Endothelin Receptor Antagonists: An Overview of Their Synthesis and Structure-Activity Relationship

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Abstract: Endothelins (ETs) are potent vasoconstrictor peptides and are associated with several disease states like pulmonary hypertension, systemic hypertension and heart failure. Endothelin-1 (ET-1) is the first member of the family and it has the receptor subtypes known as ET_A and ET_B. The receptors ET_A and ET_B are attractive new therapeutic targets for diseases associated with elevated ET-1 levels. Several studies have thus led to the discovery of selective ET_A receptor antagonists as well as non-selective ET_A/ET_B antagonists. The preclinical and clinical studies have clearly established that these antagonists are effective in the treatment of essential hypertension, pulmonary hypertension, heart failure and atherosclerosis. The advances in this area have resulted in the FDA approval of the orally active dual antagonist Bosentan for pulmonary hypertension in 2001. This review highlights the synthesis and structure-activity of the endothelin receptor antagonists and covers the literature in this area up to 2001.

INTRODUCTION

Endothelins (ETs) are a novel family of vasoconstrictor peptides, each consisting of 21 amino acid residues with two intra chain disulfide linkages between positions 1-15 and 3-11 and a hydrophobic C-terminal hexapeptide. The first member of the family identified, endothelin-1 (ET-1), was isolated by Yanagisawa [1] from supernatant of cultured porcine aortic endothelial cells. Subsequent screening of a genomic DNA library led to the discovery of other two members [2] of the family, ET-2 and ET-3 (Fig. (**1**)). ET-1 was also shown to have contractile activity on various non-vascular smooth muscles and some activity in the central nervous system (CNS) [3]. All three are products of separate genes, which have been identified in mammalian genomes [2a]. The three related homologous polypeptides differ by 2 and 6 amino acids respectively and bear 70% homology with one another. ET-1 is the most powerful pressor peptide isolated, and is a potent mitogen too. In addition to that ET has potent renal, pulmonary and neuroendocrine actions and has been implicated in a wide variety of human diseases including Ischemia [4a], cerebral vasospasm [4b], stroke [4c], renal failure [4d], hypertension [4e], heart failure [4f], pulmonary hypertension [4g], and restenosis [4h]. These observations suggest that specific blockage of ET actions at receptor level can be potential treatment of disease states caused by elevated levels of ETs (Table **I**).

Although no splice variant of the pre-pro ET-1 m-RNA is known to exist, several alternatively spliced forms of pre-pro ET-2 and ET-3 m-RNAs have been reported to be present in different human tissues. The exact significance of these is yet not clear [5]. In addition to three known peptides; gene of another ET like peptide was reported in mouse and rat genome. This particular peptide, referred to as vasoactive intestinal constricting peptide (VIC), is expressed exclusively in intestines. The gene sequence representing VIC is believed to be an isoform of the ET-2 gene [6]. Synthesis and release of all three ETs are transcriptionally regulated [6]. A variety of stimuli can induce the expression of ET genes with resultant transcription of pre-pro ET m-RNAs, which in turn are translated into respective proteins. For example, the gene for pre-pro ET-1 can be induced by thrombin, angiotensin II, cyclosporin, low-density lipoprotein (LDL) and hypoxia etc. Pre-pro ET-1, a 212 amino acid precursor protein, is cleaned and transported across the nuclear membrane.

Under the influence of an endopeptidase, pro-protein convertase, pre-pro ET-1 is converted to pro ET-1 (big ET-1), a 38 (human)/ 39 (porcine) amino acid polypeptide. ET-1 converting enzyme, a neutral metallo protease, converts pro ET-1 to mature ET-1, which in turn is released outside the cell and exerts various biological effects. The biological potencies of ET have been attributed to the following features. a) Sequence heterogeneity in the *N*-terminal region of the peptides, specifically between residues 4 and 7; b) four cysteine residues at positions 1,3,11 and 15; c) carboxyl terminal hexapeptide region [16-21]; d) aromatic dipeptide at positions 13 and 14; and e) charged loop region spanning Asp^8-Lys^9-Glu^{10}.

Biological actions of endothelin are mediated through specific cell surface receptors. Using unlabelled endothelin to displace radiolabelled ET-1 from sarcolemmal preparation of different tissues [7] as well as using cloned receptors [8], two distinct receptor-binding sites have been identified. The receptor that binds ET-1 with high affinity (K_d = 0.2 nM) is called ET_A and the other with non-selective affinity (K_d = 0.2 nM) for ET-1, ET-2, ET-3 is known as ET_B receptor. In terms of relative selectivity while ET-1 is equiactive at both ET_A and ET_B sites, ET-2 is 3 times and ET-3 is 1000 times more selective for ET_B than ET_A. More recently, a related family of peptides has been identified and isolated from the

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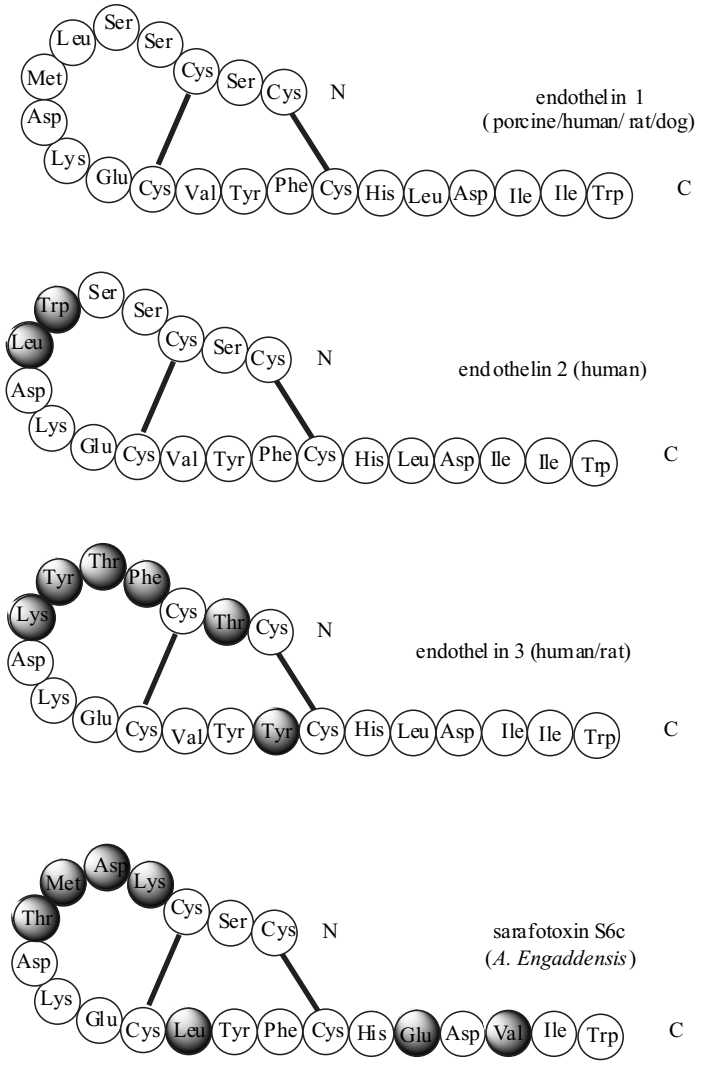


Fig. (1). Peptides of Endothelin and Sarafotoxin family. Dark circles represent amino acid residues different from ET-1.

venom of Israeli burrowing asp known as Sarafotoxins [9]. A member of this family Sarafotoxin S6c has 300,000 fold more selectivity towards ET_B (K_d = 0.03 nM) when compared to ET_A receptors [10].

ET_A and ET_B receptors are widely distributed not only in vascular [1,11] but also in nonvascular [12] tissues and possess different functions depending on species and location. ET_A receptors are predominantly found in peripheral tissues, especially in vascular smooth muscle tissues to mediate vasoconstriction though they are also present in certain regions of the brain [8b,13]. In contrast, ET_B receptors are thought to be exclusively localized in the endothelium and nonvascular tissues such as the liver, kidneys and brain [14]. Endothelial ET_B receptors are functionally linked to vasodilation, possibly through the release of endothelium derived relaxing factor [15]. However, it has been confirmed that ET_B receptors are also located in certain vascular and airway smooth muscle tissues, mediating their constriction [16]. The cDNAs of ET_A and ET_B receptors have been cloned and these receptor genes have been identified in human genome. Deduced amino acid sequence of these receptors predict 427 and 442 amino acids respectively [8b,13,17]. Hydropathy analysis of these sequences suggests that these receptors belong to a superfamily of seven transmembrane domain proteins. Overall, human ET_A and ET_B receptors share 63% sequence homology, while the human ET_B receptor amino acid sequence exhibits 85% sequence homology with rat or bovine ET_B receptors. In addition to ET_A and ET_B receptors, two other cDNAs representing ET_C and ET_{AX} receptors have

Table I. Pathophysiological Conditions Involving ET

a) Cardiovascular	Essential Hypertension, Myocardial Ischemia, Myocardial Infraction, Unstable Angina, Congestive Heart Failure, Atherosclerosis
b) Extra cardiovascular	Pulmonary Hypertension, Bronchial Asthma, Glomerular Inflammation, Acute and Chronic Renal Failure, Migraine, Ischemic Stroke, Depression, Diabetes Mellitus, Ulcerative Colitis, Liver Cirrhosis, Breast Cancer

been cloned from *Xenopus* melanophores [18] and heart [19], respectively. Cloned ET_C receptor recognizes ET-3 as a ligand and its deduced amino acid sequence predicts a 454 amino acid peptide. This receptor is 52% identical to rat ET_A receptor. ET_{AX} receptor recognizes ET-1 as a ligand with the same affinity as mammalian ET_A receptors, however, ET-1 cannot be displaced from this receptor by selective ET-1 receptor antagonists. Neither ET_C nor ET_{AX} genes have so far been identified. Pharmacological studies, using ET_A and ET_B receptor selective agonists and antagonists, also point towards ET receptor heterogeneity. The existence of ET-3 receptor [20] and distinct subtypes of ET_A and ET_B receptors, namely ET_{A1} [21], ET_{A2} [22], ET_{B1} and ET_{B2} [21-23] have been suggested. Of course, it remains to be seen whether the data, obtained from pharmacological and molecular biology experiments, can be reconciled.

However, the physiological significance of ET-1 in adult life is not very clear. Although circulating level of ET-1 is very low, it has been proposed that ET-1 may act in both endocrine and paracrine fashion [22,24]. It is believed that continuous albuminal release of small amount of ET-1 from endothelial cells may contribute to the maintenance of vascular tone by constricting vascular smooth muscle as well as releasing endothelium derived relaxant factor. Pharmacologically ET-1 exerts a variety of effects mediated through ET_A or ET_B receptors. These effects include positive inotropy, vasoconstriction, sodium and water retention, increase in central and peripheral sympathetic activity, generation of renin, angiotensin II, aldosterone and adrenaline, migration and proliferation of vascular smooth muscle (Table II) [22,24].

The advances in biological and pharmacological studies related to endothelin has inspired medicinal chemists to search for small molecules as potential antagonists of these vasoactive peptides. Thus basic research activities in the field of ET antagonists have spilled rapidly from the academic world into new drug discovery research, indicating a strong commercial interest for the discovery of potent endothelin antagonists. Random screening of libraries of chemical compounds and fermentation broths has produced active ET receptor molecules. Thus, a number of peptidic antagonists have been synthesized and evaluated for *in vitro* and *in vivo* activity. Among these, cyclic pentapeptide (Banyu) BQ-123 (**3**, Fig. **2**) [25], cyclic hexapeptide (by Takeda) TAK-044 [26], tripeptide FR-139317 (from Fujisawa Pharmaceuticals) [27] and some of their semipeptidic analogues / mimics have been found to exhibit antagonist activities with IC_{50} ranging from low micro molar to sub-nanomolar range. For example, BQ-123 is a highly soluble, potent and selective ET_A receptor antagonist (IC_{50} for human ET_A = 8.3 nM, IC_{50} for human ET_B = 61 μM); TAK-044, an ET_A receptor-selective antagonist, possessed not only affinity similar to that of BQ-123 for ET_A but also higher affinity for ET_B than BQ-123 and inhibits [^{125}I]ET-1 binding to the ET_A and ET_B receptors with IC_{50} values of 0.082 nM and 120 nM, respectively. Most of the non-peptide compounds identified through random screening of existing chemical libraries are derivatives of indan, butenolide, tetrahydronapthalene, indole etc. Sulfonamides as a class by itself emerged as potent antagonists with the identification of Bosentan (Hoffmann-La Roche) [28] including several of its analogues. Studies on structure activity relationship were carried out extensively using isoxazolyl dansyl amides among which BMS-182874 (**29**, Fig. **6**) exhibited the best *in vivo* activity. Further extension of these earlier studies is now emerging in the literature in which attempts are being made to discover novel compounds utilizing structural mimics identified through molecular modeling.

Recently, two reviews have been published from Cheng *et al.* [29] and Liu [30] on endothelin receptor antagonists. This review is aimed at the survey of the developments in the domain of design, synthesis and biological evaluation of potential ET receptor antagonists. The following sections describe the progress on the chemistry related to ETs antagonists and the review is categorized based on the type of chemical structures. Since a large number of literatures have appeared in the past few years, and after preparation of this write-up, it has not been possible to incorporate all the references available; but a sincere attempt has been made to include as many examples as possible.

A: PEPTIDIC ET

As a result of the importance of vasoactive peptide endothelin, there have been a number of studies of the solution structure of endothelin. On one hand, elements of secondary structure have been detected in various studies [31], but on the other the notion that the peptide is conformationally flexible in some regions has also been

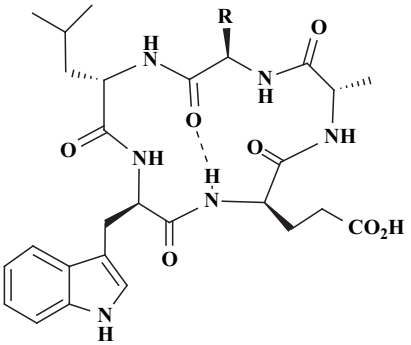
Table II. Biological Actions of ET-1

Tissue	Effect
Heart	Positive Chronotropy, Positive Inotropy, Hypertropy of Cardiac Myocytes
Blood Vessel	Vasodilation followed by constriction, Constricts coronary, renal arteries, arterioles and venules
Blood Pressure	Initial hypotension followed by sustained hypertension, Increase in Total Peripheral Resistance
Pulmonary System	Broncho constriction, Pulmonary Vasoconstriction through ET_A receptor and Vasodilation through ET_B receptor
Renovascular System	Decrease in Glomerular Filtration Rate, Increase in Renovascular Resistance, Inhibition of Sodium and Water
Endocrine System	Increased secretion of Renin, Angiotensin II, Aldosterone, Atrial Natriuretic Factor, Vasopressin, Catecholamines
Promitogenic Action	Stimulates growth of Vascular Smooth Muscle cells, Melanocytes, Tumor Cells

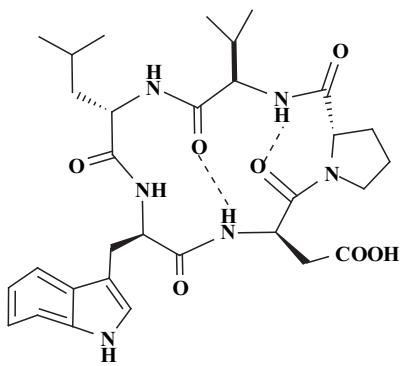
proposed [31,32]. In the design of antagonists it has therefore been of interest to consider constrained molecules, and to this end a number of cyclic peptides have been identified which have significant antagonist activity. Potent ET_A antagonists have the potential to play multiple therapeutic roles as well as to be useful research aids to assess the physiological and pharmacological roles of the endothelin peptide family. Ihara *et al.* and Miyata *et al.* independently reported the discovery of natural antagonists BE-18257A (**1**) [33] and BE-18257B (**2**) [33,34], isolated from *Streptomyces misakiensis*. Their chemical modification to a more potent antagonist BQ-123 (**3,** Fig. (**2**)) was also reported [25,35]. Both of these antagonists are specific to ET_A receptors and are cyclic pentapeptides with alternating D- and L-amino acid.

Coles *et al.* (Victoria College of Pharmacy) [36] in a bid to generate structural information that would be useful in defining the relationship between structure, conformation and activity synthesized BE-18257B (**2**) *via* solid-phase methods and examined its solution conformation by NMR spectroscopy. The conformation calculated for the peptide backbone by them, comprising a type-II β-turn and an inverse γ-turn, is one that has already been previously observed in cyclic pentapeptides. First observed in the crystal structure [37] of cyclo(Gly-Pro-Gly-D-Ala-Pro) and in the solution [38], this hydrogen bonding pattern has since been shown to be common for peptides having alternating D and L chirality amino acids [39]. They also simulated annealing calculations based on NOE constraints. Additional information used in the structure determination included coupling constants and chemical shift measurements as a function of temperature. The chemical shifts of two of the NH protons (D-Glu and D-Ile) exhibited low sensitivity to changes in temperature, indicating their involvement in hydrogen-bonded interactions. The main features of interest in the solution conformation were the presence of both type-II β-turn and an inverse γ-turn with central hydrogen bonds between H_N of D-Glu, and the C=O of D-allo-Ile^3 and between H_N of D-allo-I1e^3 and the C=O of D-Glu^1. The backbone conformational analysis of the peptide **1**, using ^{1}H NMR techniques, revealed that it possesses a type-II β-turn in the D-Val^4-Leu^5-D-Trp^1-D-Glu^2 region and an inverse γ-turn in the D-Glu^2-Ala^3-D-Val^4 region with two intramolecular hydrogen bonds. Chirality changes at the D-Trp^1 and/or D-Glu^2 positions of **1** destroyed the β,γ-backbone conformation and abolished ET_A receptor binding affinity.

