B) respectively[69]. Structure–activity relationship studies of the nonpeptide orally-active PD 156707 series of ET antagonists have led to compounds with selectivity ratios for ET_A and ET_B receptors ranging from >2000- to <10-fold[69].

Diketopiperazines

A series of weakly-active nonpeptide ET antagonists (**24**, **25**; Box 4) exemplified as derivatized diketopiperazines has been described[73]. These derivatives appear to be ET_A-selective, but minimal data have so far been reported.

Substituted imidazole thiazoles and oxazoles as ET_A-selective antagonists

Peptidomimetic analogs containing substituted imidazoles, thiazoles and oxazoles (Figure 3) have recently been reported as ET_A-selective antagonists (ET_B receptor binding affinity not reported) with IC_{50} values ranging from 0.9 nM to 53 nM for inhibition of ET-1-induced phosphatidylinositol hydrolysis[74].

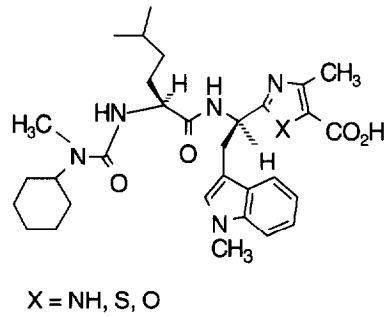


Figure 3. *Peptidomimetic analogs with substituted imidazoles, thiazoles and oxazoles are ET_A-selective antagonists.*

Benzofuran carboxylic acids and 9-substituted acridines as ET_A-selective antagonists

Benzofurancarboxylic acids (**26**) and 9-substituted acridines (**27**) have also been described as ET_A-selective antagonists with micromolar activity in inhibiting ET-1-induced arachidonic acid release in rabbit renal vascular smooth muscle cells (Figure 4)[75,76].

Substituted pyrrolidine carboxylic acids as ET_A-selective antagonists

Very recent disclosures include a series of substituted pyrrolidine carboxylic acids as ET_A-selective antagonists (Figure 4). A-127722 (**28**)[77], which was derived from the indane series of antagonists developed earlier by SmithKline Beecham (see SB 209670 below), is an *N,N*-dibutyl acetamide with IC_{50} value of 0.15 nM for the rat ET_A receptor.

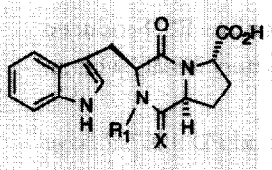
Substituted phenyl derivatives as ET_A-selective antagonists

Substituted phenyl derivatives exemplified by **29** (Figure 4) have been reported in the patent literature by Rhône-Poulenc Rorer. These compounds are reported to be ET_A-selective, although no biological data have been reported. The compounds are structurally related to the potent Merck ET_A/ET_B antagonist, L-749,329 (discussed below)[78].

