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Potent Nonpeptide Endothelin Antagonists: Synthesis and Structure–Activity Relationships of Pyrazole-5-carboxylic Acids

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Abstract—We have previously reported the identification of pyrazole-5-carboxylic acids as a new class of endothelin antagonists from low affinity pyrazol-5-ol ligands, which were obtained by random screening assays.[1] We describe herein the synthesis and the structure–activity relationships (SARs) of these pyrazole-5-carboxylic acids with potent ET_A selective, mixed ET_A/ET_B or moderately ET_B selective antagonist activities.

Introduction

The endothelins (ET-1, 2 and 3) are 21-amino-acid peptides, with ET-1 being one of the most potent vasoconstrictors known.[2] The endothelins exert their biological effects by interacting with two specific G-protein coupled membrane receptors (ET_A and ET_B).[3] Elevated levels of endothelins have been associated with a certain number of diseases, such as myocardial infarction, hypertension, heart failure, athero-sclerosis, cerebral and coronary vasospasm, renal failure and asthma.[4] Several studies have shown that the mode of action of ET-1 as related to the aforementioned pathological conditions is mediated through the ET_A receptor, which is mainly found in the vascular smooth muscle tissues. The ET_B receptor is expressed on both vascular endothelial and smooth muscle cells, but its role remains poorly understood. Therefore selective ET_A receptor antagonists may have beneficial effects for patients with those disease states. In addition, all types of antagonists (ET_A and ET_B selective: mixed ET_A/ET_B) should be very useful tools for understanding the roles of both ET receptor subtypes in normal physiological and pathological conditions.

A large number of potent, nonpeptide ET_A selective (Ro61-1790, L-754,142, SB217242, BMS-182874, PD156707 and TBC11251),[5] ET_B selective (Ro46-8443 and A-308165)[6] and ET_A/ET_B mixed antagonists (Bosentan, SB209670, L-749329, and LU302872)[7] have been described in the literature. We have previously reported the preparation of pyrazole-5-carboxylic acids (i.e. **1**) as a new class of endothelin antagonists from low affinity screening hits, pyrazol-5-ols.[1] We wish to describe herein the synthesis and the SAR of these pyrazole-5-carboxylic acids, which resulted in potent ET_A selective and mixed ET_A/ET_B antagonists, but also in some moderately ET_B selective antagonists.

Chemistry

The structures of the pyrazole-5-carboxylic acids described in this study are depicted in Tables 1–3. The synthesis of compound **1** has already been reported. However, this compound has also been prepared according to the procedure outlined in Scheme 1, which was developed for the synthesis of pyrazole acids **7a–q** (Table 1) and **8a–d** (Table 3).[8] The commercially available ketones **2** were treated with diethyl oxalate in the presence of NaOEt in ethanol to give quantitatively the corresponding α,γ-diketoesters **3** according to a literature method.[9] Reactions of α,γ-diketoesters **3** with 6-chloro-piperonyl chloride were performed in the presence of EtONa and NaI in DMF. The crude

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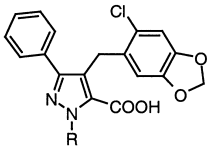
compounds **4** were allowed to react directly with hydrazine monohydrate in ethanol to afford the desired pyrazole esters **5**. Regioselective alkylation of **5** with the requisite alkyl halide or alkyl tosylate in the presence of NaH in DMF at room temperature gave compounds **6**. Finally, the pyrazole acids **7a**–**q** and **8a**–**d** were obtained after saponification in good yields.

Table 1. In vitro endothelin receptor binding affinity (IC_{50} (nM)) for compounds **1**, **7a**–**q**



No.	R	IC_{50} (nM) ET_A[a]	ET_B[b]
1		18	34
7a		28	9.7
7b		17	11
7c		34	26
7d		15	31
7e		3.6	2.6
7f		3.4	4.9
7g		226	131
7h		70	953
7i		4.9	810
7j		2.5	14.8
7k		20.5	3.2
7l		11	2045
7m		6.2	89
7n		2.5	44
7o		79	440
7p		59	3.3
7q		89	4.0

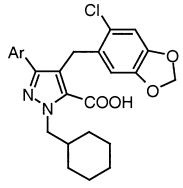
[a]Rat heart ventricles.
[b]Rat cerebellum.

Pyrazole-5-carboxylic acids **17a**–**f** (Table 2) were obtained by a new route outlined in Scheme 2. Wittig–Horner reaction of 6-chloropiperonal **9** with triethyl phosphonoacetate in DME in the presence of NaH, afforded unsaturated ester **10**. After reduction of the double bond with $NaBH_4/Cu_2Cl_2$ in a mixture of THF/EtOH,[10] the saturated ester derivative **11** was obtained in modest yield. Reaction of **11** with diethyl oxalate in toluene in the presence of NaH gave **12**, which was cyclized into the hydroxypyrazole **13** by the reaction with cyanoethylhydrazine in AcOH. Bromopyrazole **14** was obtained after OH–bromine exchange in pure $POBr_3$ at 60 °C. The deprotection of the bromopyrazole **14** was realized by a retro-Michael-type reaction on the cyanoethyl substituent by treatment with NaH in DMF. The so-obtained ionic pyrazole intermediate was regioselectively alkylated in situ with cyclohexylmethyl bromide, which afforded the desired compound **15**. Suzuki reaction or Stille coupling of **15** with requisite aryl boronic acids or aryl stannanes gave the corresponding pyrazoles **16a**–**f**, which after saponification under standard conditions yielded the desired pyrazole-5-carboxylic acids **17a**–**f**.