Coles and coworkers [36] found the backbone conformation of BE-18257B (**2**) and BQ-123 (**3**) similar and suggested that these peptides might mimic structural features of the C-terminal tail of the endothelins. This proposal that the cyclic pentapeptide antagonists of ET_A derive their activity from their ability to mimic the C-terminal of the endothelins is preferred over alternative hypothesis raised by Satoh and Barlow (King's College London) [40]. It became evident that the studies of three-dimensional conformation of these peptides should assist in a better understanding of the structural requirements at the ET_A receptor and hence in the development of both non-peptidic and peptidic antagonists. The solution structure of BQ-123 (**3**) was generated using molecular dynamics constrained only by experimental heteronuclear $^3J_{NH\text{-}C\beta}$ and homonuclear $^3J_{NH\text{-}H\alpha}$ values. A stable structure was determined by the NMR spectroscopy and molecular modeling by Reily *et al.* (Parke-Davis, Warner Lambert) [41]. Their studies included amide protons likely to be involved in hydrogen bonding, temperature dependent shifts of exposed amides protons and observed NOEs. They demonstrated that the ^{13}C-edited TOCSY experiment at natural abundance and ~30 mM peptide concentrations can reduce the ambiguity in φ angle determination which exists when only homonuclear coupling constants are used. Although hydrogen bonds were not used in the modeling calculations, it is clear from the model that the D-Val amide proton is internal and is involved in a trans-annular hydrogen bond to the carbonyl oxygen of the D-Asp residue. Molecular modeling studies showed that **2** and **3** have very similar structures to that reported for ET-1. Parts of their 3D structures were also shown to match closely with that reported for residues 6-8 in ET-1. On the basis of these similarities, Satoh and Barlow [40] had proposed a structural determinant for ET_A receptor binding and suggested novel designs for providing more potent and selective ET_A receptor antagonists by using a minimum of 3 residues - *Leu*, *Ala* and *Asp*. Also, since the conformation of the molecule must be arranged so that the two-peptide CO groups are available for intermolecular hydrogen bonding, there must be some constraints employed to ensure that the structure forms a loop. Peishoff *et al.* (Smithkline Beecham) [42] co-related the functionally important regions of the cyclic pentapeptide **3** with the structure of the C-terminal tail



1. BE-18257A: cyclo-(-D-Trp-D-Glu-Ala-D-Val-Leu)
2. BE-18257B: cyclo-(-D-Trp-D-Glu-Ala-D-allo-Ile-Leu)
IC_{50} (hET_A)=590 nM; IC_{50} (hET_B)=>100000 nM

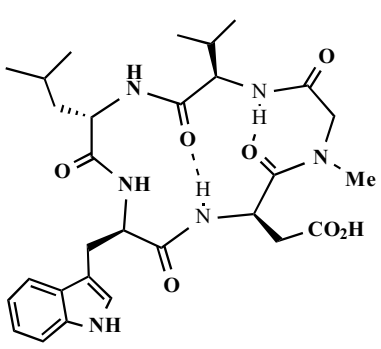


3. BQ-123: cyclo-(-D-Trp-D-Asp-Pro-D-Val-Leu)
IC_{50} (hET_A)=8.3 nM; IC_{50} (hET_B)=>61000 nM

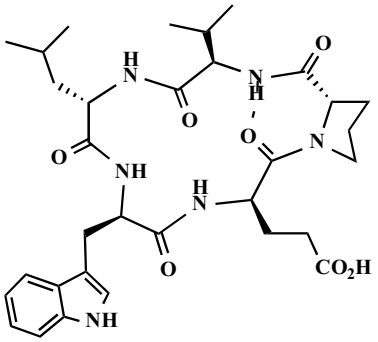
Fig. (2). Chemical structures of BE-18257A, BE-18257B and BQ-123.

of ET-1 during X-ray crystal structure study. Residues 18 and 21 of ET-1 were spatially juxtaposed such that they superposed extremely well with D-Asp and D-Trp of the antagonist, consistent with the residues on this surface of the endothelin helix being important for binding. The study provided new information on the three dimensional nature of ET_A receptor binding site which might prove useful for rational drug design. Ishikawa *et al.* (Banyu) [43] further attempted the modification of these lead peptides **1** and **2** and were successful in identifying more potent and more soluble antagonists with high ET_A receptor selectivity.

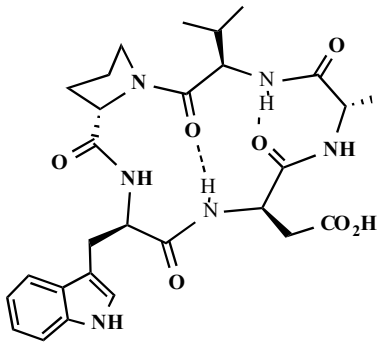
Fukami *et al.* [44] prepared analogues of ET_A receptor antagonists **1** and **2** and tested for inhibitory activity against ET-1 binding to protein ET_A receptors. They synthesized cyclic pentapeptide analogues **4-9** (Fig. (**3**)) by deprotection following cyclization of side chain-protected linear pentapeptide hydrazides having appropriate amino acid sequences. These hydrazides were prepared by Fmoc solid-phase peptide synthesis. Compound **4** was synthesized as shown in scheme 1. The method for preparing most of the cyclic pentapeptides involved formation of precursor side chain-protected linear peptide hydrazides, conversion to acyl azides, cyclization and deprotection of side chain protecting groups. The DDLDL chirality sequence was thus proved to be very important for the activity of this series of compounds. Systematic modifications at each position of the analogues clarified the SAR and led to highly potent and selective ET_A receptor antagonists. Most replacements of D-Trp^1 and Leu^5 with other amino acids caused a significant loss of inhibitory activity. In contrast, replacement of D-Glu^2 with D-Asp^2 enhanced the activity. With regard to the Ala^3 position, all analogues with imino acids, independent



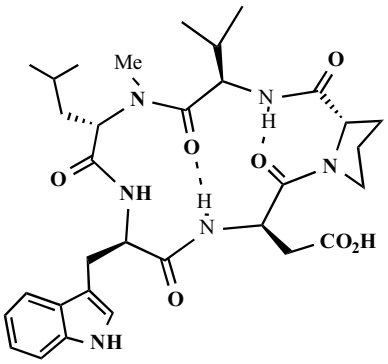
4: cyclo(-D-Trp-D-Asp-Sar-D-Val-Leu-)
IC_{50} (pET_A) = 32 nM; IC_{50} (pET_B) = 30 μM



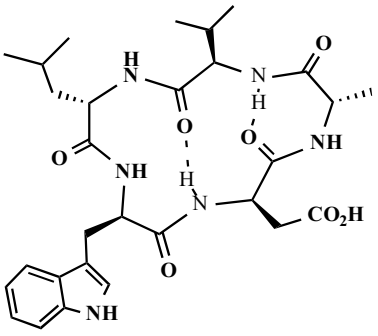
5: cyclo(-D-Trp-D-Glu-Pro-D-Val-Leu-)



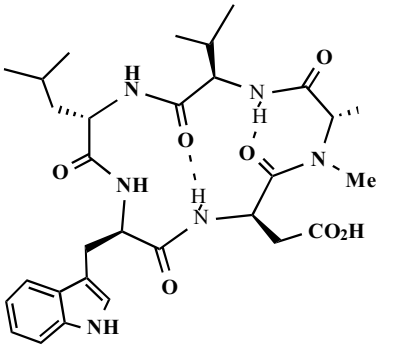
6: cyclo(-D-Trp-D-Asp-Ala-D-Val-Pro-)



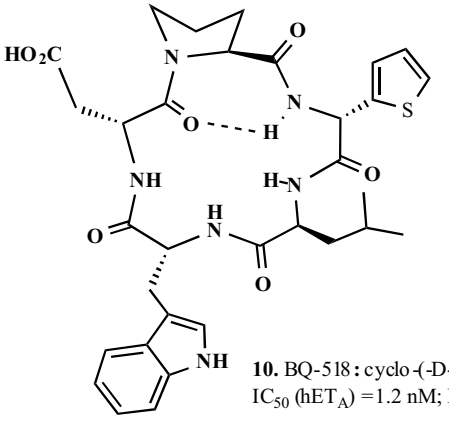
7: cyclo(-D-Trp-D-Asp-Pro-D-Val-MeLeu-)



8: cyclo(-D-Trp-D-Asp-Ala-D-Val-Leu-)

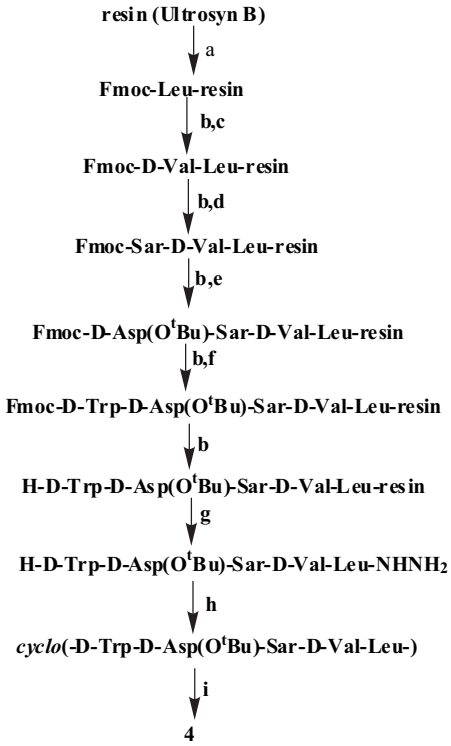


9: cyclo(-D-Trp-D-Asp-MeAla-D-Val-Leu-)



10. BQ-518 **:** cyclo-(-D-Trp-D-Asp-Pro-D-Thg-Leu)
IC_{50} (hET_A) = 1.2 nM; IC_{50} (hET_B) = 55000 nM

Fig. (3). Chemical structures of cyclopeptides **4-10.**



***Reagents**: a) (Fmoc-Leu-)$_2$O and DMAP/DMF; b) 20% piperidine/DMF; c) Fmoc-D-val-OH, HOBt, and DIPC/DMF; d) Fmoc-Sar-OH, HOBt, and DIPC/DMF; e) Fmoc-D-Asp(O^{t}Bu)-OH, HOBt, and DIPC/DMF; f) Fmoc-D-Trp-OH, HOBt, and DIPC/DMF; g) (i) 10% H_2NNH_2, dioxane-MeOH (9:1), RT, 2 h, (ii) dry ice; h) (i) 3.1 M HCl-dioxane/DMF, -50 °C, (ii) isoamyl nitrite, -30 to -20 °C, 1.5 h, (iii) dilution with DMF, -60 °C, (iv) Et_3N, -60 to -20 °C, overnight, (v) evaporation followed by preparative TLC on SiO_2 ($CHCl_3$-MeOH 10:1, R_f = 0.68; i) (i) TFA/EDT, 0 °C to RT, 1 h, (ii) evaporation followed by trituration with ether.*

Scheme 1.

of being cyclic or acyclic showed higher affinities than did the amino acid analogues. In addition, most replacements with amino acids, which had various functional groups on their side chains, did not significantly modify ET_A binding affinity. The D-Val^4/D-alloIle^4 was very important for inhibitory activity, and a β-position branched D-amino acid

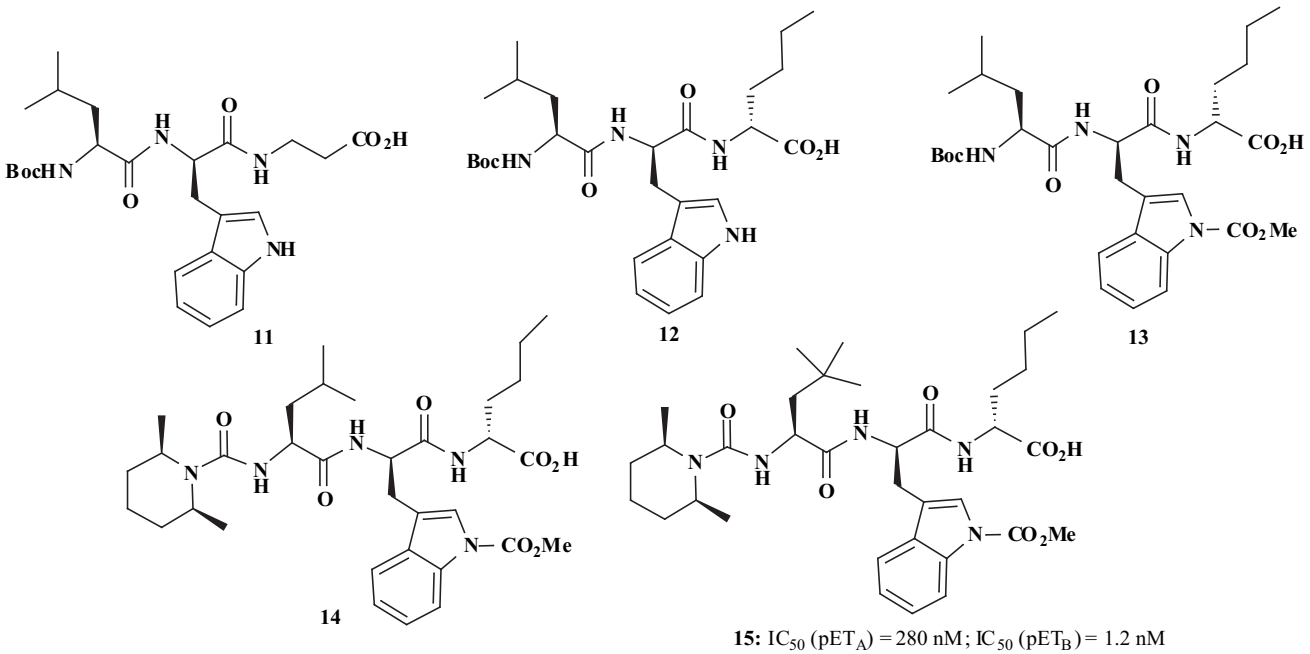
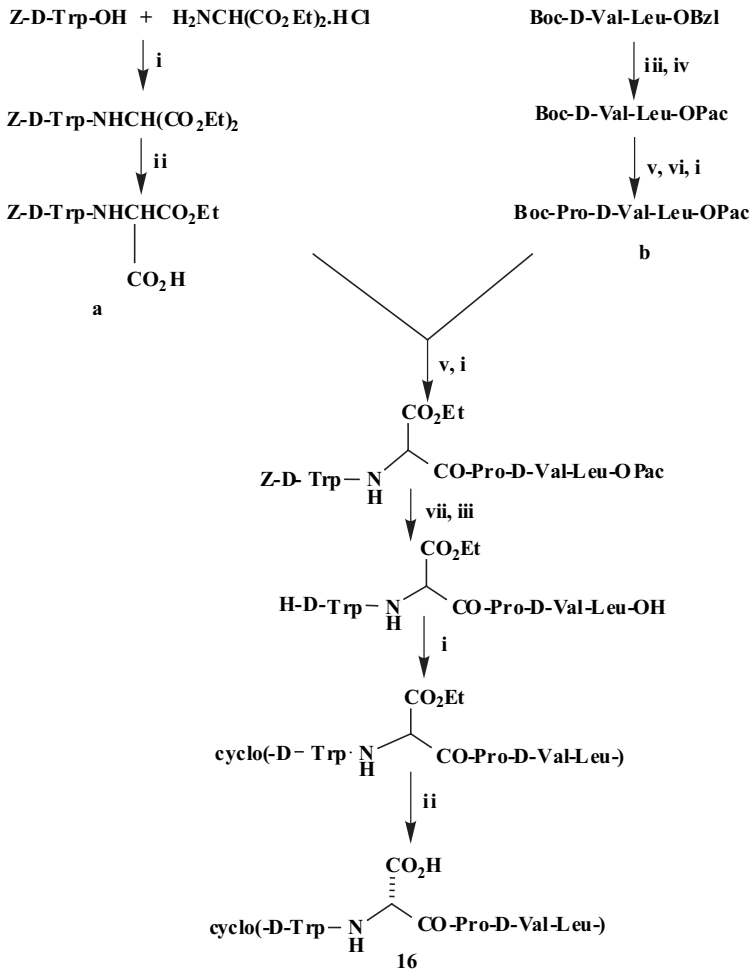