Box 4. Weakly-active diketopiperazine ET antagonists

Compound	R_1	X	Binding IC_{50} (μM)[a] ET_A	ET_B
24	H	O	22.5	–
25	BOC	H_2	31.1	–

[a]TE671 cells



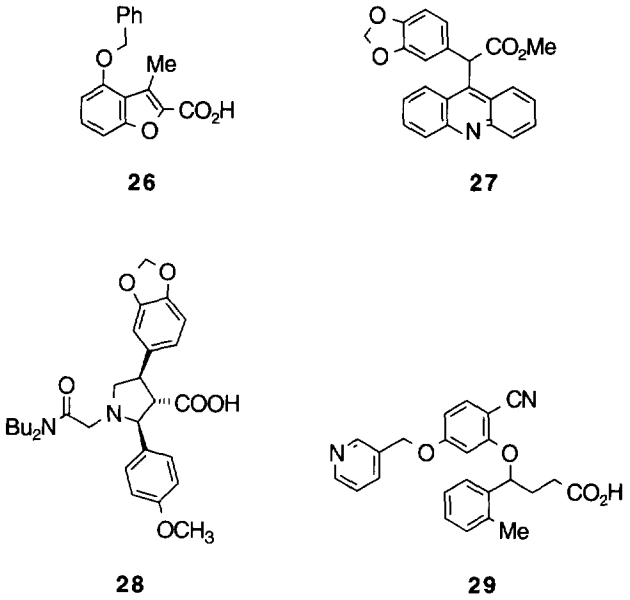


Figure 4. *ET_A-selective antagonist activity has been demonstrated for benzofurancarboxylic acids (**26**) and 9-substituted acridines (**27**) in rabbit renal vascular smooth muscle cells, and has recently been reported for the N,N-dibutyl acetamide A-127722 (**28**)[77]. Substituted phenyl derivatives exemplified by **29** are reported to be ET_A-selective, although biological data are not yet available.*

Phenyl sulfonamide ET_A/ET_B antagonists

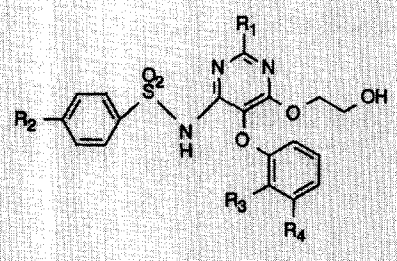
Several nonpeptide ET_A/ET_B antagonists have been reported in the literature including *N*-(4-pyrimidinyl)phenyl sulfonamides[15,79–81]; specifically, the nonselective ET_A/ET_B antagonist Ro 46-2005 (**30**)[15,77] and Ro 47-0203 (**33**) (Refs 78,79). Thus, a series of trisubstituted and/or tetrasubstituted pyrimidines, which are balanced ET_A/ET_B antagonists (**30–33**) discovered by SAR of leads from compound library screening, are shown in Box 5 (Refs 15,79). Ro 46-2005 has ET_A receptor binding IC_{50} value of 216 nM in human smooth muscle cells and ET_B receptor binding value of 221 nM in porcine cerebellum. Ro 46-2005 inhibited the ET-1-induced contraction in rat aortic rings (ET_A) with a pA_2 of 6.5 and SRTX-6C-induced contraction in mesenteric arteries (ET_B) with a pA_2 of 6.5. This agent is a selective ET receptor antagonist because it does not antagonize contractions caused by potassium, serotonin, angiotensin II, prostaglandin $F_{2\alpha}$, thromboxane A_2 analog or acetylcholine[79]. The addition of a fourth substituent at the 2-position of the pyrimidine ring seems to impart an approximate tenfold increase in ET_A potency. In addition, the electronic properties of substitution on the phenoxy group also play an important role on receptor affinity.

The *in vivo* efficacy of Ro 46-2005 has been reported in several animal models. Ro 46-2005 prevented postischemic renal vasoconstriction in rats (3 mg kg^{-1} intravenous dose) by causing a 66% increase in renal blood flow versus control[15]. The decrease in cerebral blood flow caused by subarachnoid hemorrhage (SAH) in rats was also alleviated by Ro 46-2005. In this model, a 3 mg kg^{-1} dose of Ro 46-2005 dramatically increased blood flow in comparison to controls when rats were subjected to SAH by injection of autologous blood in the cisterna magna. Although no significant effect on mean arterial blood pressure (MABP) was seen in the rat renal or SAH models, Ro 46-2005 (10–100 mg kg^{-1}) exhibited a dose-dependent reduction in MABP in sodium-depleted squirrel monkeys[15].

By comparison of the oral and intravenous administration of Ro 46-2005, an oral bioavailability of 30% has been observed in rats, with peak blood levels achieved 15 min after

Box 5. Series of tri- and/or tetrasubstituted pyrimidines discovered by SAR of leads from compound library screening

Compound	R_1	R_2	R_3	R_4	Binding IC_{50} (μM) ET_A	ET_B
30	H (Ro 46-2005)	*t*-Bu	H	OCH_3	0.216[a]	0.221[c]
31	H	*i*-Pr	OCH_3	H	0.073[a]	nr[d]
32	CF_3	*i*-Pr	OCH_3	H	0.05[a]	nr
33	3-pyrimidyl (Ro 47-0203)	*t*-Bu	OCH_3	H	0.020[b]	0.150[b]



