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Endothelins – an overview

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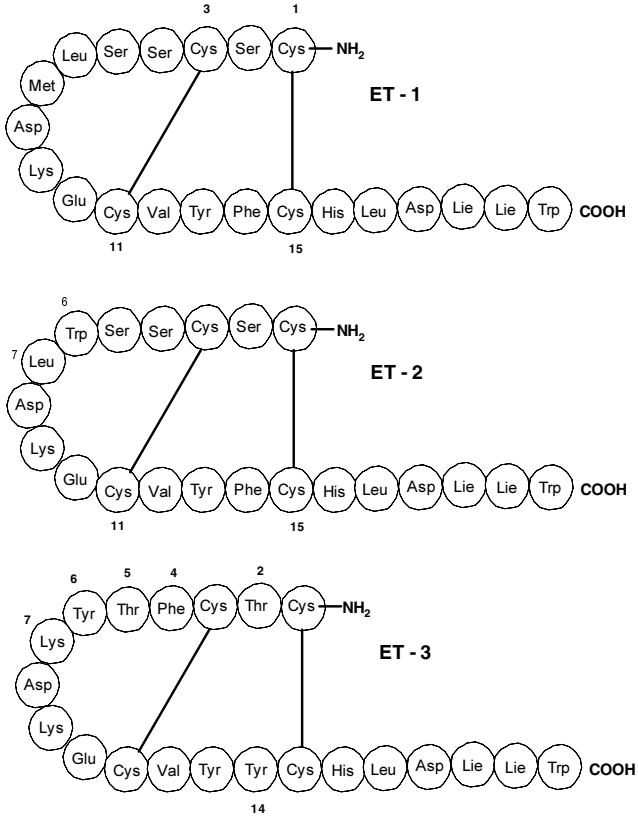
1. Introduction

A polypeptide composed of 21 amino acids, named endothelin (ET), discovered in 1988 [1] is suggested to play a active role in the etiology of various diseases. ET is the expression product of the prepro ET gene. The prepro ET gene produces prepro ET, which is proteolytically cleaved to form pro ET also known as big ET-1 [2, 3]. A protease enzyme called "ET converting enzyme" (ECE) cleaves Trp^{21}-Val^{22} of big ET to form the mature ET peptide. Patients with hypertension, coronary vasospasm or acute myocardial infarction have increased levels of ET in their blood stream [4, 5]. ET concentration is also found to be high in patients having bronchial asthma [6] and it has also been proved to be one of the most potent constrictors of vascular smooth muscle. ET receptors are present in high concentration in the peripheral tissues and also in the central nervous system. The recent availability of potent and selective antagonists of the endothelin receptors has allowed a more convincing case for the involvement of endothelin in disease to be developed, and therefore it follows that endothelin receptor antagonists will ultimately emerge as therapeutic agents. Advancement in the cloning of ECE [7] (endothelin converting enzyme) and the recent studies of endothelin-1 (ET-1), a potent vasoconstrictor from endothelial cells may help in new drug discovery. The endothelium has been proposed to mediate vasoconstriction via the production of endothelium-derived vasoconstriction factor(s) (EDCF) in response to various chemical and physical stimuli [8, 9].

2. Conformational studies

The endothelin isopeptides are a family of three peptides: endothelin-1, endothelin-2, and endothelin-3. Each peptide is composed of 21 amino acids with disulfide bridges between Cys^1-Cys^{15} and Cys^3-Cys^{11}. The primary sequence of human endothelin has been deduced from a human placental cDNA library and found to be identical to that of



porcine endothelin, now referred to as endothlin-1 (ET-1)[2]. Other than ET-1, two other peptides have been reported and designated as endothelin-2 (ET-2) and endothelin-3 (ET-3), differing by 2 and 6 amino acid residues, respectively [10, 11]. The sarafotoxins (cardiotoxic peptides) isolated from the venom of the burrowing asp, *Atractaspis engaddensis*, show a remarkable sequence homology with the endothelin peptides [12, 13]. NMR studies of human ET-1 in DMSO-d6 have suggested a well-defined conformation in the N-terminal core region (residues 1–15). Three-dimensional NMR and distance geometry calculations have indicated an alpha helix comprising residue Lys^9 to Cys^{15}. Circular dichroism CD studies support the helical structure of ET-1 as determined by NMR and indicate that ET-1 is about 30–35% helical, and generally considered to exist between residue Lys^9 and Cys^{15}. The helicity is believed to be induced by the disulfide arrangement even though the fully linear peptide [Ala [1, 3, 11, 15]] ET-1 shows no helical character. Most studies are unable to define the apparently flexible C-terminus portion of ET-1 region [14, 15].