Fig. (4). Chemical structures of peptides **11-15.**

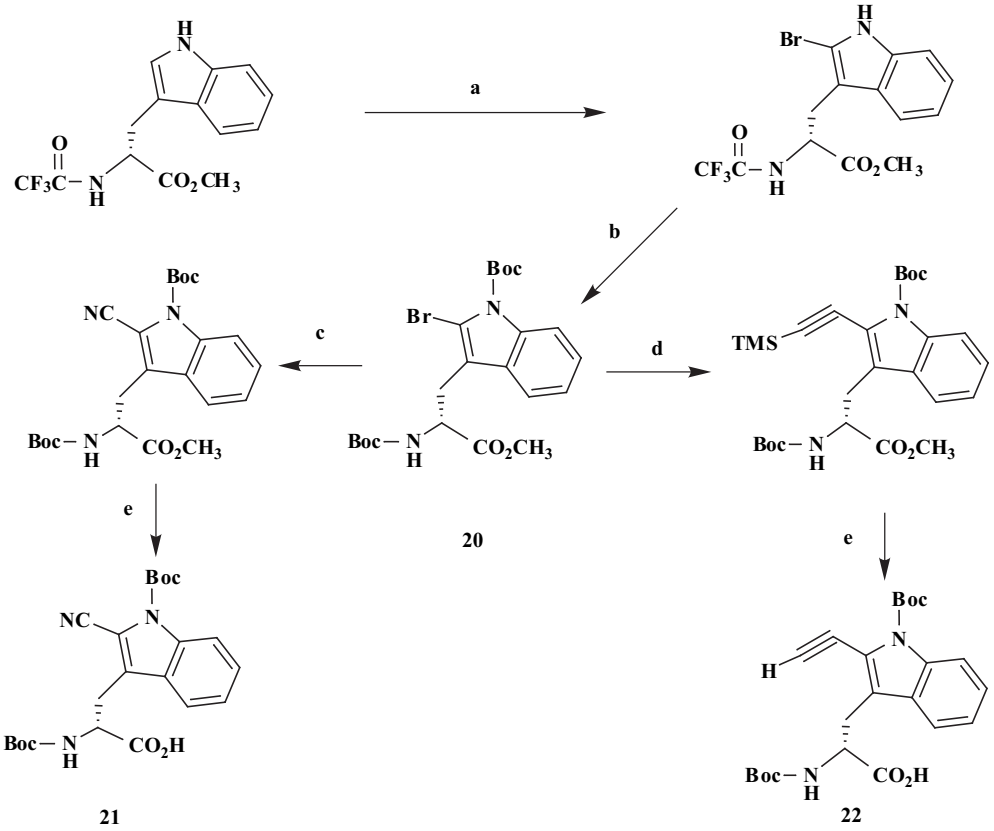
or a D-heteroarylglycine was preferable at this position. Among the synthesized cyclic pentapeptides, BQ-518 (**10**) (Fig. (**3**)) was found to be the most potent ET_A receptor antagonist showing the highest selectivity between ET_A and ET_B receptors. In contrast, compound **3** is a potent and highly soluble antagonist with a high ET_A / ET_B selectivity ratio. These compounds are useful tools for *in vitro* and *in vivo* pharmacological studies of endothelin and endothelin receptors. SAR study [45] for the identification of potent and selective tripeptide ET_A antagonists revealed that some structural alterations intensified an ET_B rather than ET_A affinity of some of the analogues **11-15** (Fig. (**4**)) synthesized by them. The synthesis of an aminomalonic acid (Ama) containing analogue, compound **16**, is illustrated in scheme 2. The replacement of the C-terminal β-alanyl residue in **11** with a D-norleucyl residue resulted **12**, which considerably increased ET_B affinity but, at the same time, decreased ET_A affinity. The introduction of a methoxy carbonyl group onto the indole nitrogen of the D-tryptophanyl residue in compound **12** resulted in an analogue **13** with a markedly increased ET_B affinity. Subsequent modification of **13** revealed that the replacement of the *N*-terminal *tert*-butoxycarbonyl (Boc) group with a *cis*-2,6-dimethylpiperidinocarbonyl group further improved ET_B affinity (**14**). Likewise, the replacement of the leucyl residue in **14** by a γ-methylleucyl residue finally yielded **15** (BQ-788) (Fig. (**4**)), a potent and selective inhibitor of ET binding to ET_B receptors. During the investigation of structure-activity relationships of the cyclic pentapeptides (**1-3**) Nagase *et al.* [46] revealed that all the peptides possessing different amino acid residues (third residue in the sequence), had similar ET_A antagonistic activity. Optimization of each amino acid residue then led to the potent ET_A antagonist BQ-485 (**17**) (Fig. (**5**)) or a selective ET_B receptor antagonist BQ-788 (**15**).

Fukami *et al.* [47] further studied the SAR of 2-substituted D-Tryptophanyl residues in both linear tripeptide derivatives and cyclic pentapeptide ET antagonists for the discrimination of ET_A/ET_B receptor subtype selectivity. The synthesis of the tripeptide derivatives and the cyclic pentapeptides with 2-substituted D-tryptophans involved the synthesis of D-tryptophan analogues with C-2 substituents and their subsequent derivatisation. The D-tryptophan analogues with 2-halo (**20**), 2-cyano (**21**), and 2-ethynyl (**22**) were synthesized as in scheme 3. The 2-methyl D-tryptophan analogues (**23** and **24**) were prepared by Sato and Kozikowski [48], as shown in scheme 4. The introduction of 2-halo and 2-methyl-D-tryptophans produced combined ET_A/ET_B receptor antagonists while the introduction of 2-cyano-D-tryptophan afforded ET_B antagonists decreasing



***Reagents**: i) NMM, EDCI, HOBt; ii) NaOH (aq); iii) Pd-C, H_2; iv) phenacyl bromide, Cs_2CO_3; v) TFA; vi) Boc-Pro; vii) Zn-powder, 90% AcOH*

Scheme 2.

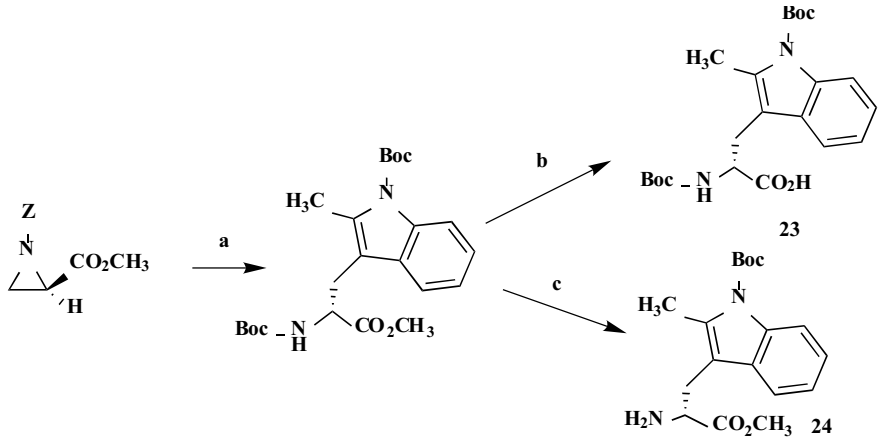


***Reagents**: a) CCl_4, NBS, Δ; b) (i) CH_3CN, Boc_2O, DMAP, (ii) 3-(dimethylamino)propylamine; c) DMF, CuCN; d) (trimethylsilyl)acetylene, $(Ph_3P)_4Pd$, CuI, Et_2NH; e) MeOH, aq NaOH.*

Scheme 3.

ET_A affinity. Therefore, the C-2 substituent of the D-tryptophanyl residue appears to be very important for the determination of ET_A/ET_B subtype selectivity of the antagonists. The presence of a group like methoxycarbonyl on the indole nitrogen of the D-tryptophanyl residue of BQ-788 (**15**) was found to be very effective for both strong ET_B antagonistic activity and ET_A/ET_B receptor subtype selectivity. Of the derivatives comprising D-tryptophan analogues with modifications on the indole ring, potent ET_A/ET_B-nonselective and ET_B-selective receptor antagonists were found in 2-substituted D-tryptophan containing tripeptide derivatives. For example, the 2-bromo-D-tryptophan containing linear tripeptide derivative BQ-928 (**18**) (Fig. (**5**)) is a combined ET_A/ET_B receptor antagonist while the 2-cyano-D-tryptophan containing derivative BQ-017 (**19**) is an ET_B selective antagonist [49].

Spatola and Crozet (University of Louisville) [50] reported solid phase synthesis and bioassay of a cyclic pentapeptide library designed to validate the efficiency of



***Reagents**: a) 2-methylindole, $Zn(OTf)_2$, $CHCl_3$; b) MeOH, aq NaOH; c) MeOH, Pd-C, H_2*

Scheme 4.

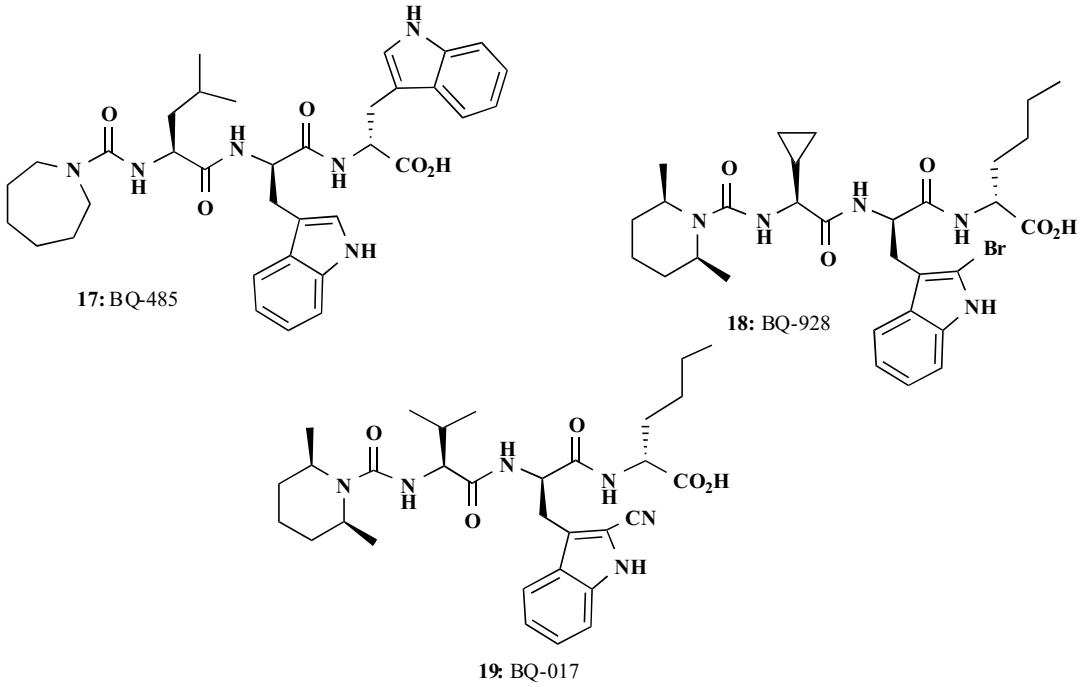


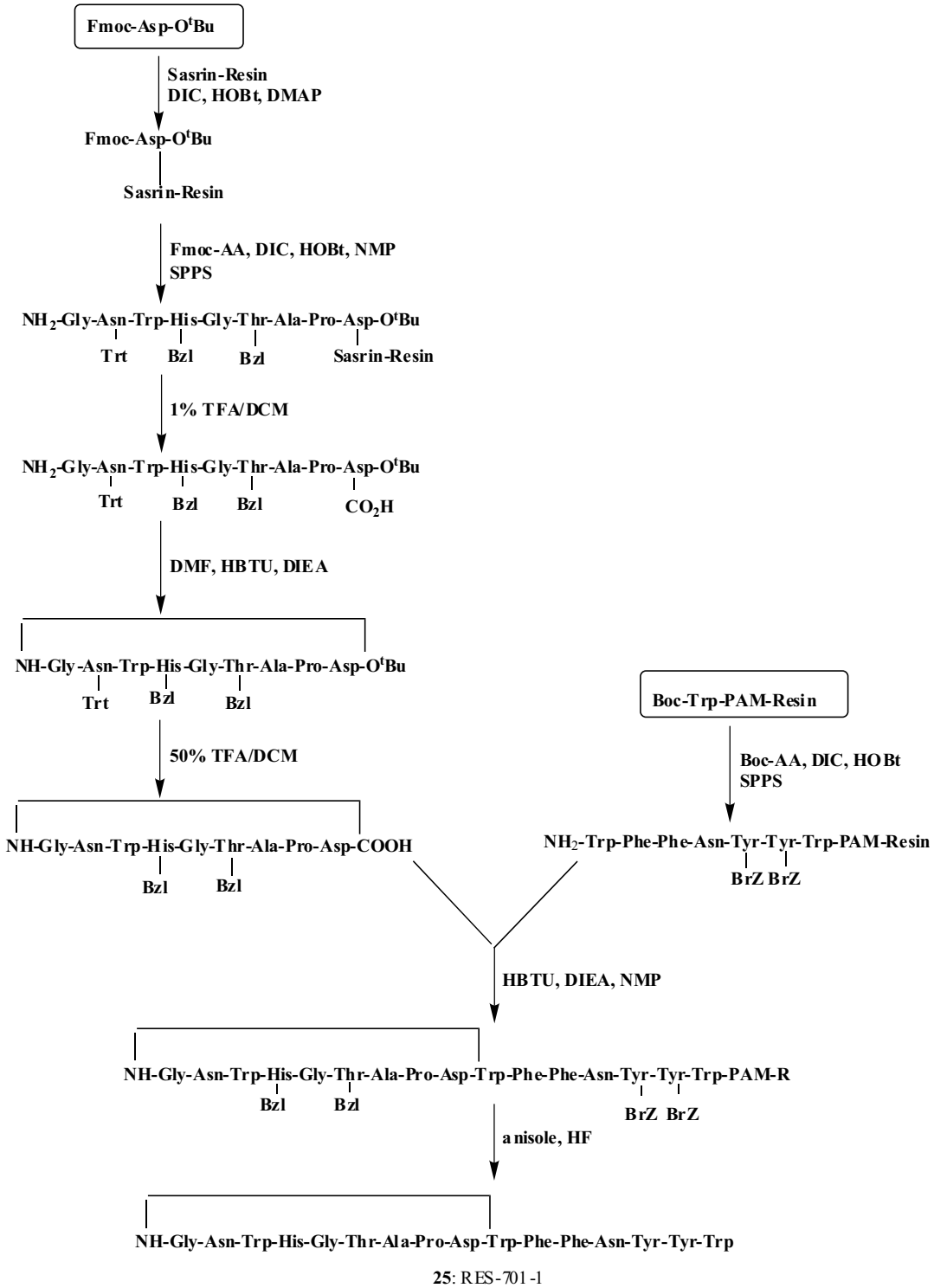
Fig. (5). Chemical structures of peptides BQ-485, BQ-928 and BQ-017.

moderately large mixtures combined with a positional scan approach as applied to cyclic peptides and related compounds. The major synthetic feature was side chain attachment of the first amino acid to the resin, and on-resin cyclization appeared reasonable and effective for the preparation of head to tail cyclic peptide libraries. Although they could not prepare better antagonist than BQ-123 (**3**) their self deconvoluting library confirmed that the strategy was useful for the rapid identification of suitable targets for new receptor classes where no prior constrained leads were available.

In an attempt to develop specific antagonists for the ET_B receptor, Urade *et al.* (Ciba-Geigy) [51] realized that this receptor had recognized the structure common to all the three ET isopeptides. They, therefore, synthesized a peptide – IRL-1038 – corresponding to the C-terminal half (residues 11-21) of ET-1 because this segment was conserved in all the three ET isopeptides. The structure of the peptide is [Cys^{11}-Cys^{15}]-ET-1(11-21). It had one Cys^{11}-Cys^{15} disulfide bond instead of two disulfide bonds at Cys^{1}-Cys^{15} and Cys^{3}-Cys^{11} in ETs. By using this antagonist they had shown that contraction of guinea pig ileal and tracheal smooth muscle was, at least in part, mediated by the ET_B receptors. Earlier studies [52] of a series of linear peptide corresponding to the C-terminal position of ET-1 and their N^{α}-succinyl derivatives revealed that Suc-[Glu^{9}-$Ala^{11,15}$]-ET-1(8-21), IRL-1620, was the most potent and specific ligand for the ET_B receptor. IRL-1620 is actually an agonist and induces contraction of guinea pig tracheal muscle with an efficiency comparable to that of ET-3 [52]. Its cluster of charged residues in the *N*-terminal portion seems to be involved in the agonist activity to contribute to the high selectivity for the ET_B receptor, in view of the fact that the selectivity of IRL-1038 to the ET_B receptors is markedly lower than that of IRL-1620. Nevertheless, IRL-1038 when used at appropriate concentrations is a specific antagonist for the ET_B receptor. When the disulfide bond of IRL-1038 was replaced by Cys-Ala substitution (IRL-1443), the affinity and selectivity for the ET_B receptor decreased markedly in porcine lung [45] and in various tissues of rats, guinea pigs and human beings, indicating that the disulfide bond in IRL-1038 is important to maintain its high affinity and selectivity for the ET_B receptors. The ET_B receptor was recently deduced to be significantly involved in positive inotropy [53], inflammation [54], renal functions [55], asthma [56], ocular functions [57], venous constriction [16c], vasodilation [58] and also in several central nervou functions [59]. Thus, IRL-1620 and IRL-1038, an ET_B receptor-selective agonist and antagonist, respectively, will be useful to clarify the role of ET_B-mediated responses in these biological functions.

Cody and co-workers (Parke-Davis) [60] presented the first known functional antagonist of endothelin-stimulated vasoconstriction Ac-D-Dip-Leu-Asp-Ile-Ile-Trp; PD-142893 (D-Dip = D-diphenylalanine) which showed high affinity for both ET_A and ET_B receptor subtypes. PD-142893 was a potent non-selective ET receptor antagonist with IC_{50} values 15 nM and 150 nM for ET_A and ET_B, respectively, with limited tolerance for modification. SAR [61] has revealed the importance of the C-terminal hexapeptide (residues 16-21) of ET (His^{16}-Leu^{17}-Asp^{18}-Ile^{19}-Ile^{20}-Trp^{21}) to the development of potent antagonists at both receptor subtypes. Position 17 (Leu) can incorporate acidic as well as basic residues maintaining affinity for both receptor subtypes [61]. Position 18 only tolerates acidic and aliphatic substitutions without loss of ET_A receptor affinity; however the rat ET_B receptor allows all modifications. Thus compounds Ac-D-Dip^{16}-Leu-Lys-Ile-Ile-Trp^{21}, Ac-D-Dip^{16}-Leu-Phe-Ile-Ile-Trp^{21} and Ac-D-Dip^{16}-Leu-Tyr-Ile-Ile-Trp^{21} are rat ET_B selective ligands but Ac-D-Dip^{16}-Leu-Lys-Ile-Ile-Trp^{21} does not bind to human ET_B receptor at concentration up to 10 μM.