[a]human placental membranes
[b]human CHO expressed ET_A or ET_B receptors
[c]porcine cerebellum membranes
[d]nr, not reported

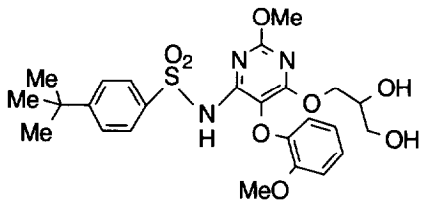


Figure 5. *Ro 46 8443 (**34**), an ET_B-selective nonpeptide antagonist discovered following SAR studies around the bosentan series.*

intravenous administration and approximately 4 h after oral administration.

Bosentan (**33**) has improved *in vitro* binding affinity at both receptor subtypes [ET_A, IC_{50} = 20 nM and ET_B, IC_{50} = 150 nM (human)][80,81]. It inhibits contraction in isolated rat aorta (ET_A) pA_2 = 7.4 and in rat tracheal contraction (ET_B) pA_2 = 6.8. In a rabbit SAH model, a 30 mg kg^{-1} dose reversed (rather than prevented) vasoconstriction[81]. The same dose lowered MABP in stroke-prone spontaneously hypertensive rats and in salt-depleted animals but not in normotensive rats. An infusion of the peptide antagonist BQ-123 produced a 20-mmHg drop in MABP in spontaneously hypertensive rats, and an additional infusion of bosentan lowered the MABP by 40 mmHg.

Bosentan has been shown to antagonize the vasomotor effect of topical endothelin (10 nM) on feline pial arteries *in situ*, after either topical (1 μM) or intravenous administration (17 μMol kg^{-1})[82]. This compound has been studied in several other disease models, including models of pulmonary hypertension[83] and myocardial ischemia[84]. In rats exposed to hypoxia for 15 days and simultaneously treated with bosentan, pulmonary arterial pressure was lower and right ventricular hypertrophy was less severe than in control animals. In the rat model of ischemia–reperfusion, bosentan appeared to exert no effect on myocyte or coronary endothelial injury. Bosentan has been selected for clinical studies and is reported to be in clinical trials for SAH, hypertension and congestive heart failure[85].

An ET_B-selective nonpeptide antagonist, Ro 46 8443 (**34**; Figure 5), discovered by performing SAR studies around the bosentan series has been reported by the Roche group[86].

Dihydropyridine anhydrides as ET_A/ET_B antagonists

The dihydropyridine anhydride CGS 27830 (**35**; Box 6) evolved from the calcium ion modulators of similar structure, presumably by compound library screening[87]. The anhydride was inactive as a calcium channel antagonist but showed ET_A (porcine thoracic aorta membranes) and ET_B (rat cerebellum membranes) receptor binding affinity, with IC_{50} values of 15.9 nM and 295 nM respectively. CGS 27830 also produced a dose-dependent inhibition of ET-1-induced contraction in isolated rabbit aorta, with a maximum attenuation of 77% at 10 μM. The compound was ineffective at altering the effects of KCl or phenylephrine, suggesting that CGS 27830 is a specific antagonist of ET receptors. A 5-min pretreatment (10 mg kg^{-1} intravenous dose) in a conscious rat abolished the pressor response caused by an ET-1 challenge and attenuated the depressor response. The half-life of this antagonist was however found to be rather short ($T_{1/2}$ < 60 min). The two optical centers in the anhydride appear to be important. The SS derivative was inactive at both receptors and the RR derivative was much less active (ET_A, IC_{50} = 422 nM and ET_B, IC_{50} = 2.7 μM)[87]. No additional ring substitutions have been published.