3. Biosynthesis of endothelin peptides

Apart from endothelial cells, from which endothelin obviously derives its name, ET-1 is produced by mesangial,

kidney and epithelial cells and also by various human cancer cell lines and human macrophages [16–20]. ET-1, derived from a 203 amino acid peptide precursor known as preproendothelin, is cleaved after transition by endopeptidases specific for the paired dibasic studies to form a 38 (human) or 39 (porcine) amino acid peptide, proendothelin or big ET [21]. Big ET is then converted to active ET by a putative endothelin converting enzyme (ECE) [1, 22]. In addition to the ET gene transcription which occurs in the human brain especially in the hypothalamus [23], transcription and expression of the ET-3 gene happen in the human placenta along with ET-2 in human tumor cell [24, 25]. In the intact circulation, thrombin and A23197 have been demonstrated to enhance ET-1 while endothelium-derived relaxing factor (EDRF) inhibits its production [26].

4. Endothelin receptors

ET receptors are classified into two categories namely ET_A and ET_B. ET_A receptors were found to be specific for ET-1 and cause vasoconstriction when bound to ET in vascular smooth muscle [27–31]. The ET_A receptors of cDNAs from bovine lung and from smooth muscle cell line have been found to be highly specific for ET-1 (ET-1 = ET-2 > ET-3). ET_B receptor is commonly found in the lung parenchyma and in many other tissues including brain, kidney, and liver [32] and has also been found in human and porcine endothelial cells and has evoked the production of EDRF [33–35]. ET_B receptor is nonselective for ET-1 (ET-1 = ET-2 = ET-3) and binds with equal affinity to all the three Ets [36–40].

5. Structural activity relationship of endothelins

Endothelins and sarafotoxins consist of more than eight compounds forming a super family with four isopeptides in each of the two families. A mono D-amino acid scan of ET (16–21) has revealed that substitution at His^{16} gives rise to analogues with significant binding affinity [41]. Structure-activity studies of the C-terminal hexapeptide of ET have also been carried out to elucidate the amino acids that are important for receptor binding and agonist or antagonist activity. Replacement of L-Trp^{21} by D-Trp^{21} in the intact molecule markedly reduces binding and contractile activity [42]. The replacement of the Trp^{21} residue by other aromatic amino acids such as Phe or Tyr is poorly tolerated [43]. Experimental studies indicated that the reduction of the disulfide bonds themselves is not the absolute requirement for receptor-recognition. It has been found that sequential removal of C-terminal residues in ET decreases receptor binding and vasoconstrictor activity. ET (1–15) has been found to be totally inactive [44]. Removal of the C-terminal tryptophan residue reduces *in vitro* functional activity by nearly three orders of magnitude [45]. Changing Trp^{21} to either Phe^{21} or Tyr^{21} or blockade of the N or C termini of endothelin-1 had a deleterious