Morishita *et al.* [62] isolated a novel cyclic ET_B selective antagonist RES-701-1 (**25**) the primary structure of which was determined by the sequencing of peptide fragments obtained by limited chemical hydrolysis and FAB-MS [63]. RES-701-1 (**25**) possesses a novel internal linkage between β-carboxyl group of Asp^9 and α-amino group of Gly^1. Recently, **25** has been reported to inhibit ET-1 induced increase in cytosolic Ca^{2+} levels in transfected COS cells expressing the human ET_B receptor [62]. In order to further study the pharmacological role of the ET_B receptor, He *et al.* [64] synthesized **25** by a combined solution/solid phase synthetic strategy. They found that the peptide was not selective for the human ET_B receptor over that of the ET_A receptor. Rather, it only possesses micro molar affinity for both the receptor subtypes. They suggested that the reported amino acid sequence of **25** might either be contaminated with a very minute quantity of a highly potent compound or be incorrect. Katahira and co-workers [65] synthesized **25**



25: RES-701-1

Scheme 5.

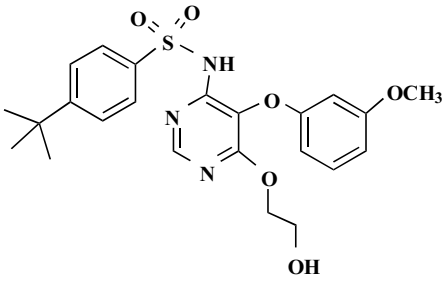
and examined it by ^{1}H NMR and FAB-MS and compared the results with those for authentic **25**. They realized that their synthetic **25** also exhibited much lower receptor binding activity compared to the authentic **25** as was reported by He *et al.* [64] (for synthesis see scheme 5). However, they confirmed the amino acid sequence of **25** reported by Yamasaki [63]. Secondary structure of the authentic **25** was found remarkably different from the synthetic one, which could be the reason for the difference in the receptor binding activity. It was considered that due to the extraordinary folding adopted by authentic **25** it is difficult to synthesize such a peptide by ordinary method.

B: NON-PEPTIDIC ET

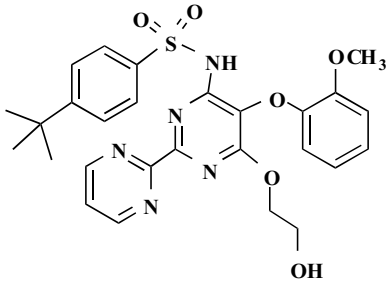
A large number of non-peptide ET antagonists of different subtype selectivity have been discussed in the literature and used as pharmacological tools to further increase the understanding of the physiological importance of ET [66]. Mimicking binding of a peptide to its receptor or blocking of such binding by small molecule antagonists is an essential first step in drug discovery. We will discuss different types of non-peptidic ETs based on particular functional groups present in the structure.

1. Sulfonamides

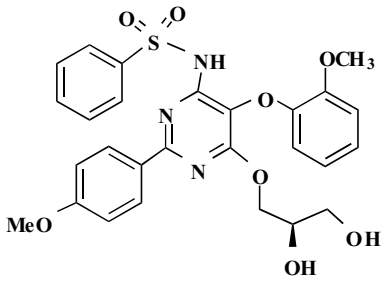
The development of non-peptide, small molecule antagonists that could be orally administered became an important objective for the chemists ever since the discovery of endothelin. Several thousands of compounds from a chemical library were screened for their capacity to inhibit specific ^{125}I-ET-1 binding in a human placenta membrane preparation. This screening led to the identification of a class of pyrimidinyl sulfonamides that had been synthesized as a part of an antidiabetic project and were noted to be weak inhibitors of ^{125}I-ET-1 binding. Structural modification of these compounds by chemical synthesis led to the discovery of Ro-46-2005 [67] [4-*tert*-butyl-*N*-[6-(2-hydroxyethoxy)-5-(3-methoxyphenoxy)-4-pyrimidinyl]benzenesulfonamide (**26**)] (Fig. (**6**)), the first orally active non-peptide endothelin receptor antagonist. Clozel *et al.* [68] provided evidence for the pathophysiological role of ET-1 as brought by this potent inhibitor. The efficacy of **26** in different experimental models showed the potential of this new therapeutic concept of ET receptor blockade in the treatment of vasoconstriction. Breu and coworkers [67] performed competition and saturation binding studies on tissues or cells containing ET_A or ET_B receptors and also analyzed the effect of Ro-46-2005 on the release of arachidonic acid, one of the second messengers involved in the action of ET. Bosentan or Ro-47-0203, 4-*tert*-butyl-*N*-{6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2,2'-bipyrimidin-4-yl}benzenesulfonamide (**27**), a new non-peptide mixed antagonist of ET receptor was obtained by structural optimization [28] of the less potent **26**. Clozel and coworkers [28] described the *in vitro* and *in vivo* effects of Bosentan and its capacity to inhibit the biological consequences of stimulation of the ET receptors. The selectivity, inhibitory and oral activity along with its duration of action made Bosentan to be approved in 2002 for use in pulmonary arterial hypertension. Actelion in Europe and Genentech in the US market it under the trade name Tracleer. Breu *et al.* [69] described *in vitro* characterization of Ro-46-8443 (**28**), the first non-peptide antagonist selective for the endothelin ET_B receptor. It is a sulfonamide with central pyrimidine moiety and is structurally related to **26** and Bosentan (see Fig. (**6**)), which bind to both ET_A and ET_B receptors with comparable potency. However, replacement of the second pyrimidine, found in the Bosentan structure, by a *p*-methoxyphenyl moiety and replacement of



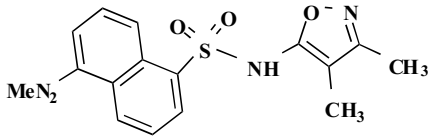
26: Ro-46-2005



27: Bosentan (Ro-47-0203)

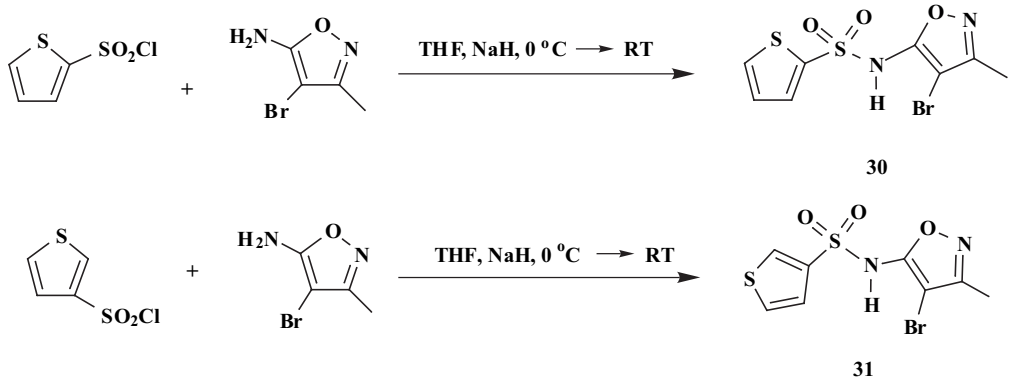


28: Ro-46-8443



29: BMS-182874

Fig. (6). Structures of Ro-46-2005, Ro-46-8443, Ro-47-0203 and BMS-18274.

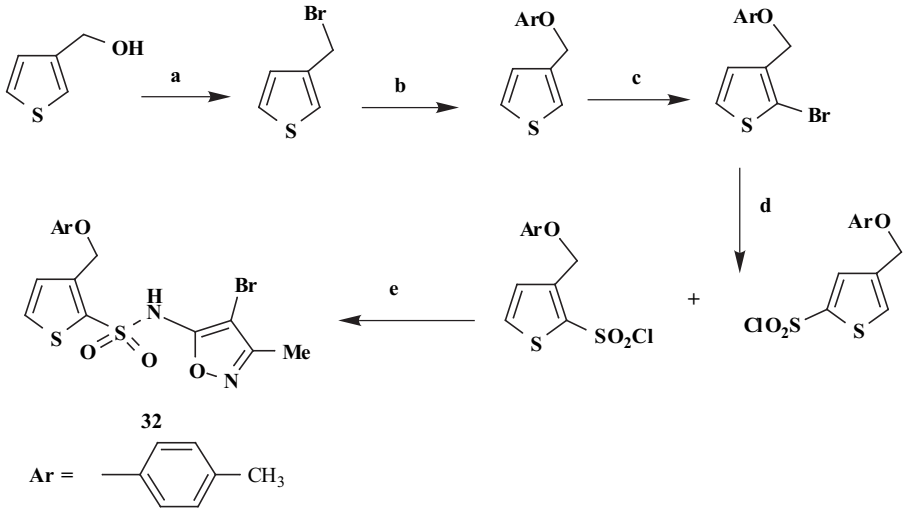


Scheme 6.

the hydroxyethoxy side chain by a glycerol residue resulted in an ET_B receptor selective antagonist with 100-fold selectivity for ET_B over ET_A receptors.

Stein *et al.* (Bristol-Myers Squibb) [70] described the discovery of benzenesulfonamide ET_A receptor antagonists and SAR studies which led to the identification of BMS-182874, 5-dimethylamino-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide (**29**); a potent, orally active, highly selective ET_A receptor antagonist. The poor affinity of 4-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-methylbenzenesulfonamide indicated that unsubstituted sulfonamide nitrogen was critical to the receptor affinity of this class of ET antagonists. Studies of the isoxazole substituents using 4-amino-*N*-(5-isoxazolyl)-benzenesulfonamide indicated that the 4-methyl group is required for potent binding while replacement of the 3-methyl group with larger substituents led to large losses in affinity [18,71]. Using *N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide, the effects of phenyl substituents were studied and *N*-(3,4-dimethyl-5-isoxazolyl)naphthalenesulfonamide containing aromatic nitrogen substituents were prepared for all possible substitution patterns. The 1,5-substitution pattern provided the most potent analogues. Stein *et al.* [72] further described in detail the SAR of sulfonamide ET_A antagonists. Random screening of compounds in an ET_A receptor-binding assay led to the discovery of a class of benzenesulfonamide ligands. Optimization led to the discovery of 5-amino-*N*-(3,4-dimethyl-5-isoxazolyl)-1-napthalenesulfonamides, which were functional antagonists. Structural features of this class of molecules that contributed to receptor affinity included a 1,5-napthalene-substitution pattern, a 5-amino-substituent, a relatively acidic sulfonamide NH and a dimethylisoxazole.

In order to understand the structural and conformational requirements for its endothelin receptor affinity, Krystek *et al.* [73] used a molecular modeling based three-dimensional quantitative structure activity relationship (QSAR) to study the naphthalenesulfonamide ET_A receptor antagonists. They applied steric and electrostatic fields (comparative molecular field analysis, CoMFA) to 36 aryl sulfonamides assayed for endothelin receptor ET_A antagonism, and provided predictions, which agreed well with experimental values. CoMFA was used to discriminate between possible bioactive conformations and between different molecular overlays, which superimposed functional groups giving slightly worse results supporting a hypothesis that suggests



Reagents: a) CH_2Cl_2, Py., Br_2, PPh_3, 0 °C; b) THF or DMF, NaH, ArOH, 0° C to RT;
c) $CHCl_3$-AcOH (1:1), NBS, RT; d) THF, n-BuLi, -78° C, SO_2, NCS, RT; e) NaH, THF, 5-amino-4-bromo-3-methylisoxazole, 0° C to RT

Scheme 7.

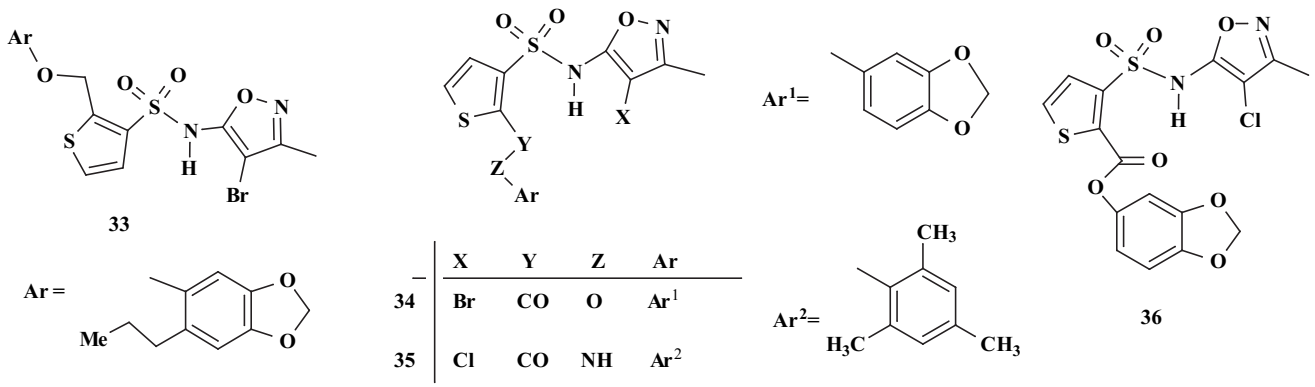


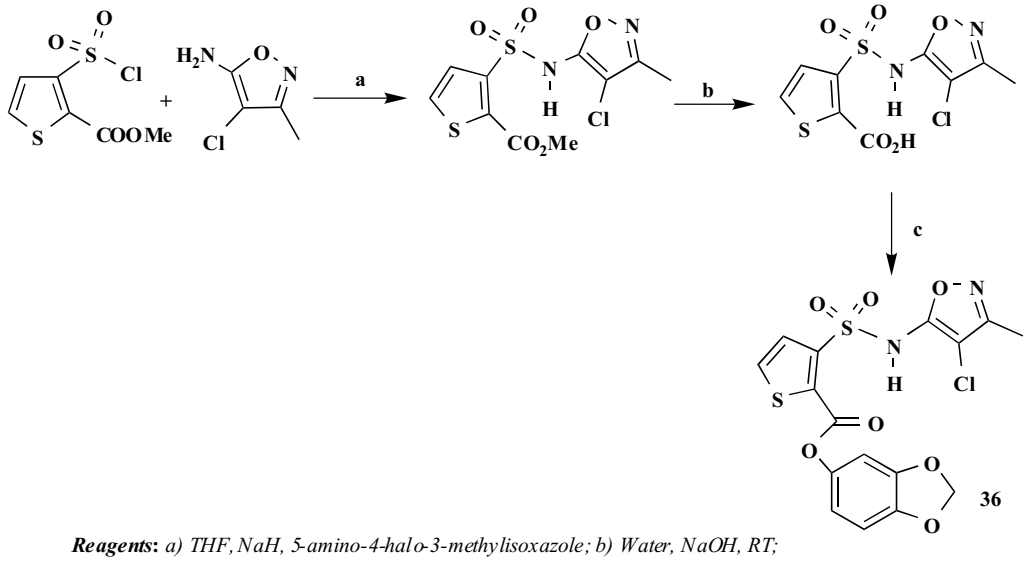
Fig. (7). Chemical structures of **33-36.**

a binding role for the orientationally variable Tyr^{129} in the ET_A receptor. The significance of the CoMFA results was validated through randomization trials of both biological activities and the molecular superposition, both of which yielded insignificant cross-validation results. Significant CoMFA results were obtained even with crude geometry and simple partial charge schemes. That the results improved substantially when the charges and geometry were refined further validating the physical significance of the results. Refining the charges improved results more than did refinement of geometry.

Chan *et al.* (ImmunoPharmaceutics/Texas Biotechnology) [74] reported that replacement of the 4-methyl group in a series of *N*-(3,4-dimethyl-5-isoxazolyl)benzenesulfonamide endothelin antagonists with a bromine or chlorine atom resulted in a three to 10-fold increase in the binding affinity for both ET_A and ET_B receptors accompanied by a potentiation of the antagonistic effect as measured by the attenuation of the ET-1 induced rat aortic smooth muscle contraction. This potentiation in activities was also observed for naphthalene- and biphenylsulfonamide endothelin antagonists.

