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THIOL INHIBITORS OF ENDOTHELIN-CONVERTING ENZYME

Pierre Deprez*, Jacques Guillaume, Jacques Dumas and Jean-Paul Vevert.

Hoechst Marion Roussel, Centre de Recherches Roussel Uclaf

102, route de Noisy, 93235 Romainville Cedex, France.

Abstract : Synthesis and structure activity relationships of a series of thiol inhibitors of the endothelin-converting enzyme (ECE) are presented. Optimisation of the stereochemistry as well as of the P'_1 and P'_2 residues led to inhibitors with similar potency to that of phosphoramidon. Copyright © 1996 Elsevier Science Ltd

The 21 amino acid peptide endothelin-1 (1-21), ET-1,[1] is a potent vasoconstrictor which has been implicated in a range of diseases from hypertension to renal failure and stroke.[2] Its biosynthetic precursor is a 203 amino acid peptide which is converted to big endothelin-1 (1-39), big ET-1. This peptide is in turn cleaved at the Trp^{21}-Val^{22} bond by a specific membrane bound zinc metalloprotease called the endothelin-converting enzyme (ECE) to yield ET-1.[3]

Recently, ECE has been purified[4] from various sources (cells and tissues). Cloning[5] has also been achieved indicating that ECE is not a single enzyme but a group of related proteins (named ECE-1a, ECE-1b and ECE-2). In general terms, these ECE are membrane-bound proteases with structural homology to neutral endopeptidase NEP 24.11 and Kell blood group protein. They consist of a transmembrane domain associated with a short N-terminal cytoplasmic tail and a large extracellular fragment including a zinc catalytic domain and many N-glycosylation sites. They are inhibited by phosphoramidon, one of the most potent ECE inhibitors known to date, but not by captopril or thiorphan.

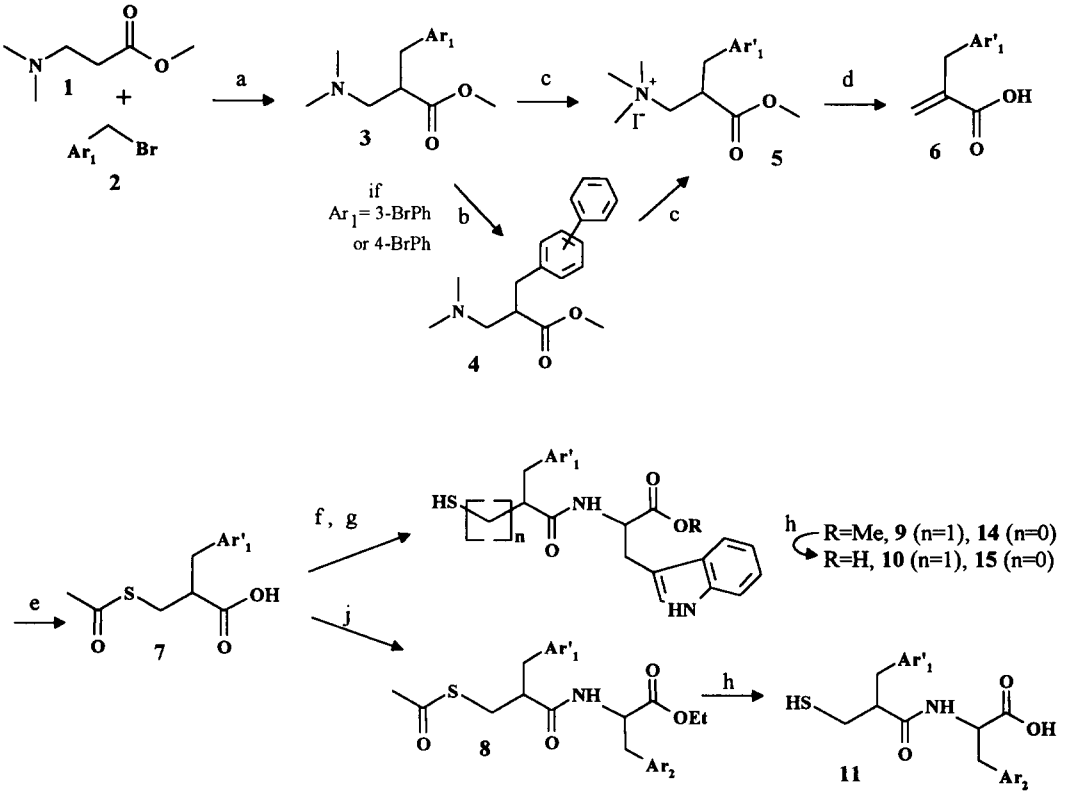
Since big ET-1 is almost devoided of *in vitro* activity (less than 1 % of that of ET-1), blockade of the ET biosynthesis by inhibition of ECE has opened a new alternative to ET antagonists.[6] However, mainly due to the difficulties encountered with the purification and characterization of the enzyme responsible for the *in vivo* cleavage of big ET-1, few synthetic inhibitors have been reported yet,[7] most of them being analogs of phosphoramidon. We wish to report herein the synthesis and *in vitro* activity of new thiol inhibitors of ECE, with structures not directly related to phosphoramidon.[1]