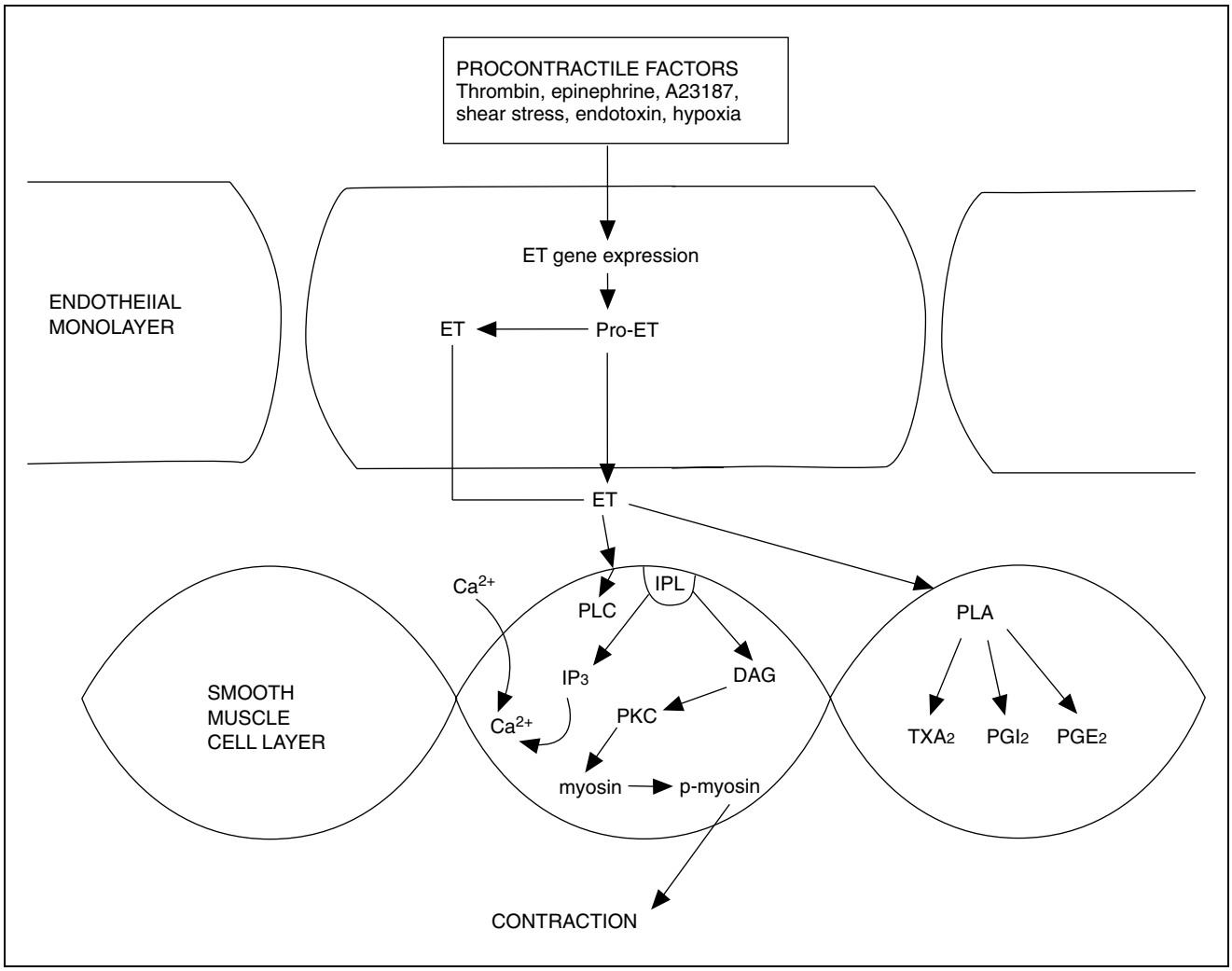


Fig.: Pathways of intracellular transmembrane signalling

effect on functional activity [43]. Disulfide bridge residues Asp^{8}, Try^{13}, Iie^{20}, and Trp^{21} are implicated as being critical to binding to endothelin receptors and while the affinity of analogs substituted at Glu^{10}, Phe^{14}, Leu^{17}, and Asp^{18} for endothelin receptors is appreciable their agonist potencies are substantially less than the respective parent compounds. The dominant structural feature is a helix extending from Lys^{9} to Cys^{15} and although this region has been described as alpha helical [46], most of the data indicate an irregular helical conformation with Lys^{9} as the predominant initiation site and residues 15, 16, or 17 as the termination point. The crystal structure of endothelin-1 consists of an N-terminal β-strand followed by a turn region described as a "bulge" between residues 5 and 7, followed by a hydrogen-bonded loop between residues 7 and 11 [47]. Based on the high degree of secondary structure observed in the crystal structure of endothelin-1, particularly in the C-terminal hexapeptide, it has been argued that the crystal conformation represents a likely bioactive conformation. In fact, based on the electron cryo-microscopy data available for the G-protein-coupled receptor rhodopsin [48], and the structurally related proton pump bacteriorhodopsin [49–51], and from endothelin receptor mutagenesis results [52–54], it is reasonable to postulate that, when bound to the endothelin receptor, endothelin-1 may be entirely surrounded by the receptor.

6. Biological importance of endothelins

6.1. Cardiovascular action and mitogenic action

Endothelins produce long-lasting vasoconstriction in almost all arteries and veins [55]. Ca^{2+} channel blockers and the K^{+} channel opener, cromakalim reduce the pressure responses of ET-1 [56]. The contraction potentiation of mammary artery rings by ET-1 in the presence of norepinephrine and serotonin, suggests that ET may play an important role in acute ischemic disorders associated with platelet activation [57]. In addition ET-1 is reported to have potent inotropic and negative chronotropic effects on isolated perfused hearts and to induce coronary vasospasm, severe arrhythmia, atrioventricular block, and lethal ventricular fibrillation [58]. Administration of ET-1 as an infusion to humans resulted in increases of mean blood pressure and serum potassium concentration [59], while plasma concentrations of renin, ANP, and aldosterone remained unchanged. But low doses of ET-3 cause continuous vasodilation of mesenteric arteries preconstricted with norepinephrine accompanied by elevation of cyclic nucleotides [60]. The concentration of ET is found to be increased during the period of congestive heart failure and myocardial ischemia [61–63] and monoclonal studies revealed the pathophysiological significance of ET in essential hypertension. Proteinkinase C (PKC) [64–65] will become stimulated as a result of a steep increase in the concentration of inositol tris- and biphosphates (IP), and 1,2-*O*-diacyl-glycerol (DAG), because of Et-1 binding to its G-protein coupled receptors' ultimately ending up in a initial rise of intracellular calcium and phosphorylation of myosin light chains leading to the vascular contractile response of ET (Fig.). It is not known whether ET activates PLA_2 directly via a G-protein or indirectly by increasing intracellular Ca^{2++} but the initial transient vasodilator action of the ETs has been attributed to the release of PGI_2 (prostaglandin I_2) and/or EDRF [66]. Different receptors are expected to be associated with the vasodilator and vasoconstrictor actions.