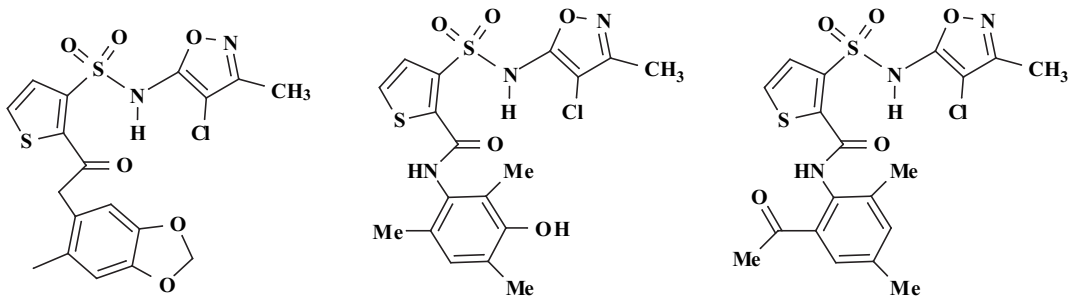
Raju *et al.* [75] designed a series of thiophene-sulfonamides as ET_A selective endothelin receptor antagonists based on molecular modeling and isosteric replacement of the phenyl ring in benzenesulfonamide endothelin receptor antagonists with a thiophene ring. They identified **30** and **31** (Scheme 6) as promising leads, which led them to develop SAR studies of the thiophenesulfonamides. The substitution pattern between the sulfonylisoxazole and aryl moieties on the thiophene ring determined the ET_A or ET_B selectivity of the phenyl thiophenesulfonamides. Raju and co-workers [76] used aryloxymethylene group as a replacement for an ester or an amide bond present in a series of ET_A selective endothelin antagonists. The ether linkage provided information on the effect of the carbonyl group in 2-aryloxycarbonylthiophene-3-sulfonamides on the binding affinity. The synthesis of 3-aryloxymethylthiophene-2-sulfonamide (**32**) is given in scheme 7. On one hand there was a 100-fold loss in ET_A binding by substituting the carbonyl group, present in 2-aryloxycarbonylthiophene 3-sulfonamides or 2-arylaminocarbonylthiophene-3-sulfonamides by a methylene group. On the other hand there was a substantial improvement in the ET_B binding as in analogue **33** (Fig. (**7**)) compared to the corresponding ester derivative.

The potential proteolytic instability of the amide bond present in some ET_A selective thiophenesulfonamide endothelin antagonists exemplified by TBC-10708 led Raju



Reagents: *a) THF, NaH, 5-amino-4-halo-3-methylisoxazole; b) Water, NaOH, RT; c) THF, Carbonyldiimidazole, Et3N, Carbondiimidazole, ArOH, Reflux.*

Scheme 8.



37: TBC-11251
IC_{50} (hET_A) = 1.7 nM;
IC_{50} h(ET_B) = 9.8 μM

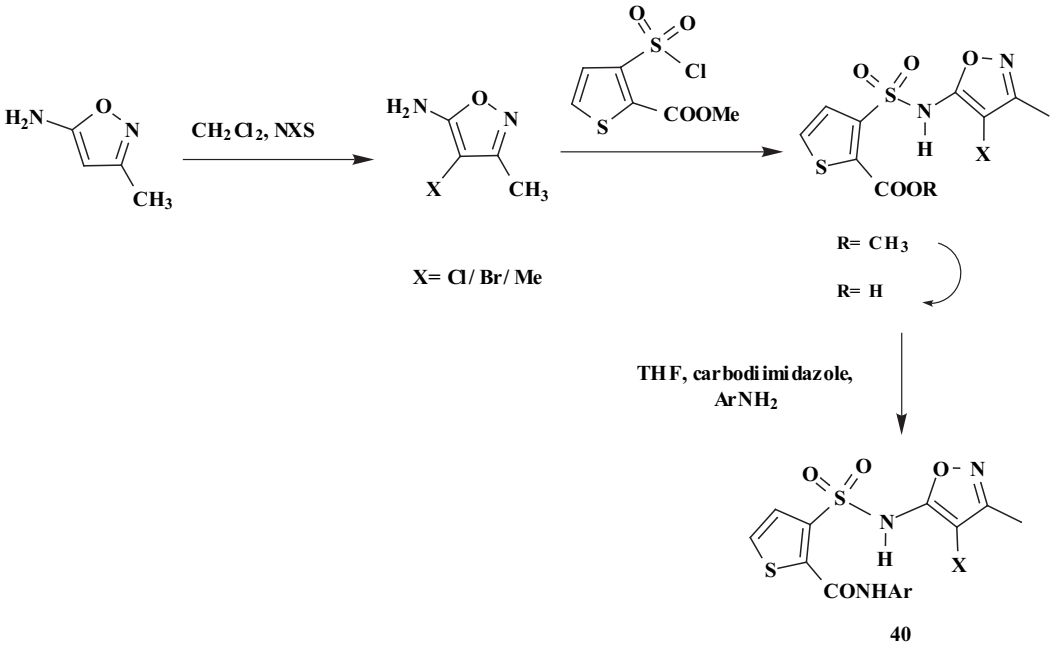
38: TBC-2576

39: TBC-3214

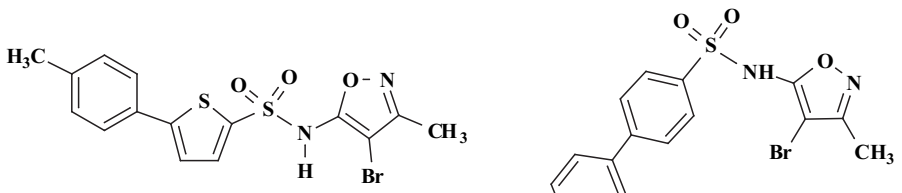
Fig. (8). Chemical structures of TBC-11251, TBC-2576 and TBC-3214.

and coworkers [77] to investigate its replacement with stable amide bond surrogates such as a *trans* double bond and an ethylene spacer. They proposed that the carbonyl group in **34** and **35** (Fig. (**7**)) might impose a conformational preference for the spatial display of the phenyl ring for optimum binding and/ or the carbonyl group might have favorable interaction with receptor elements. Their studies showed that there was no significant difference in the ET_A binding affinity of arylethyl- and aryloxymethyl thiophene-sulfonamides. Therefore, the oxygen atom in aryloxymethyl thiophenesulfonamides might not be a necessary element for highly potent inhibitory activity. Also there was no significant difference in the ET_A binding affinity of arylethyl- and cinnamylthiophenesulfonamides, which suggests that the conformation alone could not significantly influence the binding affinity. In continuation of the SAR studies on thiophenesulfonamides Raju *et al.* [78] further synthesized a series of 2-aryloxycarbonylthiophene-3-sulfonamides and identified *N*-(4-chloro-3-methyl-5-isoxazolyl)-2-[(3,4-methylenedioxy)phenoxycarbonyl]thiophene-3-sulfonamide (chloro compound **36**, Fig. (**7**), Scheme 8) as a highly selective, potent and low molecular weight ET_A non-peptide antagonist. They reported that the insertion of a carbonyl function between the phenyl and the thiophene rings resulted in dramatic improvement in ET_A potency.

In extension of the study of amide sulfonamides as ET_A selective endothelin receptor antagonists to improve the profile of the compounds, particularly regarding oral bioavailability and half life, Wu *et al.* [79] investigated replacement of the amide bond with various linker groups like imide, urethane, ureido and other extended amide linkages. They identified carbonyl group as the optimal spacer, leading to the discovery of TBC-11251 (**37**) (Fig. (**8**)), a compound with good potency, long duration of action and good bioavailability. Wu *et al.* [80] further presented the SAR of one particular thienylsulfonamide series: N^2-aryl-3-(isoxazolylsulfamoyl)-2-thiophenecarboxamides, a novel series of ET_A receptor antagonists consisting of three units - an isoxazole, a thiophene and an aryl moiety. A sulfonamide group tethers the isoxazole and the thiophene moieties while an amide linkage connects the aryl group and the thiophene. They had already shown that isoxazoles with dimethyl substituents are preferred for binding affinity [70,81] and a



Scheme 9.



41: TBC-10894 **42:** TBC-10950

Fig. (9). Chemical structures of TBC-10894 and TBC-10950.

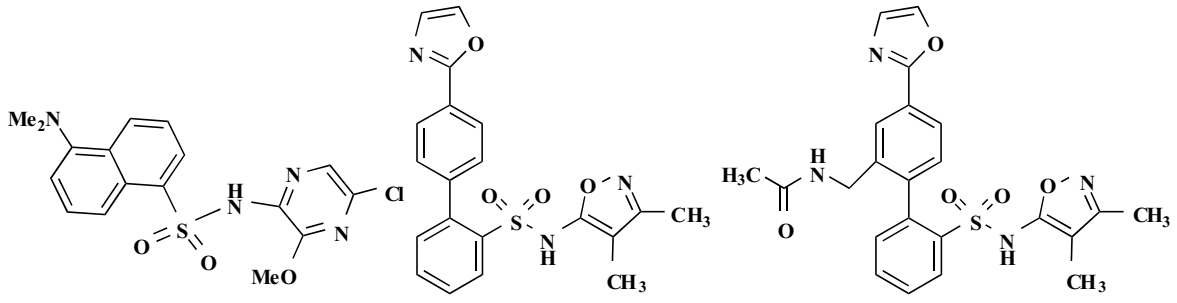
halogen substitution on the 4-position increases the binding affinity by 3 to 5-fold [18,82]. They, therefore, synthesized [78,80] isoxazolylsulfonamides with chloro, bromo and methyl group at the 4-position of the isoxazole ring as in scheme 9. The aryl group was subjected to extensive structural modification. With monosubstitution, the *para* position was most useful in increasing potency, with methyl being preferred. With disubstitution, 2,4-disubstitution further enhanced activity with methyl or cyano groups being preferred at the 2-position. Wu *et al.* [83] disclosed a new series of compounds with higher potencies by just putting an additional substituent at the 6-position of the anilino ring. It was also found that a wide range of functionalities at the 3-position of the 2,4,6-trisubstituted ring increased ET_A selectivity by ~10-fold while maintaining *in vitro* potency, therefore rendering the compounds amenable to fine tuning of pharmacological and toxicological profiles with enhanced selectivity. The optimal compound was found to be TBC-2576 (**38**) (see Fig. (**8**)), which has ~10-fold higher ET_A binding affinity than **37**, high ET_A/ET_B selectivity, and a serum half-life of 7.3 hours in rats as well as *in vivo* activity. The orally active **37** has demonstrated efficacy in a phase-II clinical trial for congestive heart failure [84] and attenuates pulmonary vascular hypertension and cardiac hypertrophy in rats [85].

In both the papers [79,80], Wu *et al.* had shown that an acyl group at 2-position of the anilide of the thiophene sulfonamides improved oral bioavailibility. As a result of their continuing exercise [86], **39** was identified as a **37** (trade name - Sitaxsentan, Fig. (**8**)) follow-on candidate. It was very potent (IC_{50} for ET_A = 0.04 nM) and highly selective for ET_A vs ET_B receptors (400000-fold), with a half-life of >4 hours and an overall good bioavailibility.

The reversal in selectivity was brought about by substitution of the 4-position with aryl and substituted aryl groups. Chan *et al.* [82] reported the systematic modification of the ET_A selective *N*-(5-isoxazolyl)benzenesulfonamide endothelin antagonists to 4-biphenyl and 5-aryl-2-thienylsulfonamide ET_B selective antagonists, TBC-10894 (**41**) and TBC-10950 (**42**), respectively (Fig. (**9**)). Of all the aromatic substituents studied, the *p*-tolyl group gave rise to the most active and selective ET_B antagonist. Larger substituents caused a decrease in both ET_B activity and selectivity. A similar trend was observed by substitution at the 5-position of the *N*-(5-isoxazolyl)-2-thiophene-sulfonamide ET_A receptor antagonists. The *p*-tolyl group was again found to be favorable for the ET_B activity and selectivity. The structural features that were found to be favorable for binding to the ET_B receptor, that is, the presence of linear, conjugated π-system of definite shape and size had been successfully incorporated into the design of ET_B selective polycyclic aromatic sulfonamide antagonists.

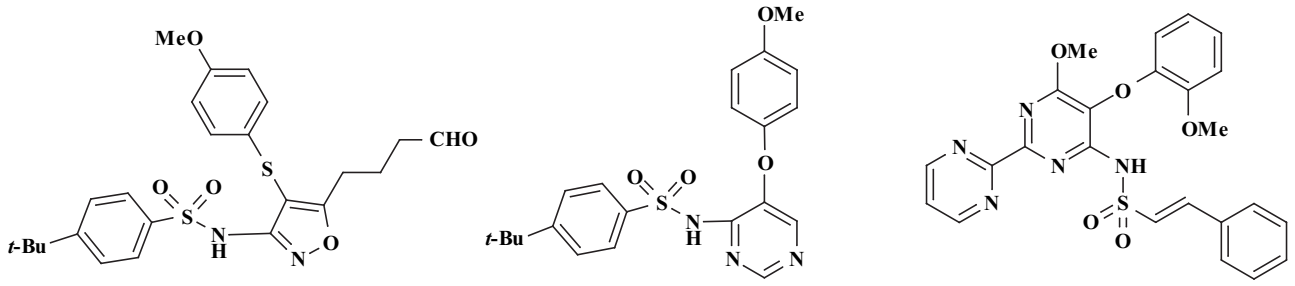
Use of automated synthesis to explore variation of the *N*-isoxazolyl substituent in the 1-napthalenesulfonamide ET_A antagonist BMS-182874 (**29**) (Fig. (**6**)) led Bradbury *et al.* (ZENECA Pharmaceuticals) [87] to discover several 6-membered nitrogen heterocycles as replacements for the isoxazole moiety. In each of these heterocycles, a small substituent such as halogen *para* to the position of attachment to the sulfonamide nitrogen atom was found to be advantageous for ET_A receptor affinity. Of these heterocycles, 5-(dimethylamino)-*N*-pyridyl-, -*N*-pyrimidinyl-, -*N*-pyridazinyl- and -*N*-pyrazinyl-), 2-pyrazines offered the greatest scope for improving receptor affinity. Optimization of the substituents at the 3- and 5-positions in the pyrazine ring led to potent ET_A selective compounds such as 5-(dimethylamino)-*N*-(5-chloro-3-methoxy-2-pyrazinyl)-1-napthalenesulfonamide (**43**) (Fig. (**10**)).

Recently Murugesan and co-workers (Bristol-Myers Squibb) [88] presented some findings in the area of 4'-isobutyl series of biphenylsulfonamides where they had



43 **44** **45**

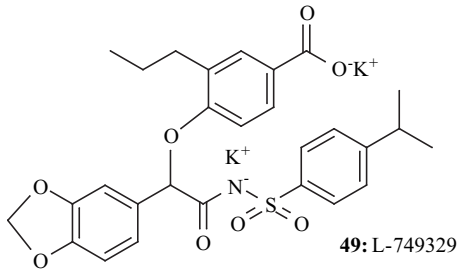
Fig. (10). Structures of **43**-**45**.



46 **47** **48**

Fig. (11). Chemical structures of compounds **46**, **47** and **48.**

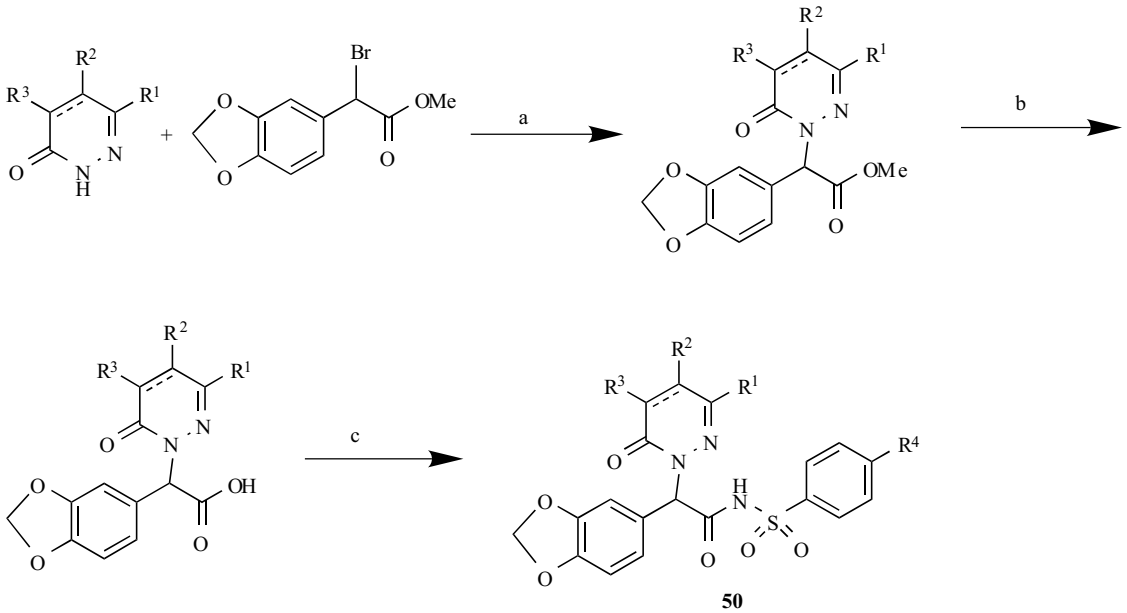
shown that addition of an appropriate 2'-substituent, such as an amine, hydroxyl, or acylaminomethyl group, resulted in substantial improvement in activity. Among the substituents, the acylaminomethyl one was found to be optimum. Very recently, Murugesan *et al.* [89] reported the synthesis and structure-activity studies of a series of 4'-oxazolyl-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1'-biphenyl]-2-sulfonamide derivatives as ET_A receptor antagonists. Among them, *N*-(3,4-dimethyl-5-isoxazolyl)-4'-(2-oxazolyl)[1,1'-biphenyl]-2-sulfonamide (**44**), had the optimum pharmacological profile [ET_A K_I = 1.4 nM, ET_B K_I = 18700 nM]. The potency and metabolic stability improved remarkably when the 2'-position was additionally substituted with acylaminomethyl group. This change further improved the binding affinity and provided ET_A selective antagonist, 2'-acylaminomethyl-*N*-(3,4-dimethyl-5-isoxazolyl)-4'-(2-oxazolyl)[1,1'-biphenyl]-2-sulfonamide (**45**) [ET_A K_I = 0.2 nM, ET_B K_I = 1700 nM]. Very recently Kanda and co-workers [90] discovered a new type of sulfonamides. In this paper they also disclosed the synthesis and SAR of this series of ET_B antagonists. The most potent compound **46** (Fig. (**11**)) displayed IC_{50} values of 1.7 μM and 0.002 μM to ET_A and ET_B receptors, respectively. The isosteric replacement of the isoxazole ring of the lead compound **30** with a pyrimidine ring led to the discovery of another highly potent ET_B selective antagonist **47** [91] with oral bioavailibility, although the modification of the terminal aldehyde group at the 6-position of the pyrimidin ring did not improve the potency further.