Results and Discussion

We have previously proposed a pharmacophore model for ET antagonism that contains a central pyrazole-5-carboxylic acid flanked by a piperonyl moiety, a second benzyl group and an additional hydrophobic substituent located next to the piperonyl (i.e. **1**).[1] In order to increase the ET antagonist potencies, we performed

Table 2. In vitro endothelin receptor binding affinity (IC_{50} (nM)) for compounds **7e**, **17a**–**f**

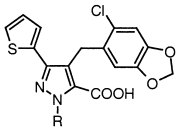


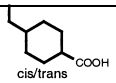
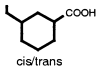
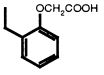
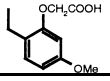
No.	Ar	IC_{50} (nM) ET_A[a]	ET_B[b]
7e		3.6	2.6
17a		4.7	3.4
17b		17.8	6.3
17c		2300	178
17d		1.3	9.8
17e		1.1	3.2
17f		1.1	1.7

[a]Rat heart ventricles.
[b]Rat cerebellum.

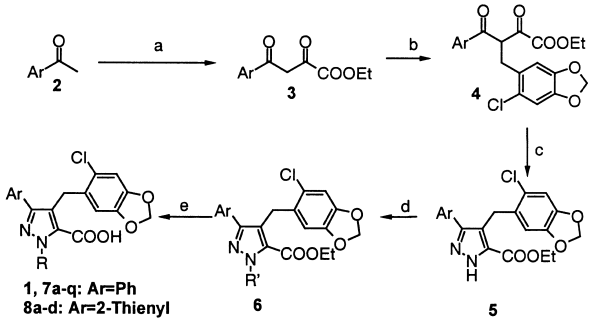
further SAR studies on the two later substituents while the carboxylic acid function and the 6-chloropiperonyl were kept constant. As in our previous study,[1] the inhibition of endothelin binding to ET receptors was measured using [^{125}I]-labelled ET-1 competition assays.[8] Table 1 summarizes the results of this study for the 1-position of these pyrazoles (Scheme 1). Obviously, hydrophobic benzylic substituents (compounds **7a–c**) gave balanced ET antagonists, but no significant difference in potency has been observed as compared to the lead compound **1**. Interestingly, aliphatic hydrophobic substituents (compounds **7d–f**) were well accepted by the receptors with one exception for the secondary cyclohexyl in compound **7g**. Introduction of a heteroatom like oxygen into these aliphatic substituents resulted in a significant loss in affinities for both ET receptors (compound **7h**). The best affinities were obtained with the cyclohexylmethyl group (**7e**), which resulted in a 5-fold improved affinity for ET_A and a 10-fold improved affinity for ET_B receptor compared to compound **1**. However, all these compounds showed mixed ET antagonism, and in our effort to search for ET subtype selective antagonists, additional compounds (**7i–q**) containing a second acid functionality were prepared. Amazingly, very good ET_A selectivity was observed following a minor change of the position of the acid group as demonstrated with the compound **7i** with the carboxylic acid at the 4-position of cyclohexylmethyl. Indeed, this compound showed an ET_A/ET_B ratio of 1/165. A carboxylic acid functionality at the 4-position seems crucial for ET_A selectivity, since the corresponding aromatic derivative **7l** displayed the same kind of selectivity (1/186). Surprisingly, changing the position of this second acid function to the 3-position in **7j** (cyclohexylmethyl) or in **7m–o** (benzyl group) led to poor ET_A selectivity (ET_A/ET_B ratio varying from 1/6 to 1/14). Finally, ET_B selectivity was obtained when this second acid was introduced to the 2-position of the cyclohexylmethyl (**7k**) or the *ortho*-position of the benzyl group (**7p,q**), with **7q** being the most ET_B selective antagonist obtained (ET_A/ET_B: 22/1).