ET-1 has been reported to be a potent mitogen in fibroblasts and rat aortic smooth muscles cells and has been implicated in the pathophysiology of atherosclerosis [67, 68]. But some studies have indicated that ET is in fact a co-mitogen in the presence of platelet derived growth factor [69]. ET-1 is not directly involved in inducing or modulating the aggregation of human platelets *in vitro* [70] but can inhibit platelet aggregation *in vivo*, presumably via release of EDRF or prostacyclin [71]. Platelets can directly stimulate ET expression and biosynthesis in *in vitro* culture [72]. Thus platelets appear to regulate endothelium-dependent constricting factors and indeed there may be a feed-back mechanism from endothelial cells to platelets. ET agonist may be beneficial, but its chemoattractant properties suggest a possible role in wound-healing processes.

6.2. Effect on renal function

ET-1 causes a reduction in renal blood flow and urinary sodium excretion [73] and intense long-lasting renal vasoconstriction will occur in the presence of even very low concentrations of ET-1. Platelet activating factor antagonists have been reported to inhibit the effects of ET on renal function and mesangial cell contraction [74]. In the kidneys mRNA for ET has been detected in the cortical and medullary regions along with the localization of ET receptors in the rat renal system. In patients with chronic renal failure the level of ET-1 and ET-3 has been found to be increased, indicating their involvement, and ET also plays a major role in studies involving cyclosporine-induced renal failure, its level being increased by cyclosporine [75]. Anti-bodies to deactivate endogenous ET in an anti-ischemic kidney model indicated the peptide's involvement in acute renal ischemic injury [76].

6.3. Bronchopulmonary and gastrointestinal effects

ET-1 is one of the more potent contractile agonist, known in human isolated bronchus and pulmonary artery. Human bronchial smooth muscle cells possess separate binding sites for ET-1 and human bronchial epithelium cells have been shown to secrete an ET-like material. ET immunoreactivity has been localized to pulmonary endocrine cells, especially in the fetal lung and in non-stop carcinomas of the lung [77]. Local intraarterial infusion of ET causes hemorrhagic and necrotic damage in rat mucosa, and thus ET has been implicated in the pathogeneses of ulcerative diseases of the stomach [78].

6.4. Neuroendocrine and endocrinological effects of endothelins

ET peptides and their precursors present in human cerebrospinal fluid along with big ET-1, -2, and -3 and ET-1 and/or ET-3 have been suggested to act as neuropeptides, playing an important role in the control of neuronal action. ET-1 has been shown to release vasopressin from the perfused rat hypothalamus [79]. Intracerebrocutricullar (icv) injection of ET-1 to conscious rats caused the dependent elevation of arterial pressure. Endothelin levels (both ET-1 and ET-3) in the cerebrospinal fluid of cerebral ischemia patients are indeed substantially elevated, indicating ET's possible involvement in cerebral vasospasm and the subsequent neurologic deterioration [80]. Micro-autoradiography studies with high-density iodinated ET-1, -2 and -3 in the human uterus revealed the possible involve-

ment of endothelins in the control of menstruation [81] and it is also believed to play a role in pregnancy-induced hypertension.

6.5. Diabetes

ET is a potent agonist in the liver eliciting both a sustained vasoconstriction of the hepatic vasculature and a significant increase in hepatic glucose output [82]. Insulin stimulates ET-1 gene expression in endothelial cells [83] resulting in the elevation of sugar levels.