49: L-749329

Fig. (12). Chemical structure of L-749329.

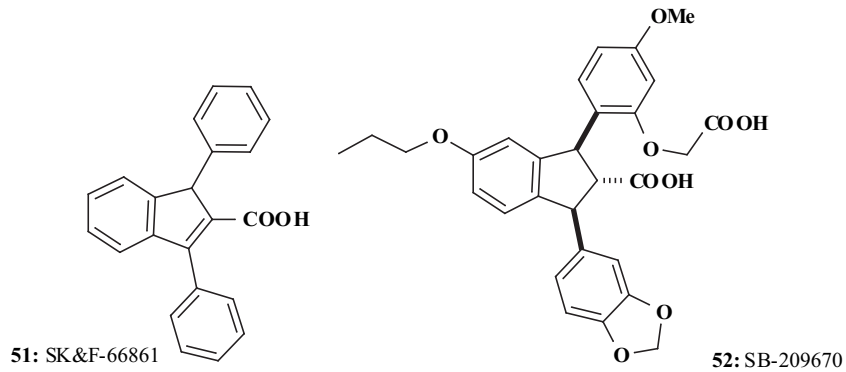
Harada and co-workers (Yamanouchi) [92] have shown recently that 2-phenylethenesulfonamide possesses potent affinity for ET_A receptor with high ET_A selectivity. Compound had a long acting oral activity in the inhibition of the pressor response caused by a big ET-1 infusion in both pithed and conscious rats. Monopotassium salt of compound **48** showed excellent pharmacokinetic profile with



50

Reagents: *a) $CsCO_3$, DMF; b) NaOH, MeOH; c) CDI, THF, 60 °C, 2 h, then* p-$R^4PhSO_2NH_2$, *DBU, 60 °C, 3 h.*

Scheme 10.

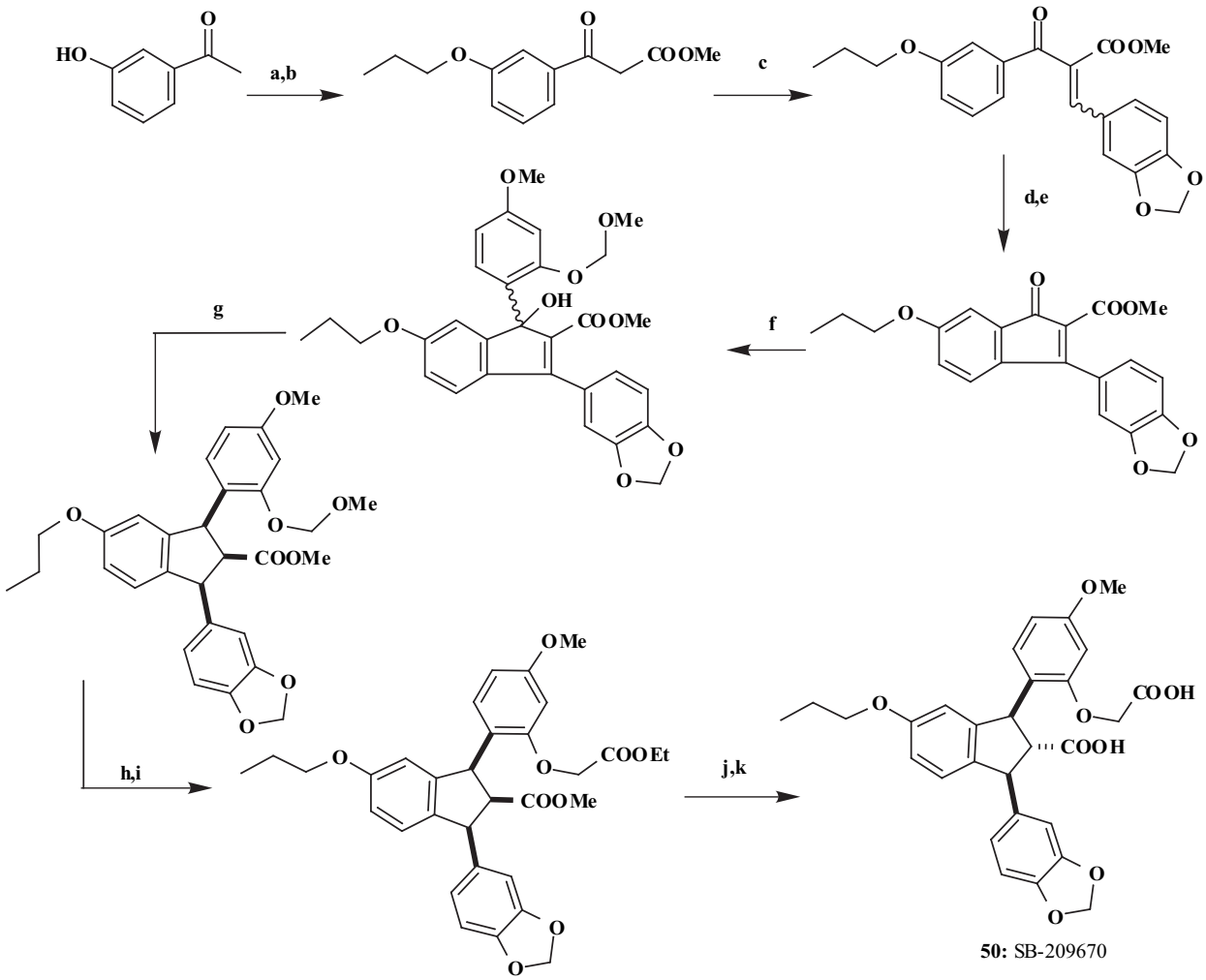


51: SK&F-66861 **52:** SB-209670

Fig. (13). Chemical structures of SK&F-66861 and SB-209670.

bioavailabilities of 89% in the rat and 97% in the dog (0.3 mg/kg). The monopotassium salt of **48** (YM598) is now in clinical trials.

One of the most important sulfonamide antagonists is the acylsulfonamide L-749329 (**49**) [93] (Fig. (**12**)). Dorsch and co-workers (Merck) [94] determined the minimum



50: SB-209670

Reagents: *a) DMF, K_2CO_3, n-PrI (quant.); b) dimethyl carbonate, NaH (quant.); c) benzene, piperidine, AcOH, 3,4-(methylenedioxy)benzaldehyde, reflux (48%); d) TFA (87%); e) dioxane, DDQ (44%); f) ether, [4-methoxy-2-(methoxymethoxy) phenyl]magnesium bromide (91%); g) EtOH-EtOAc, 10% Pd-C, H_2, 50 psi, 50 °C (96%); h) cat. HCl, MeOH, H_2O (78%); i) DMF, NaH, $BrCH_2COOEt$ (71%); j) resolution on chiralpak AD column; k) NaOH, H_2O, dioxane, then HCl, H_2O (71%).*

Scheme 11.

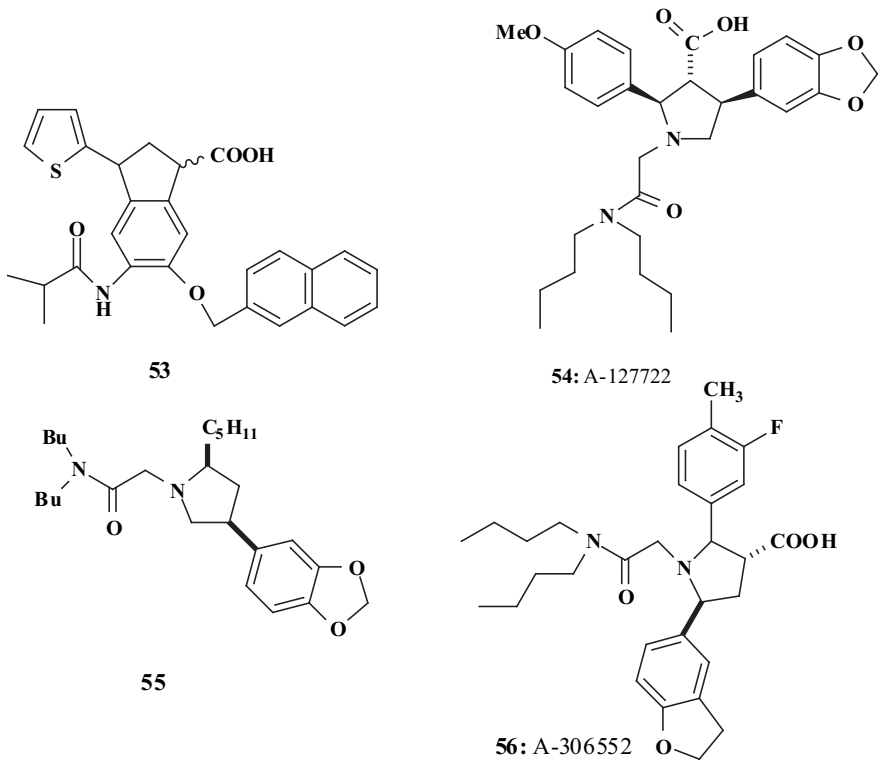
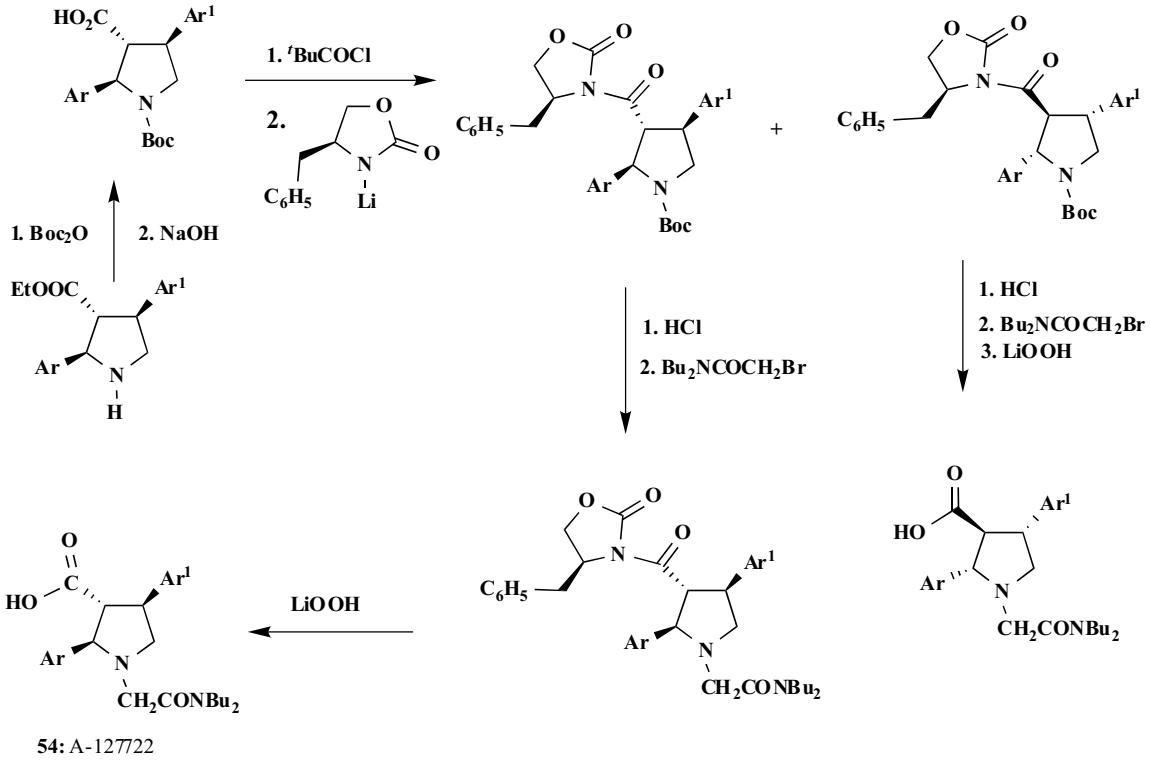


Fig. (14). Chemical structures of compounds **53-56.**

energy conformations of several derivatives of **49** where the aryloxy group was replaced by different heterocycles using the molecular modelling. The best overlap with **49** was achieved by 4-arylpyridazone derivatives **50**, which was synthesized [94] subsequently (Scheme 10).

L-744453, (±)3-[4-{1-carboxy-1-(3,4-methylenedioxyphenyl)methoxy}-3,5-dipropylphenylmethyl]-3*H*-imidazo-[4,5-*c*]pyridine (Fig. (**12**), **51**), is an endothelin receptor antagonist from a new structural class, the dipropyl-α-phenoxyphenylacetic acid derivatives [95]. Compound **51**

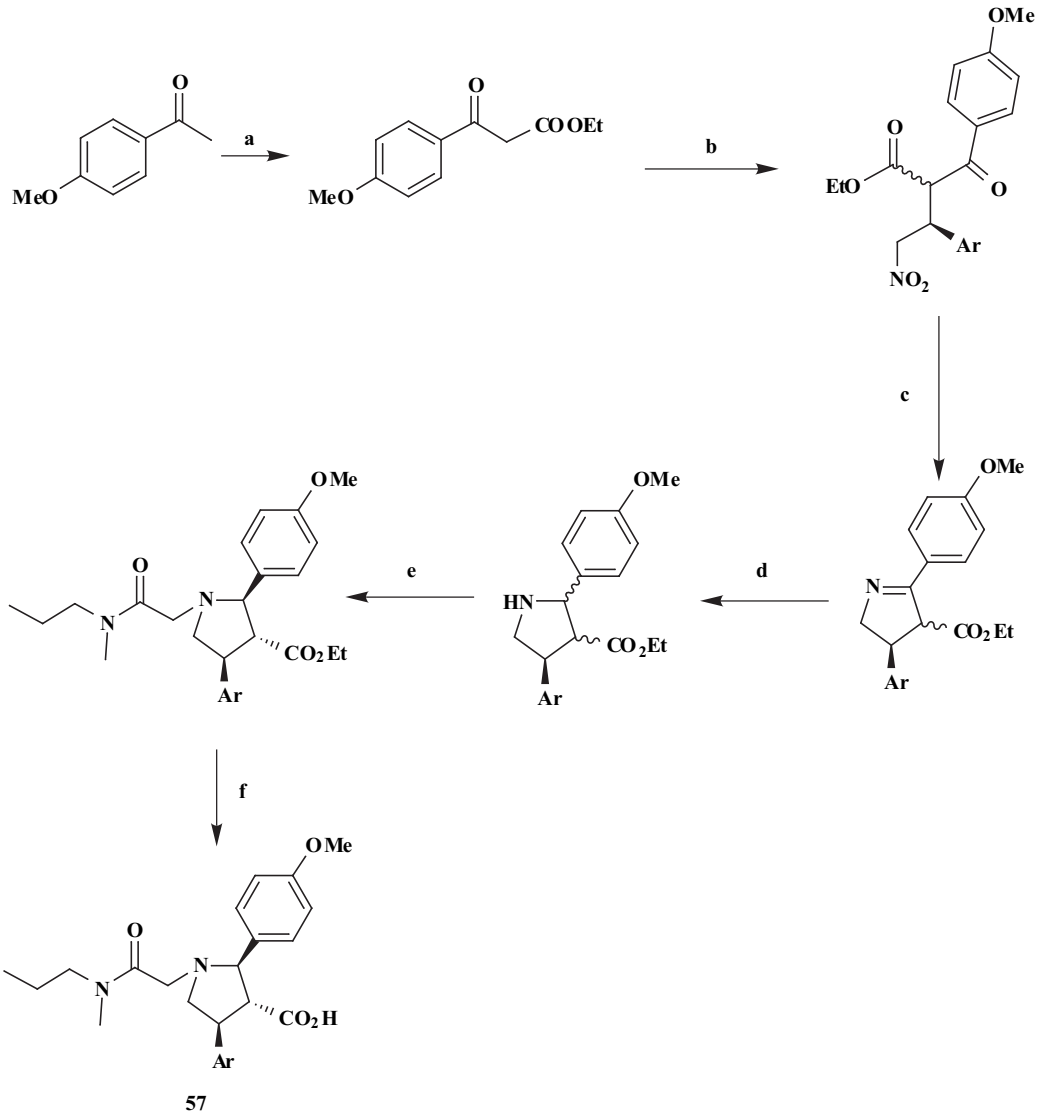


Scheme 12.

competitively and reversibly inhibits [^{125}I]ET-1 binding to Chinese Hamster Ovary cells expressing cloned human receptors (K_Is: hET_A = 4.3 nM; hET_B = 232 nM), and is selective for endothelin receptors compared to other peptide receptors. This compound inhibits ET-1 stimulated contraction of rat aortic rings with a K_b value of 50 nM. Compound **51** protects against ET-1 induced lethality in mice after i.v. (AD_{50} = 13 mg/kg i.v.) or oral administration. This compound also antagonises ET-1 induced increase in diastolic blood pressure in conscious normotensive rats [95].