Box 6. The dihydropyridine anhydride CGS 27830 (35) is inactive as a calcium channel antagonist but has been shown to possess ET_A and ET_B receptor binding affinity

Compound	#	*	Binding IC_{50} (μM) ET_A	ET_B
CGS 27830 (**35**)	S	R	0.0159[a]	0.295[b]
	S	S	ia	ia[c]
	R	R	0.422[a]	2.7[b]

[a]porcine thoracic aorta membranes
[b]rat cerebellum membranes

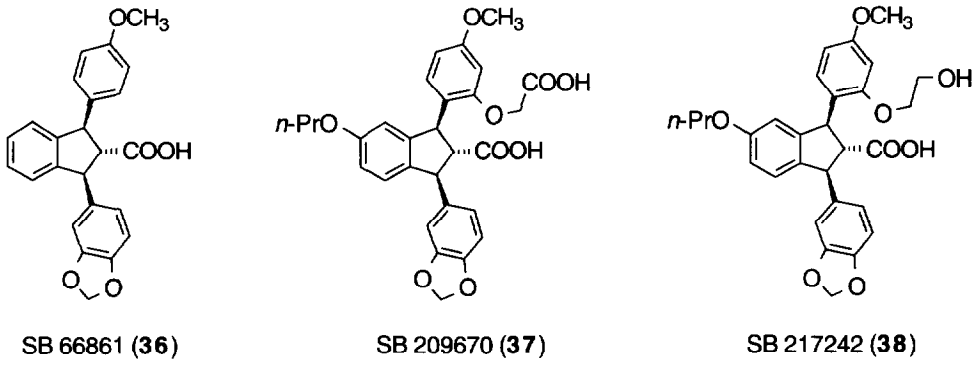


Figure 6 *Series of trisubstituted indane carboxylic acids discovered recently by researchers at SmithKline Beecham.*

Indane carboxylic acids as ET_A/ET_B receptor antagonists

A series of trisubstituted indane carboxylic acids have also been discovered recently by researchers at SmithKline Beecham (Figure 6); SB 209670 (**37**) is representative of this series[84,85]. This compound was rationally designed using [^{1}H] NMR-derived conformational models of ET-1. Thus the 1- and 3-phenyl groups of a lead structure SB 66861 (**36**) discovered from compound library screening were considered to be mimics of a combination of two of the aromatic side-chains of Tyr13, Phe14 and Trp21 in ET-1. The carboxylic acid group was proposed to mimic either the Asp18 or C-terminal carboxyl in ET-1. (+)-SB 209670 has potency at both ET_A and ET_B receptors; K_i = 0.2 nM and 18 nM, respectively (human cloned). SB 209670 did not affect the basal hemodynamic parameters, with continuous intravenous or bolus intravenous administration, in male Sprague Dawley rats. However, a 5-min pretreatment of SB 209670 (1 mg kg^{-1}) selectively attenuated the initial depressor response caused by an intravenous bolus injection of ET-1 (0.3 nM kg^{-1}); the secondary pressor response was unaffected by this dose. At a dose of 10 mg kg^{-1}, SB 209670 abolished both responses caused by ET-1. SB 209670 has further been shown to be effective at reversing the effects of ischemia-induced acute renal failure in the rat[90]. At a dose of 30 μg kg^{-1} min^{-1}, SB 209670 induced a significant change in several renal functions and resulted in a 75% increase in survival. SB 209670 was also shown to be an effective antihypertensive agent in spontaneously hypertensive rats and to have no effect in normotensive rats[91]. MABP was reduced at doses of 10 μg kg^{-1} min^{-1} infusion and 10 mg kg^{-1} intraduodenally, but cardiac output and heart rate were unaffected.

SB 209670 has also been shown to protect from ischemia-induced neuronal degeneration in a gerbil stroke model[88] and to attenuate neointima formation following rat carotid artery balloon angioplasty[89]. The compound also inhibited the cerebral vasospasm in the basilar and anterior spinal arteries on the seventh day in a canine SAH model[93].

A closely related hydroxyl analog (**38**) of SB 209670 has been reported to exhibit improved pharmacokinetics and oral bioavailability (Figure 6)[94].

Several patents displaying variations of the ring system around SB 209670 have been reported recently, including cyclopentanes and bicyclic 6,6- and 6,7-ring systems (Figure 7)[95–98].