[1] Telefax +33 (1) 49 91 50 87 E-mail : Deprez @ msmdrs.romainvill.rousseluclaf.fr

Chemistry

Thiols **9**, **10** and **11** listed in Table 1 and 2 have been prepared from methyl dimethylaminopropanoate **1** according to the synthetic pathway depicted in the scheme. Deprotonation of **1** with LDA followed by alkylation with a range of arylmethylbromides **2** in the presence of DMPU afforded **3** (in 30 to 80% yield depending on the electrophile). Biphenyl compounds **4** have been synthesized from **3** (with Ar_1 = 4-Br phenyl or 3-Br phenyl) *via* a Suzuki coupling reaction[8] with phenylboronic acid using $PdCl_2(PPh_3)_2$ as a catalyst in 80 % yield. Quaternarisation of the tertiary amine **3** or **4** with methyliodide in isopropanol afforded in quantitative yield a white precipitate of the salt **5** which, after filtration, underwent an Hofmann elimination under basic conditions in 80% yield. The resulting acrylic acid **6** was then reacted with thioacetic acid (without solvent) to provide the Michael adduct **7** which was used without purification after removal of the excess of thioacetic acid. All compounds **7** have been obtained as racemates except compound **7** with Ar'_1= phenyl where both enantiomers have been resolved with norephedrine as described by Duhamel.[9] The final thiols **9**, **10** and **11** (n=1) have been

Scheme



(a) **1** in THF, LDA, 30 mn at -78°C then DMPU (1.2 eq), then **2** (1.2 eq) in THF, 3h at-78°C and 1h at rt. (b) $PhB(OH)_2$ (1.5eq), $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (3 eq), toluene, EtOH, 100°C, 2h. (c) MeI, (3 eq), iPrOH, overnight. (d) NaOH 1N (2 eq),100°C, 2h. (e) CH_3COSH (2.5 eq), 1.5h at rt and 1h at 50°C. (f) L-Trp OMe HCl, BOP (1 eq), TEA (2eq), CH_2Cl_2. (g) NaOH (1N) , MeOH, 30mn at 0°C. (h) LiOH (2.5 eq) THF/H_2O (2:1), 1h, rt. (j) H_2N-$CH(CH_2Ar_2)$-COOEt, BOP (1 eq), TEA (2eq), CH_2Cl_2.

synthesized through a peptidic coupling between the acid **7** and the requisite aminoester[10] using BOP as a coupling reagent, followed by a joint saponification and thiol deprotection procedure using LiOH in a THF/H_2O mixture without epimerisation of the chiral centers. Selective thioacetate cleavage was achieved with NaOH in methanol to yield thiol ester **9**.

Thiols **14** and **15** (n=0) were prepared similarly to thiols **9** and **10** from optically pure 2-(benzoylthio)-3-phenyl-propanoic acid[11] through a peptidic coupling with tryptophan methylester followed by deprotection of the thiobenzoate with NaOH in MeOH and subsequent saponification of the ester with LiOH in THF/H_2O.