2. Carboxylic Acid

The approach toward non-peptide endothelin receptor antagonists included the screening of compounds selected for their similarity to antagonists of other G-protein coupled receptors and containing features of ET-1 known to be important to receptor binding [96]. As a result of this effort, SK&F-66861 (**51**) (Fig. (**13**)) was identified and found to bind ET_A receptors selectively [97]. Using ^{1}H NMR derived conformational models of ET-1, this initial lead structure was elaborated according to the hypothesis that the 1- and 3-phenyl groups of **51** were mimics of a combination of two of the aromatic side chains of Tyr^{13}, Phe^{14} and Trp^{21} in ET-1. Additionally, the carboxylic acid was proposed to mimic either the Asp^{18} or C-terminal carboxyls in ET-1. Although several combinations of these elements appeared physically possible, one of the most energetically favorable conformations matched the carboxylic acid and 1- and 3-phenyl groups of the non-peptide lead molecule to Tyr^{13}, Phe^{14} and Asp^{18} of the peptide. Subsequent rational design for compounds containing oxidatively less labile indan, unlike that present in **51**, continued with the elaboration of the pendant aromatic groups reflecting the electron-rich nature of the tyrosine aryl ring. The hypothesis that the single carboxylic acid present in **51** mimics Asp^{18} suggested the incorporation of a second carboxylic acid moiety to take



57

Reagents: *a) NaH, $CO(OEt)_2$; b) nitrostyrene, 5 mol% DBU,iPrOH, THF; c) EtOAc, W-2 Ra-Ni, H_2 (4 atm); d) THF, EtOH, $NaCNBH_3$, HCl (pH 3-4); e) MeCN, iPr_2NEt, $BrCH_2C(O)N(Me)Pr$; f) 50% aq NaOH, EtOH.*

Scheme 13.

advantage of the receptor interaction responsible for binding the critically important C-terminal carboxyl of the ET isopeptides. This effort culminated in the identification of SB-209670 (**52**) (Figure (**13**), synthesis [98] is shown in scheme 11), a highly potent antagonist selective for the endothelin receptors possessing affinity for both ET_A (K_I = 0.2 nM) and ET_B (K_I = 1.8 nM). This produces antihypertensive action and protection against neuronal degeneration following cerebral Ischemia [99].

Very recently [100] Morimoto and co-workers (Tanabe Seiyaku) reported one indan derivative, 5-isobutyrylamino-6-(1-naphthylmethyloxy)-3-(2-thienyl)-1-indancarboxylic acid (**53**) (Fig. (**14**)). Using computer assisted molecular modeling, a putative pharmacophore was constructed from the superposition of the reported three-dimensional structure of the cyclic peptide BQ-123 (**3**). Among them, compound **53** showed a moderate ET_A antagonistic activity.

Replacement of the indan ring of **52** with a pyrrolidine ring led to the discovery and synthesis of a new class of endothelin antagonists, *N*-substituted *trans*,*trans*-2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)pyrrolidine-3-carboxylic acids, one of which is A-127722 (**54**) (Fig. (**14**)), which were then also evaluated by Winn *et al.* (Abbott) [101] for binding at ET_A and ET_B receptors. Compounds with *N*-acyl and simple *N*-alkyl substituents had weak activities and compounds with *N*-alkyl substituent containing ethers, sulfoxides or sulfones showed increased activities. Much improved activity resulted from compounds where *N*-substituents were acetamides. A-127722 (**54**) with *N*,*N*-dibutylacetamide substituent, a potent and orally bioavailable [102] ET_A selective inhibitor (IC_{50} (ET_A) = 0.4 nM; IC_{50} (ET_B) = 520 nM) which is now in phase III clinical trials for hormone-refractory prostate cancer, was thus synthesized as shown in scheme 12.

Boyd *et al.* (Abbott) [103] investigated the SAR of the 2-substituent on the pyrrolidine. Compounds with alkyl groups at the 2-position possessed ET_A selectivity improved over **54** (1400-fold selective), with the best of these compounds being **55,** which showed nearly 19,000-fold selectivity. Recently, Jae *et al.* [104] have reported highly selective and very potent ET_A receptor antagonist A-306552 (**56**) (Fig. (**14**)), a pyrrolidine-3-carboxylic acid derivative. Placement of a *p*-tolyl group at the 2-position and a 5-substituted dihydrobenzofuran at the 4-position of the pyrrolidine was required for this high level of selectivity [ET_A/ET_B = 38,700] for ET_A. Introduction of fluorine at the 3-aryl increased the binding affinity and improved the bioavailibility as well.

Tasker *et al.* [105] examined the role played by the benzodioxole moiety in endothelin receptor binding. The overall concern was the propensity of benzodioxole containing compounds to undergo cytochrome P450-mediated metabolism wherein the substrate becomes irreversibly bound to the enzyme, which could thereby lead to drug-drug interactions or non-linear pharmacokinetics. Although the view of Jae and co-workers [104] is different that absence of the benzodioxole moiety eliminates the potential for inhibiting cytochrome P450-enzymes which could thereby lead to a non-linear pharmacokinetics Tasker *et al.* [102] sought to develop compounds, with their own views, devoid of this functionality and synthesized a number of compounds **57** with the replacement of benzodioxole moiety (Scheme 13). They attempted to induce A-127722 to form such P450-carbene complexes but were unable to find any direct evidence for such an event. These benzodioxole replacement studies proved to be very interesting and they developed a series of potent orally active agents possessing sub-nanomolar binding affinity for the ET_A receptor. Simple

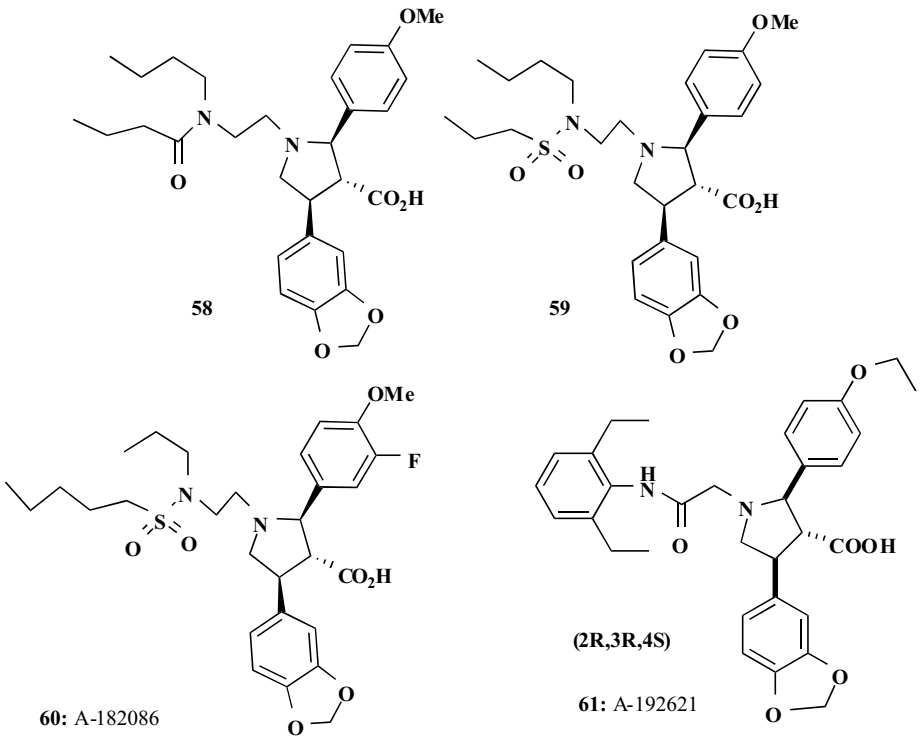
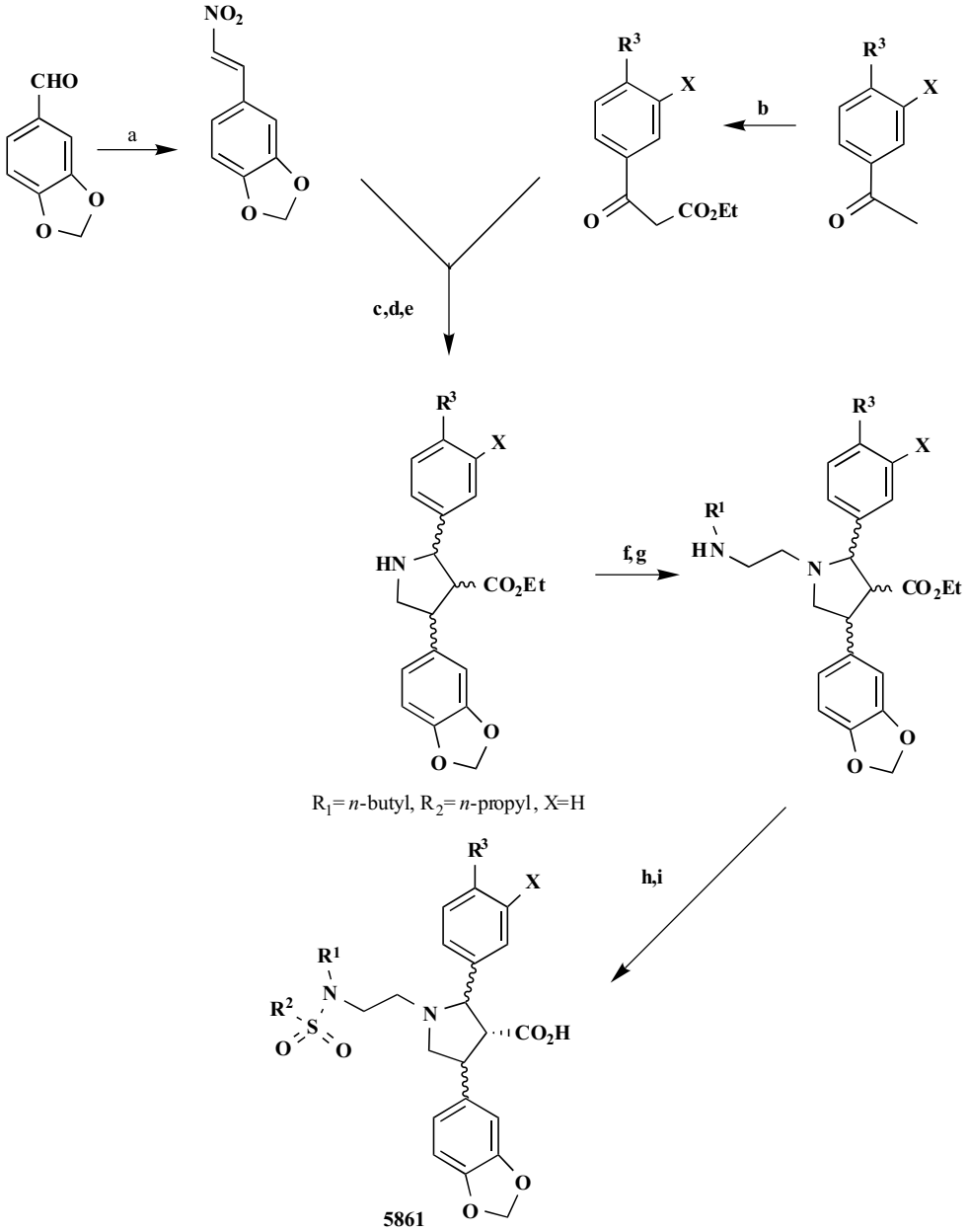


Fig. (15). Structures of compounds **58-61.**

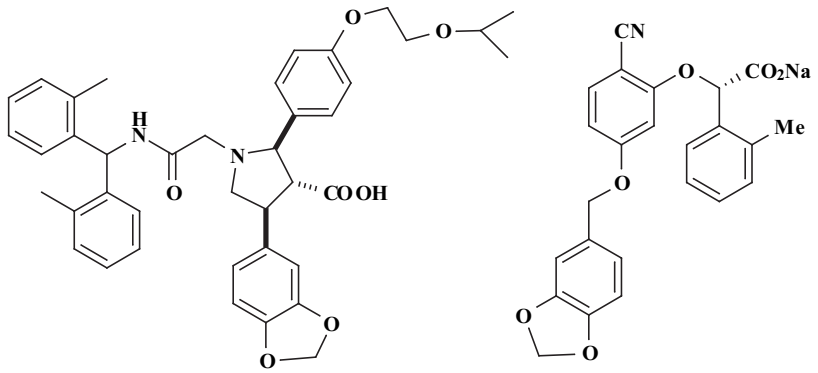
deletion of either of the two oxygen atoms (dihydrobenzofurans) provided this increased selectivity without sacrificing potency as ET_A receptor antagonist. The absorptions and oral bioavailabilities of all compounds were in moderate range (30-65%).

Jae *et al.* [106] explored the effect of reorienting the two alkyl groups by moving the H-bond donor to different positions on the side chain. The modification of the carbonyl group of the amide linkage transposed along the chain (compound **58**, Fig. (**15**)) led to a large decrease in ET_A affinity with little change in ET_B affinity. Intrigued by this observation, Jae & co-workers further experimented with other amide surrogates and discovered that the corresponding sulfonamide (compound **59**, Fig. (**15**)) was substantially more potent at both ET_A and ET_B receptors, while retaining the reduced ET_A/ET_B activity ratio of 1. In particular, the combination of an *N*,*N*-dipropyl group, an *S*-alkyl chain between four and six carbons in length, and a fluorine atom *ortho* to the aromatic-OCH_3 provides compounds with sub-nanomolar affinities for both receptor subtypes, and with ET_A/ET_B ratios close to unity. Of these, A-182086 (**60**) (Fig. (**14**)) exhibited the best combination of physical and biochemical properties. The SAR studies suggested that there was no direct mapping of the *N*- and *S*-alkyl groups of



***Reagents**: a) CH_3NO_2, AcOH, NH_4OAc, Δ; b) THF, NaH, $(EtO)_2CO$; c) THF-^{i}PrOH, DBU; d) THF-AcOH, Ra-Ni, H_2; THF-EtOH, $NaBH_3CN$, pH 5; e) THF, DBU, Δ; f) $Br(CH_2)_2Br$, cat. NaI, Δ; g) EtOH, R^1NH_2, cat. NaI, Δ; h) CH_3CN, $^iPr_2NEt$, R_2SO_2Cl; i) EtOH-H_2O, NaOH.*

Scheme 14.



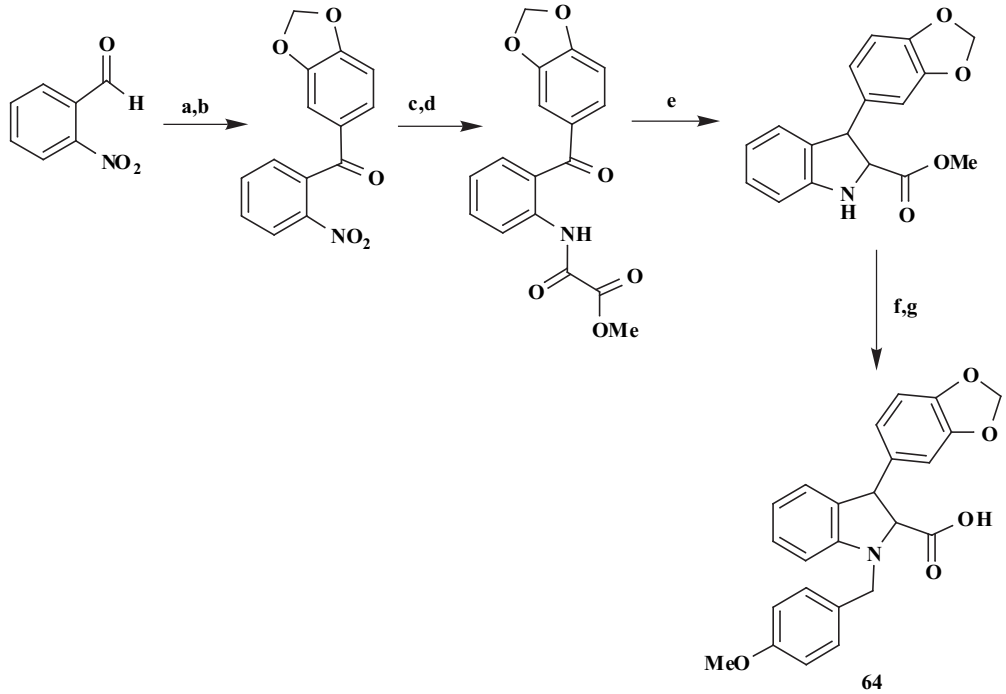
62: A-308165 **63:** RPR-118031A

Fig. (16). Structure of A-308165 and RPR-118031A.

the sulfonamide onto the two amide *N*-butyl substituents of **54** (Fig. (**14**)). Instead, while it is likely that the sulfonamide *N*-alkyl shares a hydrophobic domain with one of the amide *N*-butyls, the sulfonamide moiety served to position the longer *S*-alkyl into a domain, which is present in both ET_A and ET_B receptor subtypes. The potency/ selectivity profile of compound **59** is comparable to a number of "balanced" compounds described earlier and served as a valuable lead for further optimization. The general synthetic route to such sulfonamidoethyl-substituted pyrrolidines has been shown in scheme 14.

On the basis of the earlier studies [101,106] von Geldern and co-workers continued to explore the conformational restriction as a route to improving the selectivity of the compounds of pyrrolidine class. An unexpected result led them to observe [107] that a 2,6-dialkyl acetanilide side chain imparts ET_B selectivity to this family. Optimization of substituents on the aniline ring and the alkoxy moiety on the 2-aryl group led to the compounds with sub-nanomolar affinities for ET_B and selectivities of up to 4000-fold for ET_B versus ET_A. Studies [108] revealed that *ortho*-alkylation of phenyl rings could further increase ET_B affinity and also boost the ET_A/ET_B ratio of the resulting antagonists. Combining these features with modification of the 2-aryl group of the pyrrolidine core led to the potent antagonist A-308165 (**62**) (Fig. (**16**)) with over 27,000-fold selectivity favoring the ET_B receptor.