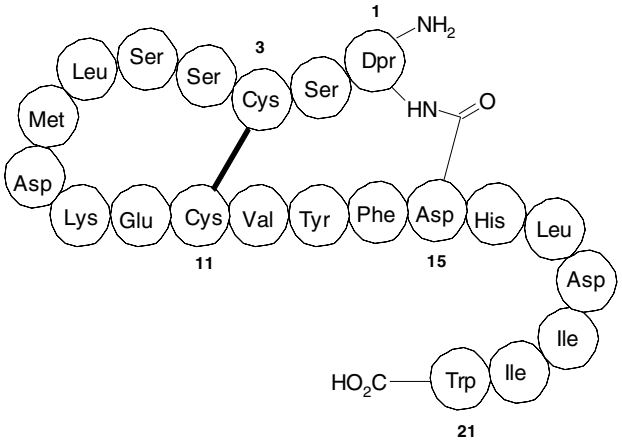
7. Endothelin receptor antagonists

7.1. Peptide antagonists

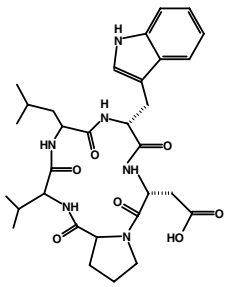
Modification of the endothelin amino acid sequence has produced a series of agonists and a number of endothelin receptor antagonists. Endothelin-1 analog [Dpr1-Asp15] endothelin-1 [84] (Dpr, dimaniopropionic acid) an endothelin receptor antagonist with the replaced amide linkage instead of cys1-Cys15 disulfide bond is a selective antagonist of the ET_A receptor, with a two fold reduced affinity compared with that of endothelin-1. Administration of [Dpr1-Ansp15] endothelin-1 in the form of an aerosol blocks endothelin-1 induced bronchoconstriction in sheep [48]. The substitution of Asp^{18} by hydrophobic amino acid yields endothelin-1 analogs that are receptor antagonists. For example, binding results [85] using porcine myocardium, a tissue rich in ET_A receptors, and bovine cerebrum, which is rich in ET_B receptors, show that [Leu18] endothelin-1 is a potent endothelin receptor antagonist with IC_{50} values of 8.60 nM (ET_A) and 0.45 nM (ET_B). It also exhibited weak inhibition of endothelin-1-induced vasoconstriction of porcine coronary artery. Endothelin-1 analogs substituted at position 18 with a hydrophilic amino acid maintain high affinity for both ET_A and ET_B receptors but substitution with threonine leads to a partial agonist, while further substitution, as [Thr^{18}, G-$MeLeu^{19}$] endothelin-1, produces an antagonist. [Thr^{18}, g-$MeLeu^{19}$] endothelin-1 which has a subtle change in the hydrophobic environment of the C-terminus, maintains high affinity for the ET_A receptor, and produces no vasoconstriction in porcine coronary artery and also blocks endothelin-1 induced vasoconstriction in isolated porcine coronary artery, showing the importance of Asp^{18} for receptor activation. The cyclic pentapeptide BE-18257B, cyclo(D-Glu-ala-D-allo-Ile-Leu), a competitive and selective ETA receptor antagonist [38] isolated from the fermented products of *Streptomyces misakiensis*, functionally antagonizes endothelin-1-induced vasconstriction in the rabbit artery. Cyclic pentapeptide BQ-123, cyclo (D-Trp-D-Asp-Pro-D-Val-Leu), a compound with improved solubility [87–88], functionally antagonizes endothelin-1-induced contraction of porcine coronary arteries with a pA_2 of 7.4 and is highly selective for ET_A receptors. BQ-123 does not antagonize vasoconstriction induced by norepinephrine or potassium chloride in the porcine coronary artery [88], indicating selectivity for antagonism of endothelin receptors.

Due to its high potency, selectivity, and aqueous solubility, BQ-123 has been widely used in the investigation of the distribution of endothelin receptor subtypes and, importantly, BQ-123 has been shown to be efficacious in *in vivo* models of disease, most notably hypertension and acute renal failure [89]. BQ-123 [90, 91] and the related compounds cyclo[D-Trp-Dval-(N-Me) Leu] [90] and cyclo[D-Trp-Cys(so3-Na^+ Pro-D-Val-Leu], BQ-153 [92] in either hydrophilic or hydrophobic solvent exhibited a highly stable backbone conformation comprised of type II b-turn containing leucine and tryptophan in the $i + 1$ and $i + 2$ positions, respectively, and a γ-turn centered on proline. WS 7338B, cyclo(D-Tryp-D-Glu-ala-D-Allo-Iie-Leu), a Cyclic pentapeptide, from a strain of *Streptomyces* [93], was the most potent of this series, structurally identical to BE-18257B, and *in vivo* evaluation showed that pretreatment of the spontaneously hypertensive rat with 10 mg/kg reduced the pressure effect of endothelin-1 (3.2 mg/kg; 55% inhibition), with no effect on the initial depressor response, indicative of selectivity for the ET_A receptor. Structure-activity studies related to BQ-123 and WS-7338B have led to a series of potent, ETA selective linear tripeptides [94–96]. The (2-pyridyl) alanine in Pya of {[N (hexahydro-1azepinyl) carbonyl]}-Leu-D-(1-Me)Trp-D-Pya [94] (FR 139317) has high affinity for the cloned human ET_A receptor and markedly lower affinity for the cloned human ET_B receptor. FR 139317 inhibits, in a dose-dependent manner, the endothelin-1-induced pressure response in conscious normotensive rats and significantly inhibits vasoconstriction of the basilar artery, suggesting a role for endothelin in this pathological condition.