Propanoic and propenoic acids

Recently, a second series of ET antagonists from this group has been disclosed. These derivatives are the tri- or tetra-substituted propanoic or propenoic acids (Figure 8) and are exemplified by compound **39** (Ref. 95). No biological data have been reported for these analogs.

Phenylacetamides as ET_A/ET_B antagonists

The nonpeptide orally-active ET antagonist L-749,329 (**40**) antagonizes the binding of [^{125}I] ET-1 in CHO cells expressing human ET receptors with IC_{50} values of 0.8 nM (ET_A) and 16 nM (ET_B) respectively (Figure 8)[99]. L-749,329 is a competitive antagonist of ET-1-induced contractions in isolated rabbit iliac artery rings, and *in vivo* blocks ET-1-induced pressor effects

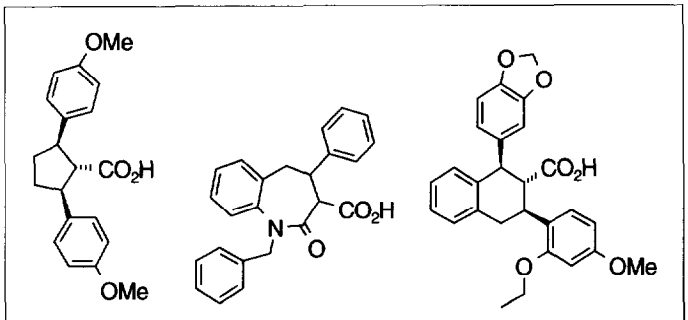


Figure 7. *Cyclopentane and bicyclic 6,6 - and 6,7-ring system variations around SB 209670.*

in ferrets and rats[100]. A closely related analog, L-751,281 (**41**), with a methylene group replacing the ether oxygen, possessing similar activity at both ET_A and ET_B receptors, has been reported (Figure 8)[101].

New series reported

Substituted 8,9-dicarboxyldibenzo[2,3:5,6]bicyclo[5.2.0]nonan-4-ones have been reported to have moderate binding affinity to both ET_A and ET_B receptors (Figure 9)[102].

A series of dibenzodiazepine-10-acetic acid derivatives (Figure 10) were designed from the cyclic pentapeptide antagonist BQ-123 and found to have moderate activity for ET_A and ET_B receptors[103].

A class of benzimidazoles with high affinity (nanomolar range), and with either ET_A or ET_A/ET_B receptor selectivities depending on the aromatic ring substitution pattern, have been reported; the class includes PD 159433 (**42**) and PD 159020 (**43**; Box 7)[104].

Several new classes of ET antagonists of unknown selectivity or potency have appeared in the patent literature very recently[105–108], and reports on the discovery, design and affinity of these compounds are expected in the near future.

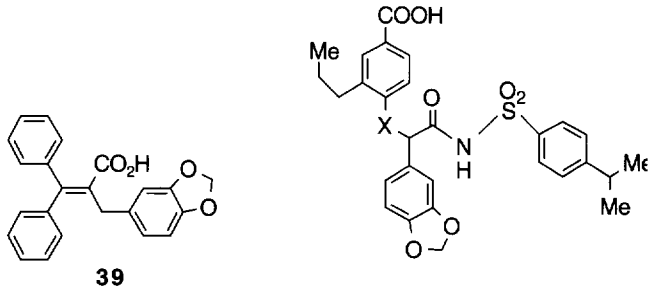


Figure 8. *An example of the tri- and tetra-substituted propenoic acid ET antagonists. (**39**) and phenylacetamide endothelin antagonists (**40**,**41**).*

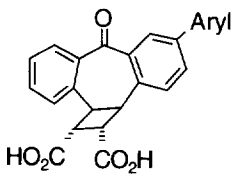


Figure 9. *Substituted 8,9-dicarboxyldibenzo[2,3:5,6]-bicyclo[5.2.0]nonan-4-ones have been reported to have moderate binding affinity to both ET_A and ET_B receptors.*

Peptidomimetic ET_B-selective antagonists

ET_B-selective antagonists (Figure 11) that are totally non-peptidic in nature have not been reported; however, two interesting peptidomimetic structures have been described, including BQ-788 (**44**)[109] and a series of tryptophan analogs exemplified by compound **45** (Ref. 110). These compounds should have applications in elucidating the role of the ET_B receptor in endothelin physiology and pathophysiology.