Results and discussion

The compounds described in this paper were tested for their ECE inhibitory activity. ECE has been purified in our laboratory from rat lungs according to the Sankyo procedure.[4b] A screening assay was then set up based on the cleavage of the radiolabelled fragment ^{3}H-propionyl-big ET-1 (19-35).[12] Indeed, besides a more rapid kinetic of cleavage, this short fragment proved to be a more convenient substrate than labelled big ET-1 itself, thanks to the easy recovery and characterization of the ^{3}H-propionyl (19-21) tripeptide resulting from the cleavage. In our hands, the IC_{50} of phosphoramidon was found to be 10 nM with this substrate (Ki = 40 nM) and 100 nM with big ET-1 itself (Ki = 40 nM), whereas captopril or thiorphan were inactive. The IC_{50} reported in this paper have been obtained with the ^{3}H-propionyl big ET-1(19-35) substrate.

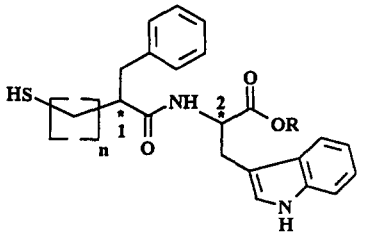
Taking into account the structure homology between NEP and ECE, we undertook a screening of NEP inhibitor libraries. It appeared that some thiols bearing P'_1 and P'_2 hydrophobic residues inhibited ECE in the micromolar range. One representative example was compound **10a** bearing the P'_1 benzyl and P'_2 indolyl methyl substituents.

This compound **10a** taken as a hit, we first turned our attention to the contribution of stereochemistry at the two stereocenters on activity. Thus, the four stereoisomers **10a-d** have been synthesized and tested. The results listed in Table 1 indicates that ECE strictly discriminates between the two stereochemistries at the P'_2 position. Thus, compound **10d** (resp. **10a**) bearing the natural ***S***-stereochemistry of L-tryptophan at P'_2 showed significant activity while the ***R***-stereochemistry-P'_2 compounds (**10b**, **10c**) were inactive, suggesting that the P'_2 side chain plays a determinant role in the enzyme binding. On the other hand, the stereochemistry at the benzyl P'_1 side chain did not seem to be so crucial with a 3-fold difference between the two active isomers (**10a** *vs* **10d**) in favour of the S stereochemistry (**10d**, IC_{50} = 180 nM).

The length of the chain bearing the thiol moiety, acting as the zinc binding group, was also investigated (compounds **15**). Comparison between the two series (n=0 and n=1, Table 1) indicated that no carbon spacer (n=0) gave lower activity, as already reported in thiol analogues of phosphoramidon.[7d] Moreover, in contrast to the n=1 series, the best affinity was observed with the (R, S) stereochemistry. Although not expected, similar results have already been described for NEP inhibitors,[13] indicating once again close similarity between the two enzymes.

The influence of the terminal carboxylic acid has also been evaluated. The 6 to 10-fold drop in potency between **10a**, **10d** or **15a** and their corresponding methylesters (**9a**, **9d** or **14a**) indicates the important role of this acidic residue, suggesting an essential ionic interaction between the carboxylate group and a putative guanidinum residue of the enzyme.

Table 1



compd	n	Stereochemistry *1	Stereochemistry *2	R	IC_{50} (nM) [a]
10a	1	R	S	H	500
10b	1	R	R	H	IN [b]
10c	1	S	R	H	IN [b]
10d	1	S	S	H	180
9d	1	S	S	Me	1 800
9a	1	R	S	Me	3 500
15c	0	S	R	H	IN [b]
15d	0	S	S	H	<10 000 [c]
15a	0	R	S	H	~400
14a	0	R	S	Me	2 200

[a] IC_{50} for inhibition of 50% of the cleavage of ^{3}H-propionyl-big ET-1 (19-35) by ECE. [b] IN=inactive compound (less than 10% of inhibition at 10 000 nM) [c] 65% inhibition at 10 000 nM.

Finally, with the optimized stereochemistry (*S*, *S*) and the (n=1) chain length in place, we examined the nature of the P'_1 and P'_2 substituents on affinity. Taking into account our screening results, we focussed essentially on aromatic rings. Structure activity relationships are summerized in Table 2.