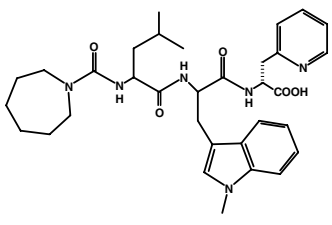
FR 139317 competitively inhibits endothelin-1 induced vasoconstriction in the guinea-pig pulmonary artery (a tissue rich in ET_A receptors) without any effect on endothelin-1



IRL 1620 and BQ 3020, ET_B selective antagonists



BQ-123



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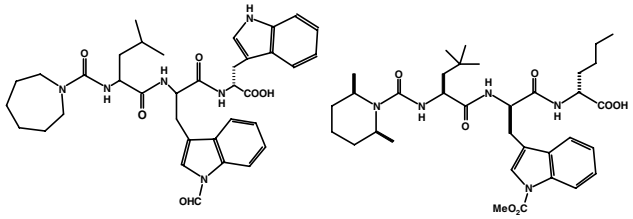
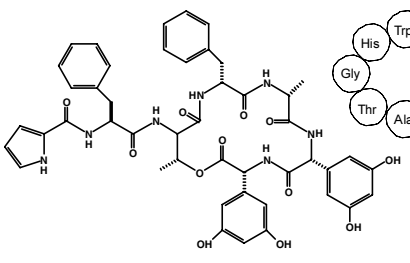


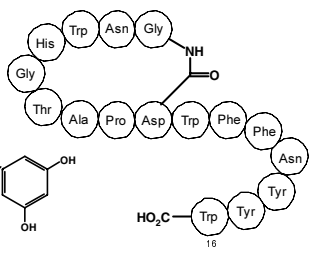
Linear tripeptide endothelin receptor antagonists

induced smooth muscle contraction in the guinea-pig trachea (an effect believed to be mediated by ET_B receptors) [97]. PD 151242 (N- (hexahydro-1azepinyl) carbonyl) L-Leu(1-Me)Trp-Tyr)49 and BQ-610 (N-hexahydro-1aepinyl) carbonyl L-Leu-D-(1-CHO)Trp-D-Trp), active linear tripeptides similar to FR 139317, possess selective ET_A receptor antagonist activity. BQ-610 functionally antagonizes endothelin-1 induced contraction in porcine coronary arteries. Potent and selective ET_B receptor antagonist BQ-788 [N-cis-2, 6-dimethylpiperidinocarbony-l-γ-meLeu-D-Tr (1-CO2Me)-D-Nle] [98], lacking an aromatic amino acid at the C-terminus competitively inhibits [^{125}I] endothelin-1 binding to ET_B receptors on human Girardi heart cells ($IC_{50} = 1.2$ nM) and has low affinity for ET_A receptors on SK-N-MC cells, a human neuroblastoma cell line (IC_{50} −1.3 μM). BQ-788 antagonizes the contractions produced by a selective ET_B-agonist and showed no effect on basal tension in this tissue. In common with the tripeptide series, the linear hexapeptides [99] contain the alkylmethyleneimino carbonyl group at the N-terminus, D-amino acids, and an aromatic side chain at selected positions, all of which appear to be important for binding to endothelin receptors. A potent compound in this series is TTA-386, {N-[(hexahydro-1-azepinayl) carbonyl]-Leu-D-Trp-D-Ala-b-Ala-tyr-D-Phe) [99] which is a selective ET_A receptor antagonist of porcine cardiac ventricular muscle. PD 145065 with a unnatural amino acid, D-Bhg, 2 (R)-10,11-dihydro-5 H-dibenzo[a, d]cycloheptен, is a competitive antagonist, inhibiting endothelin-1-and sarafotoxin 6c-induced contraction in the rabbit femoral artery (ET_A) and rabbit pulmonary artery (ET_B) with pA_2 values of 6.9 and 7.1, respectively. More recently, a combinatorial approach has been undertaken to study the contribution of each of the amino acids of the C-terminal hexapeptide common to endothelin-1, -2 and -3 [100, 101]. Multipin peptide synthesis methods allowed for the rapid preparation of 300 analogs that were subsequently screened for ET_A receptor affinity. The most potent compounds contained multiple D-acids, which should increase the resistance of these peptides to enzymatic proteolysis.