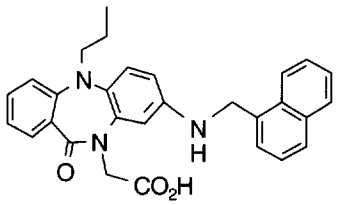
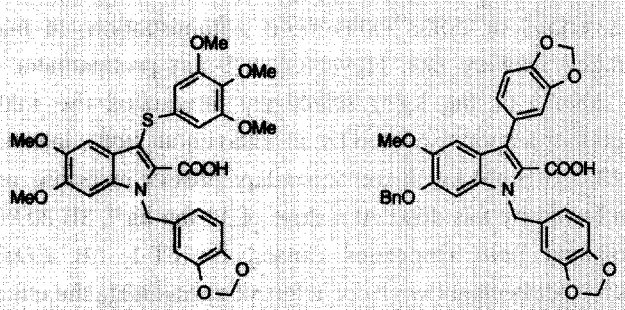


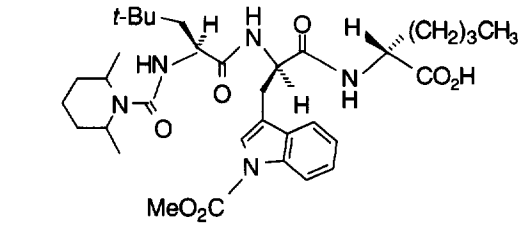
Figure 10. *Dibenzodiazepine-10-acetic acid derivatives designed from the cyclic pentapeptide antagonist BQ-123 have been found to have moderate ET_A and ET_B receptor antagonist activity.*

Box 7. High-affinity benzimidazoles with either ET_A or ET_A/ET_B receptor selectivities, depending on the aromatic ring substitution pattern



Compound	Binding IC_{50} (nM)[a]	
	ET_A	ET_B
PD 159433 (**42**)	2	380
PD 159020 (**43**)	30	50

[a]Binding data obtained using cloned human receptor assays[104]



BQ-788 (44)

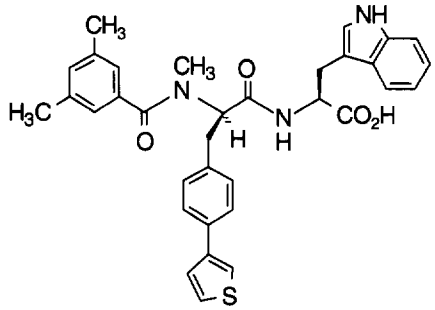


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Figure 11. *Totally nonpeptidic ET_B-selective antagonists have not yet been reported, but BQ-788 and a series of tryptophan analogs exemplified by compound* ***45*** *show promise for the elucidation of the role of the ET_B receptor.*

Conclusion

In recent years, there has been remarkable progress in the discovery of potent and selective endothelin antagonists. Several compounds are being studied intensively in different disease states, particularly the more potent compounds, such as the ET_A/ET_B antagonists bosentan (**32**), SB 209670 (**37**) and L-749,329 (**40**), and the ET_A-selective agents PD 156707 (**20**) and A-127722 (**28**). Bosentan is in early clinical development for SAH, heart failure and hypertension and possibly other indications. Some other compounds described in this review are in the late stages of preclinical evaluation and for some, toxicology studies have begun.

Disease areas of particular interest for therapeutic applications of this new class of agents include renal failure, pulmonary hypertension, heart failure, atherosclerosis, cerebral ischemia and vasospasm. The timing and therapeutic indication(s) where an ET_A and/or ET_A/ET_B antagonist will find clinical use are unclear at present. However the next few years are likely to be very exciting for those involved in both the preclinical and clinical study of this new class of pharmaceutical agents.

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