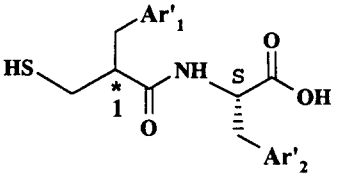
Replacement of the P'_2 indolyl moiety of **10d** (IC_{50} = 180 nM) by other aromatic groups led to compounds **11a-g**. The P'_2 phenyl group (**11a**) was found to reduce by 7-fold the inhibitory potency and the 4-hydroxy-phenyl residue (**11b**) also proved to be detrimental. However, increasing lipophilicity with O-benzylation of this latter (**11c**) improved the activity and introduction of a biphenyl moiety (**11d**) restored potency at a similar level to that of the indolyl group (**11d**, IC_{50} = 150 nM). Therefore, other compounds with a P'_2 phenyl-aryl substituent were synthesized (**11e-g**). Among them, the P'_2 4-(2-thienyl) phenyl analogue **11g** proved to be the most active with a 3-fold increase in potency (55 nM) when compared to the parent biphenyl compound **11d** (Table 2).

However, the most dramatic change was found when varying the P'_1 substituent from phenyl to substituted phenyl groups (**11h-o**, Table 2). Because we had showed that P'_1 stereochemistry was not so important in this series, we synthesized compounds **11h-o** as a mixture of diastereoisomers at this center, with the P'_2 L-Trp side chain in place. It appeared that the methylene dioxy or 4-benzyloxy substitution of the phenyl group (compound **11j** and **11k**) did not modify significantly the inhibitory potency, suggesting that large hydrophobic residues are tolerated in the S'_1 pocket. This was confirmed by the introduction of a biphenyl substituent (**11l** and **11m**) with a 9-fold increase in potency when compared to the monophenyl **11h**. Similar

biphenyl substitution had already been reported by Ciba's scientists with the discovery of CGS 26303.[7e] More surprising was the potency of either P'$_1$ 3- or 4-bromophenyl compound **11o** (RU 69296, IC_{50}= 25 nM) and **11n** (RU 69738, IC_{50}= 20 nM).

After separate identification of the best aromatic residues for the two S'$_1$ and S'$_2$ subsites, we synthesized compound **11p** which included both of them. However, no expected cumulative effect was observed, compound **11p** being slightly less active than either of the parent molecules **11g** and **11n**, even if solubility problem during the test cannot be discarded.

Table 2



compd	Stereochemistry ✳ 1	Ar'1	Ar'2	IC_{50} (nM)[a]
10d	S	Ph	Indol-3-yl	180
11a	S	Ph	Ph	1200
11b	S	Ph	4-(OH)-Ph	2800
11c	S	Ph	4- (OCH2Ph)-Ph	700
11d	S	Ph	4-(Ph)-Ph	150
11e	S	Ph	4-[4'-OMe-Ph]-Ph	800
11f	S	Ph	4-(2-naphtyl)-Ph	300
11g	S	Ph	4-(2-thienyl)-Ph	55
11h	(S, R)	Ph	Indol-3-yl	340 [b]
11j	(S, R)	6-Cl-1,3-benzodioxol-5-yl	Indol-3-yl	400
11k	(S, R)	4-OCH2Ph-Ph	Indol-3-yl	800
11l	(S, R)	4-Phenyl-Ph	Indol-3-yl	40
11m	(S, R)	3-Phenyl-Ph	Indol-3-yl	40
11n	(S, R)	4-Br-Ph	Indol-3-yl	25
11o	(S, R)	3- Br-Ph	Indol-3-yl	20
11p	(S, R)	3- Br-Ph	4-(2-thienyl)-Ph	80

[a] IC_{50} for inhibition of 50% of the cleavage of ^{3}H-propionyl-big ET-1 (19-35) by ECE. [b] IC_{50} as a mean of both isomeres **10a** and **10d.**

In conclusion, we have studied the structure activity relationships of a series of thiol compounds for their *in vitro* ECE inhibitory potency. Optimisation of the stereochemistry and of the nature of the P'$_1$ and P'$_2$ residues led to the synthesis of inhibitors with similar potency to that of phosphoramidon. Further optimisation of these residues is still needed as well as search for additional interactions with other subsites of the enzyme (S_1, S'_3). This could help to further increase the potency of the compounds in this series.

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