A new class of cyclic depsipeptides isolated from a culture of *Microbispora* [120], named cochinmicin-1 displayed weak, nonselective binding affinity for ET_A and ET_B receptors. Cochinmicin-1 is a weak functional antagonist, blocking in a dose-dependent manner endothelin-1-stimulated phosphatidylinositol turnover in rat atrial tissue. Cyclic peptide, RES 701-1, isolated from a fermentation broth of *Streptomyces* [103] is a potent and selective antagonist of the ET_B receptor. Res-701-1 consists of 6 amino acids, and is cyclized between the β-carboxyl group of Asp9 and the α-amino group of Gly1 and is reported to be resistant to protease action. Endothelin peptides and RES-701-1 showed no significant sequence similarity, except that their terminal amino acid residue is Trp, and they both contain lipophilic amino acid in the C-terminal region.



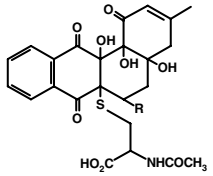
Cochinmicin-1



RES 701-1

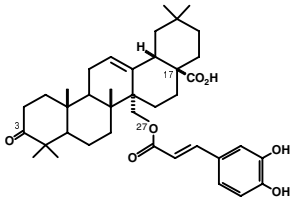
7.2. Non-peptide receptor antagonists

The discovery of non-peptide endothelin receptor antagonists helped to overcome the limitations associated with the use of peptide endothelin receptor antagonists in chronic disease models and in human therapy.

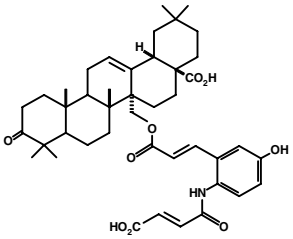


WS 009 A and B

The compounds WS009 A and B were first isolated from a *Streptomyces* strain [104, 105] and had no effect on [^{125}I] endothein-1 binding to membranes from porcine brain, a tissue in which the ET_B receptor is known to predominate. WS009 A and B are considered to be selective antagonists of the ET_A receptor and analysis of the inhibition of endothelin-1 binding to porcine aortic membrane is consistent with competitive antagonism. *In vivo*, activity for WS009 A was demonstrated in the spontaneously hypertensive rat where a small i.v. dose blocked the pressor but not the depressor response to an exogenous challenge with endothelin-1. The chemical complexity of these compounds slowed down further progress. Shionogi 50-235 [106] (caffeoyl ester of the pentacyclic triterpenoid myricerone), a natural product isolated from an extract of *Myrica cerifera* and having significant molecular complexity, has proven to be more suitable for chemical manipulation and inhibits endothelin-1 binding to rat aortic smooth muscles [107]. The compound also inhibits the mitogenic effects of endothelin-1 on A7r5 cells with an IC_{50} of approximately 100 nM [108], further supporting the notion that Shionogi 50-235 is a functional antagonist of ET_A receptors, inasmuch as the smooth muscle proliferative effects of endothelin-1 are mediated by ET_A receptors [109].

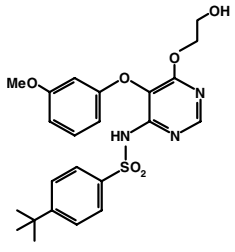


Shionogi 50-235



Shionogi 97-139

Shionogi 97-139, has approximately 5-fold higher affinity for the ET_A receptor than Shionogi 50-235 [110] and inhibits the binding of ^{125}Iendothelin-1 to rat A7r5 cells (ET_A receptors). Shionogi 97-139 is also known to block the mitogenic effects of endothelin-1 in A75 cells with a weak affinity for ET_B receptors in human Giradi heart cells. In contrast to this structural complexity, asterric acids a simple natural product from the fungus *Aspergillus*, inhibited endothelin-1 binding to ET_A receptors present in A10 cells [111]. Ro 46-2005, the first orally active endothelin receptor antagonist [112], with a secondary pyrimidinyl sulfonamide lacking a carboxylic acid moietys, possesses as high affinity for both ET_A and ET_B receptors. Ro 46-2005 was shown to inhibit the binding of ^{125}I endothelin-1 to human vascular smooth muscle cells (ET_A re-



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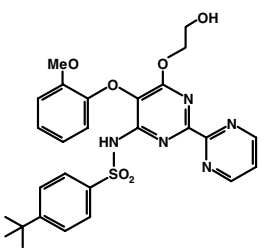