Table 3. In vitro endothelin receptor binding affinity (IC_{50} (nM)) for compounds **8a–d**



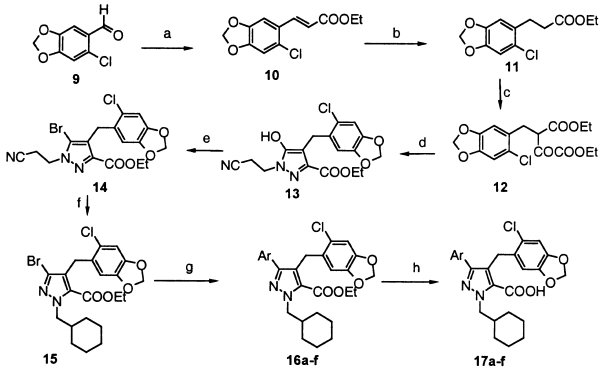
No.	R	IC_{50} (nM) ET_A[a]	IC_{50} (nM) ET_B[b]
8a		0.81	825
8b		1.3	9.9
8c		28.3	4.6
8d		14.9	8.6

[a]Rat heart ventricles.
[b]Rat cerebellum.



Scheme 1. Reagents and conditions: (a) EtO_2CCO_2Et, NaOEt/EtOH, 0 °C to rt, 20 h (100%); (b) 6-chloropiperonyl chloride, NaOEt/DMF, NaI, rt, 16 h (crude); (c) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux, 4 h (yields for steps b and c: Ar = Ph: 41%; Ar = 2-Thienyl: 30%); (d) R′X, NaH/DMF, rt, 20 h (yields: from 50 to 87%); (e) NaOH (2 N), EtOH, reflux, 2 h (yields: from 75 to 100%).

With the aim to improve even further the ET receptor binding affinity for pyrazole-5-carboxylic acids, we then decided to investigate the region occupied by the 3-phenyl group, which was identified as one of the key substituents in our previous work.[1] Compounds synthesized for this purpose are presented in Table 2 (Scheme 2). Replacement of the phenyl group by a pyridin-2-yl (compound **17a**) did not show significant change in potency, while a pyridin-3-yl (compound **17b**) led to a slight loss in ET_A binding. Introduction of a 4-methoxy substituent on the phenyl ring (compound **17c**) resulted in almost a total loss of the affinity for both ET receptors. Small lipophilic aromatic groups like furanyl and thienyl (compounds **17d–f**) slightly increased ET_A binding affinities but had little influence on ET_B affinities as compared to **7e**. Compound **17f** was the most potent mixed ET antagonist with an IC_{50} of 1.1 nM for ET_A receptor and an IC_{50} of 1.7 nM for ET_B receptor.



Scheme 2. Reagents and conditions: (a) $EtO_2CCH_2PO(OEt)_2$, NaH/DME, rt, 2.5 h, yield: 90%; (b) $NaBH_4/Cu_2Cl_2$, THF/EtOH, 0 °C, 5 h, yield: 57% (c) EtO_2CCO_2Et, NaOEt/toluene, reflux, 3 h, yield: 76%; (d) $NCCH_2CH_2NHNH_2$, AcOH, 100 °C, 1 h, yield: 55%; (e) $POBr_3$, 60 °C, 15 min, yield: 85%; (f) (i) NaH, DMF, 0 °C, 1 h; (ii) cyclohexylmethyl bromide, rt to 60 °C, 16 h, yield: 55%; (g) $ArSnBu_3$, $Pd(PPh_3)_4$, toluene, 5h (**16a,b**, yields from 56 to 67%); $ArB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, DMF, 24 h (**16c–f**, yields from 55 to 82%); (h) NaOH (2 N), EtOH, reflux, 2 h (yields: from 90 to 100%).

Since the 2-thienyl derivative gave better ET binding than the corresponding phenyl analogue, it was then chosen as a new building-block for further selectivity studies as was done previously with the phenyl series (compounds **1**, **7a**–**q**). Substituents which have shown to have the most dramatic effect on ET binding selectivity in the former series, were then incorporated into compounds **8a**–**d** (Table 3, Scheme 1). Compound **8a** showed an improved affinity for ET_A ($IC_{50} = 0.81$ nM) and unchanged affinity for ET_B, resulting in a better selectivity ratio of 1/1020 as compared to **7i**. Slightly better affinities for both ET_A and ET_B receptors were observed for compound **8b** as compared to **7j**, and therefore the selectivity was unchanged. Finally, ET_A binding affinities were also slightly increased for **8c,d** as compared to **7p,q**, while ET_B affinities remained unchanged, which led to diminished ET_B selectivities.

In summary, investigation of SARs of the pyrazole-5-carboxylic acids resulted in the synthesis of very potent ET antagonists. Representatives of this family subtype have been identified: **8a**—potent and selective ET_A antagonist (ET_A $IC_{50} = 0.81$ nM; ET_A/ET_B ratio = 1/1020); **8b**—moderately ET_A selective antagonist (ET_A $IC_{50} = 1.3$ nM; ET_A/ET_B ratio = 1/7); **17f**—ET_A/ET_B balanced antagonist (ET_A $IC_{50} = 1.1$ nM; ET_B $IC_{50} = 1.7$ nM), and **7q**—moderately ET_B selective (ET_B $IC_{50} = 4.0$ nM; ET_A/ET_B ratio = 22/1). All these compounds might provide useful tools for the evaluation of the benefit of ET_A receptor antagonism, and for the better understanding of the role of the ET_B receptor.

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