Recently, random screening of compounds in endothelin receptor binding assays led Zhang and his group [109] to the discovery of a new class of pyrazol-5-ol ligands. The SAR studies led them to design and synthesize a novel class of pyrazol-5-carboxylic acids as potent ET antagonists. Four

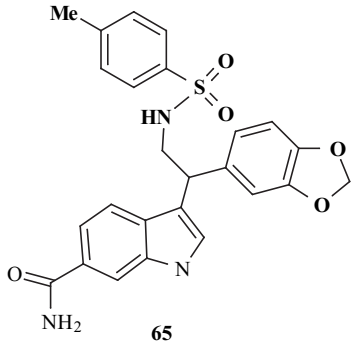


64

Reagents: *a) 3,4-methylenedioxyphenylmagnesium bromide, THF, -78 °C toRT; b) PDC, CH_2Cl_2, reflux; c) EtOAc, Pd-C (5%), H_2 (1 atm.); d)CH_2Cl_2, pyridine, Methyl oxalylchloride, RT; e) DME, $TiCl_3$, Zn, reflux; f) DMF, NaH, 4-$MeOC_6H_4CH_2Cl$, RT; g) EtOH, 2N NaOH, reflux.*

Scheme 15.

structural features on the pyrazole ring were identified as key requirements for the ET receptor binding: an acidic functionality flanked by two benzyl groups (one of them being piperonyl), and a hydrophobic substituent located next to the piperonyl. In a subsequent paper [110] they reported a potent and 1000-fold selective ET_A antagonist from the same pyrazole-5-carboxylic acid family.



65

Fig. (17). Structures of compound **65.**

1,3-Disubstituted isoindolines have been discovered by Kukkola *et al.* [111] as a new class of potent functional ET_A selective receptor antagonists through pharmacophore analysis of existing nonpeptide endothelin antagonists. The data presented in this paper clearly demonstrated the necessity of two appropriately positioned carboxylic acid groups to achieve low nanomolar binding affinity in the isoindoline class of compounds.

Fürstner *et al.* [112] described a new titanium mediated synthesis (Scheme 15) of arylated indole-2-carboxylic acid series as potential drug candidates. Their efficient approach to the target compound **64** is flexible enough to allow the formation of a series of analogues for screening purposes.

Rawson *et al.* [113] used conformational constraint as the key design element in the identification of a series of indole derived potent and selective ET_A antagonists, the most potent being **65** (Fig. (**17**)) (ET_A IC_{50} = 0.55 nM) which has a 722 fold selectivity over ET_B receptor.

3. Miscellaneous

Fujimoto *et al.* (Shionogi & Co.) [114] reported a novel non-peptide ET_A receptor antagonist, myriceron caffeoyl ester (50-235) (**66**), isolated from the bayberry extract, *Myrica cerifera*. During the testing of a large number of natural products in a high throughput screen for ET_B binding inhibitors, an extract of *Fusarium aquaeductuum* WC-5228 was found to inhibit ^{125}I-ET-1 binding to CHO-ET_B cells. Shu *et al.* [115] isolated the compound 7-chloro-1-*O*-methylemodin (**67**) (Fig. (**18**)) from this fungal strain, which is responsible for the inhibitory activity. Its non-chlorinated analogue 1-*O*-methylemodin, also isolated from the extract, was found to be inactive. It appeared that halogen substitution on the anthraquinone nucleus is essential for activity as the monochlorinated, dichlorinated and monobrominated emodins were found to be active inhibitors

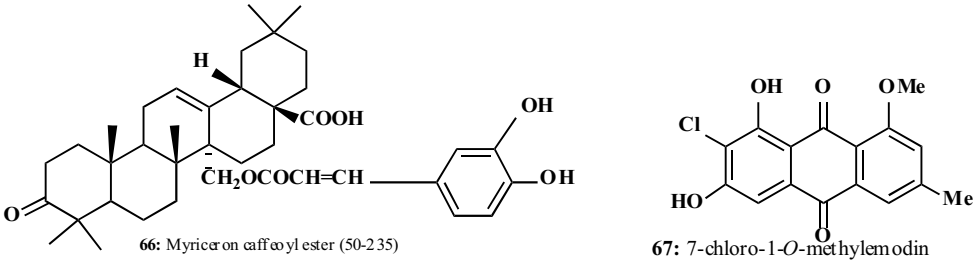


Fig. (18). Structures of Myriceron caffeoyl ester and 7-chloro-1-*O*-methylemodin.

for ET_B binding. However the 5,7-dibromoemodin was much less active and 4,5,7-tri-chloroemodin showed no activity.

Doherty *et al.* [116] described the structure activity relationships of novel butenolide series of ET_A selective receptor antagonists. They optimized the potency of an initial lead structure, PD-012527 (**68**), to develop potent orally active ET_A-selective antogonists [117]. They also

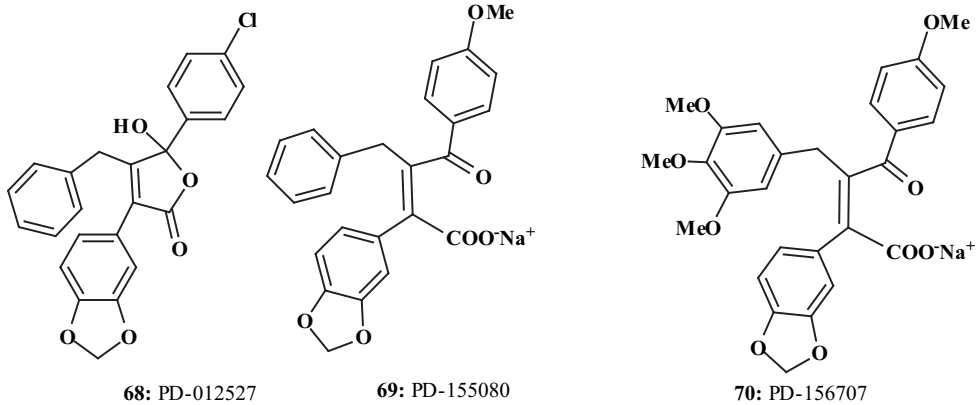
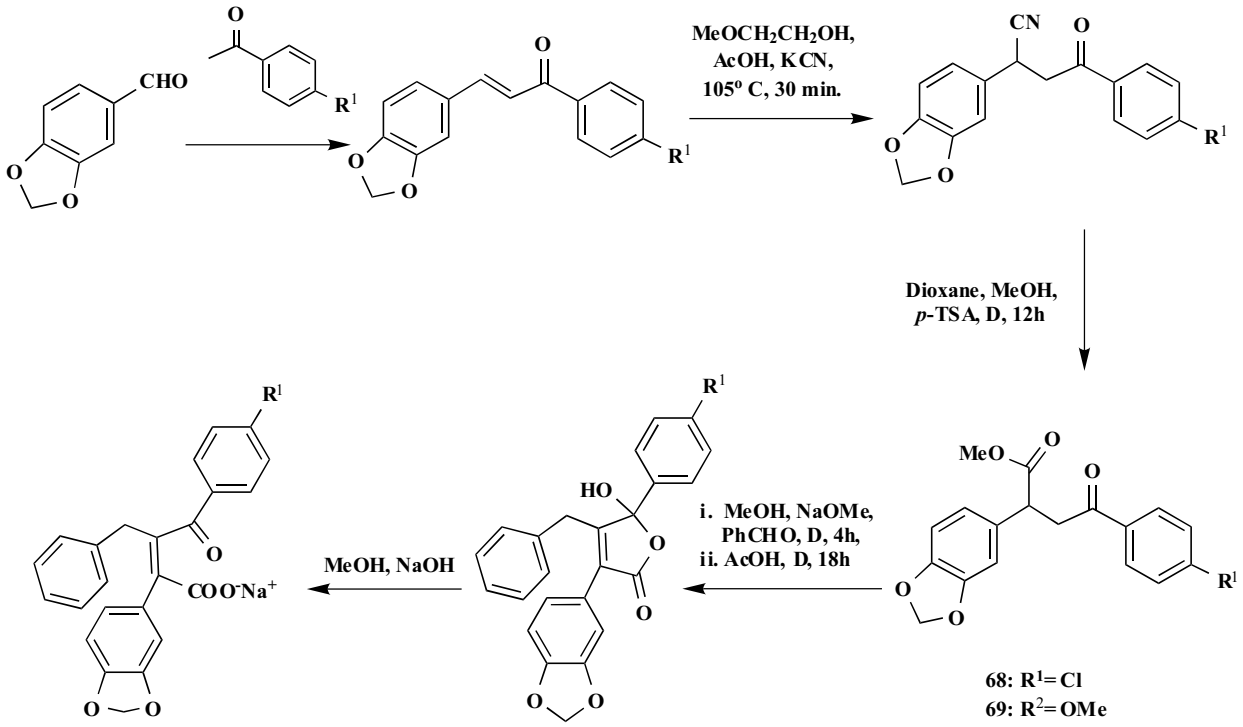


Fig. (19). Chemical Structures of PD-012527, PD-155080 and PD-156707.

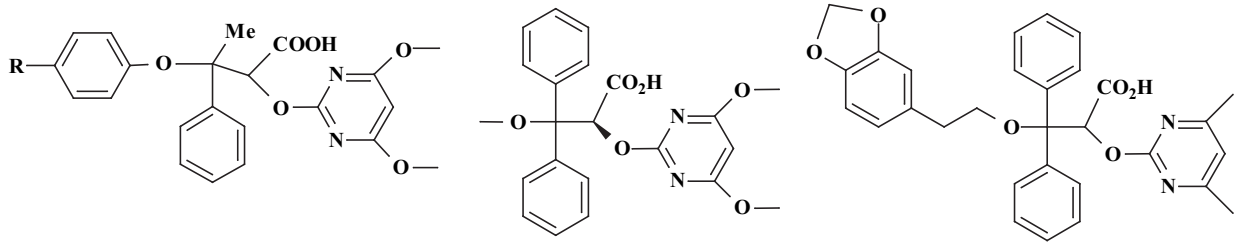


Scheme 16.

highlighted the oral activities and pharmacokinetic properties of two compounds, PD-155080 (**69**) and PD-156707 (**70**) (Fig. (**19**)). Common synthetic scheme for the preparation of compounds **68** and **69** are shown above (Scheme 16).

Patt *et al.* (Parke-Davis) [118] further developed a number of potent nanomolar ET_A selective ET antagonists using the structured Topliss tree approach, starting from the modestly active compound library screening hit (**68**). The Topliss approach takes the electronic, lipophilic and steric factors into account for substitution on a phenyl ring using basic Hansch principles in a non-computerized manner [119]. Evidence for the pH dependence of the open and closed tautomeric forms of **70** was demonstrated by an NMR study. X-ray crystallographic analysis of the closed butenolide form of **70** showed the benzylic group located on the same side of the butenolide ring as the γ-hydroxyl and the remaining two phenyl groups on the butenolide ring essentially orthogonal to the butenolide ring. Patt and coworkers [118] also presented the pharmacokinetic parameters for **70** in dogs.

Riechers *et al.* [120] reported the discovery of a novel class of non-peptidic ET_A selective antagonists. Two initial lead structures, LU-110896 (**71**) and LU-110897 (**72**), originally designed as herbicides, were discovered by screening the chemical library of BASF for compounds that bind to the recombinant human ET_A receptor (Fig. (**20**)). Compounds **71** and **72** have two stereo centers, and Riechers' main goal was to enhance the binding affinity while simplifying the structure. Their effort of synthesizing compounds with general structure **75** (Scheme 17) by introducing two identical substituents at one chiral center, resulted in the discovery of this novel series of potent ET_A selective antagonists. Structural variation of the ET_A-selective antagonist (*S*)-3-methoxy-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3,3-diphenylpropionic acid (**73**, Fig. (**20**)) led to analogues, which retained affinity but exhibited substantial ET_B affinity as well. The most active derivative, obtained by Amberg *et al.* [121], was (*S*)-3-[2-(3,4-dimethoxyphenyl)ethoxy]-2-(4,6-dimethylpyrimidin-2-yloxy)-3,3-diphenylpropionic acid (**74**, Fig. (**20**)), which has

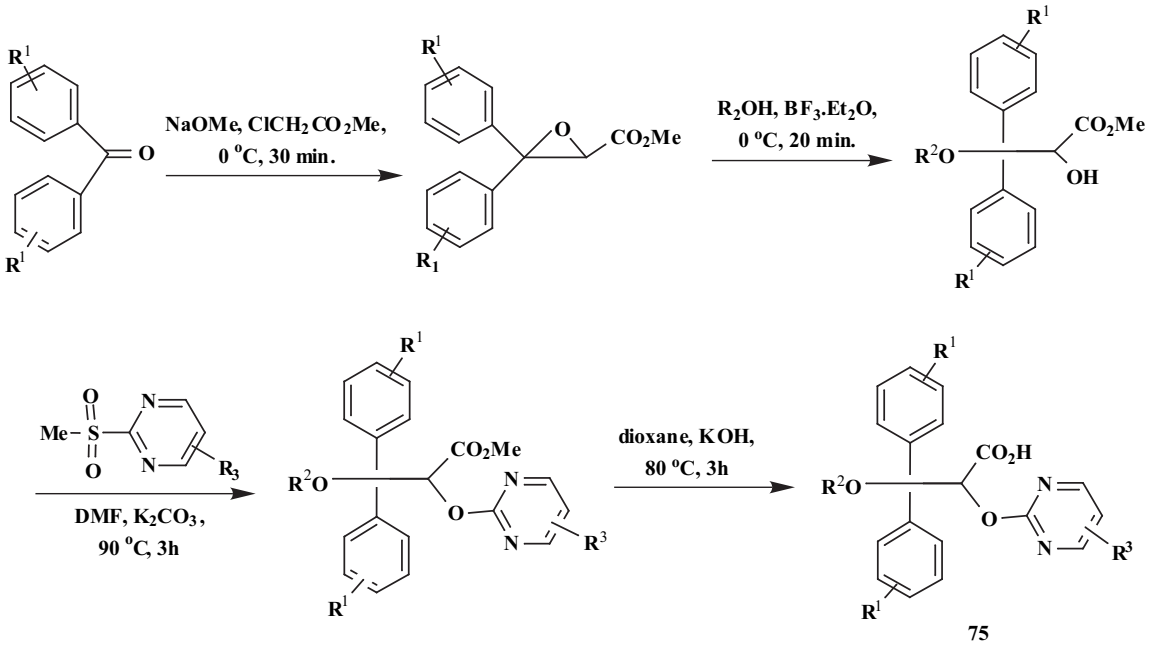


71: LU-110896, R=iPr
72: LU-110897, R=SMe

73: LU-135252

74: LU-302872

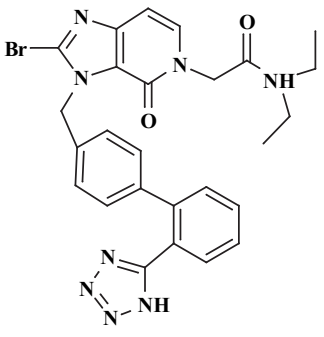
Fig. (20). Structures of LU-110896, LU-110897, LU-135252 and LU-302872.



Scheme 17.

a K_I = 2.15 nM for binding to ET_A receptor and a K_I = 4.75 nM for binding to ET_B receptor. It is orally bioavailable and antagonizes the big ET-induced blood pressure increase in rats and the big ET-induced bronchospasm in guinea pigs.

Mederski *et al.* [122] disclosed another novel non-peptide type-I subclass of angiotensin II receptor antagonists, 2,3,5-trisubstituted 4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]pyridine analogues. The most potent among them was found to be 2-butyl-4,5-dihydro-4-oxo-3-[{2'-(1*H*-tetrazol-5-yl)-4-biphenylyl}methyl]-3*H*-imidazo[4,5-*c*]pyridine-5-(*N*,*N*-diethyl acetamide) (**76**, Fig. (**21**)).



76

Fig. (21). Structure of **76.**

In an effort [123] to quickly discover a new lead based on the available information the minimum structural motif was perceived as one with a carboxylic residue suitably juxtaposed. The simplest of them was envisaged as *N*,*N*-disubstituted amino acids i.e., peptoids. Several natural and unnatural amino acids were identified for the synthesis of target peptoids. A series of compounds was prepared using L-tyrosine and L-tryptophan. Screening of these compounds revealed their affinities for endothelin receptors in terms of their abilities to competitively inhibit ET. It was clear that the carboxylic function was necessary for the activity. The tryptophan series exhibited improved activity and selectivity for ET_B while tyrosine series of compounds were generally more ET_A specific.

CONCLUSION

In conclusion, this review highlighted the research activities based on SAR being pursued in search for a potent and selective endothelin receptor antagonist. The structure based design inspired by the conformational motif of the endothelins as target is very promising and both cyclic as well as acyclic peptides, derived from a combination of D- and L-amino acids, appears as an extremely viable way to lead molecules for ET receptor antagonists. It is also evident that several sulfonamide and carboxylic acid derivatives are emerging as promising lead molecules as evidenced from their excellent pharmacokinetic and pharmacodynamic properties. The advances in this area have resulted in the FDA approval of the orally active dual antagonist Bosentan for pulmonary hypertension in 2001.

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