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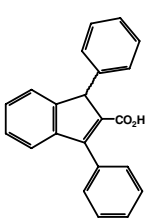

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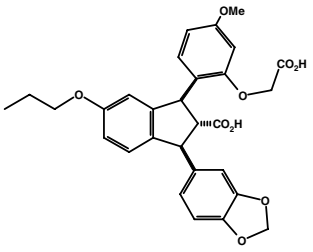
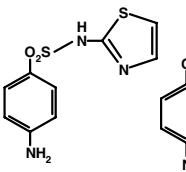


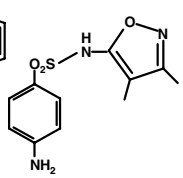
SB 209670

ceptors) and rat aortic endothelial cell (ET_B receptors) with IC_{50} values of 220 nM and 1 μM, respectively.

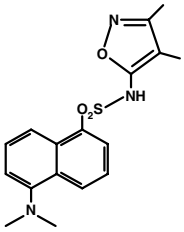
The structural activity studies in this series led to the discovery of Ro 47-0203 bosentan, a more potent, nonselective endothelin receptor antagonist than Ro 46-2005 [113], and *in vitro* functional blockade by bosentan of ET_B receptors was assessed through inhibition of sarafotoxin 6c-induced responses in isolated rat trachea, yielding a pA_2 value of 6. Sulfathiazole [114], from the sulfonamide family, had low affinity for the ET_A receptor [76] while another member of this class, sulfisoxazole, was found to have substantially higher affinity for the ET_A receptor [76]. Sulfisoxazole contains a secondary sulfonamide and lacks the carboxylic acid moiety and has proven to be a valuable lead structure. Replacement of the 4-amino substituent of sulfisoxazole with more lipophilic amines led to compounds which not only displayed somewhat greater affinity for ETA receptors, but which were also active in functional assays for antagonist activity. Naphthalene sulfonamide BM 182874 binds to A10 cells (ET_A receptors), while its binding to rat cerebellum membranes (ET_B receptors) is much weaker. BMS 182874 has been found to be valuable in elucidating the effects of endothelin that are mediated through the ET_A receptor in the pathogenesis of disease.



Sulfathiazole



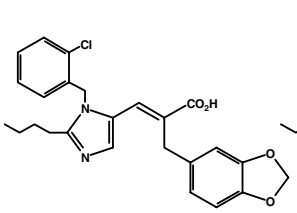
Sulfisoxazole



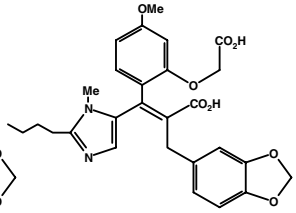
BM 182874

CGS 27830 [115] has been shown to inhibit ^{125}I endothelin-1 binding to ET_A receptors in porcine thoracic aortic membrane with an IC_{50} of 15.9 nm, whereas the affinity for the ET_B receptor subtype, present in rat cerebellum, is significantly lower ($IC_{50} = 295$ nM). SK&F 66861, a diphenylindene carboxylic acid has the ability to bind to ET_A receptors in the rat mesenteric artery but has no detectable binding to ET_B receptors in rat cerebellum [116]. The indane series of endothelin receptor antagonists structurally related to SK&F 66861 has the oxidative liability of the indane nucleus. This impeded progress and the work continued instead using trans, trans-1, 3 diphenylindan-2-carboxylic acid which has an activity profile at endothelin receptors that is similar to SK & F 66861. Substitution with oxygen-containing groups substantially increased affinity for the ET_A acceptor and led to measurable affinity for the ET_B receptor. Early structure-activity results for the ET_B receptor had highlighted the importance of the C-terminal carboxylic acid for binding to endothelin receptors [43].

Based on the overlay of the indane nucleus to the structure of endothelin-1, SB 209670 was synthesized [116]. On intravenous administration, SB 209670 was found to be effective in a number of diseased animal models, including hypertension [117], acute renal failure [118], and restenosis following percutaneous transluminal coronary angioplasty [119]. ET_A and ET_B of G-protein-coupled (seven-transmembrane-spanning) peptide receptor super family indicate a certain degree of similarity. Using this approach, combined with a similarity searching base upon the indane series of antagonists, SK & F 107328 was identified. Mutagenesis study data of the endothelin receptors through a peptidomimetic strategy resulted in the discovery of SB 209670 and SB 209834. The commonality of binding sites among structurally distinct endothelin receptor antagonists is apparent from the mutagenesis studies in that bosentan likewise does not bind to the K182A mutant [53].



SK & F 107328



SB 209834

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