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Structure–Activity Relationships of 2-Substituted 5,7-Diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic Acids as a Novel Class of Endothelin Receptor Antagonists

Kenji Niiyama,* Hirobumi Takahashi, Toshio Nagase, Hisaki Kojima, Yuka Amano, Kasumi Katsuki, Takeru Yamakawa, Satoshi Ozaki, Masaki Ihara, Mitsuo Yano, Takahiro Fukuroda, Masaru Nishikibe and Kiyofumi Ishikawa

Tsukuba Research Institute, Banyu Pharmaceutical Co. Ltd., 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan

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Abstract—Synthesis and structure–activity relationships of 2-substituted-5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acids, a novel class of endothelin receptor antagonists, were described. Derivatization of a lead structure **1** ($IC_{50}=2.4$ nM, 170-fold selectivity) by incorporating a substituent such as an alkyl, alkoxy, alkylthio, or alkylamino group into the 2-position of the cyclopenteno[1,2-*b*]pyridine skeleton was achieved via the key intermediate **8**. Introduction of an alkyl group led to the identification of potent ET_A/ET_B mixed receptor antagonists, a butyl (**2d**: $IC_{50}=0.21$ nM, 52-fold selectivity) and an isobutyl (**2f**: $IC_{50}=0.32$ nM, 26-fold selectivity) analogue. In contrast, installment of a primary amino group resulted in ET_A selective antagonists, a propylamino **2p** ($IC_{50}=0.12$ nM, 520-fold selectivity) and an isopropylamino **2q** ($IC_{50}=0.10$ nM, 420-fold selectivity) analogue. These results suggested that a substituent at the 2-position of the 5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acids played a key role in the binding affinity for both ET_A and ET_B receptors.
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Introduction

Endothelin-1 (ET-1),[1] and its closely related isopeptides (ET-2, ET-3) were identified as potent vasoconstrictor peptides consisting of 21 amino acids. The endothelins exert their diverse biological actions through distinct cell surface G-protein coupled receptors (GPCR) named ET_A and ET_B. The ET-1 selective ET_A receptor subtype mediates vasoconstriction and vascular smooth muscle proliferation. The isopeptide non-selective ET_B receptor subtype can mediate either vasodilation or vasoconstriction, depending on the tissue type. The diversity of physiological effects elicited by the endothelins has been implicated in the pathogenesis of a variety of disease states such as hypertension, renal failure, cerebral vasospasm, pulmonary hypertension, and congestive heart failure. Elevated levels of endothelins have been observed in many of these disease states. Therefore, endothelin receptor antagonists are expected to have clinical potential in the endothelin-mediated disorders that are mentioned above.[2]

A number of reports exist in the literature describing non-peptide endothelin antagonists including the ET_A selective,[3] ET_B selective,[5] and ET_A/ET_B mixed agents[4] known to date. It should be noted that these antagonists coincidentally possess a structural feature with an acidic moiety positioned between the two aromatic rings. We had an interest in the structure of SB-209670[4b] because of its high structural rigidity as well as its high binding affinity for ET receptors. Our exploration for new pharmacophores based on this structure led to the identification of a potent non-peptide endothelin receptor antagonist, 5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acid **1** shown in Figure 1.[6] A preliminary account of this work has been previously presented, and herein we describe the versatile synthetic method of 2-substituted cyclopentenopyridine derivatives and their structure–activity relationships (SARs) on the in vitro binding affinity for ET_A and ET_B receptors and selectivity for ET_A over ET_B receptors.

*Corresponding author. Tel.: +81-298-77-2220; fax: +81-298-77-2027; e-mail: niiymakj@banyu.co.jp

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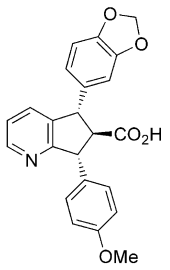

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Figure 1. New class of non-peptide endothelin receptor antagonist.

Results and Discussion

Chemistry

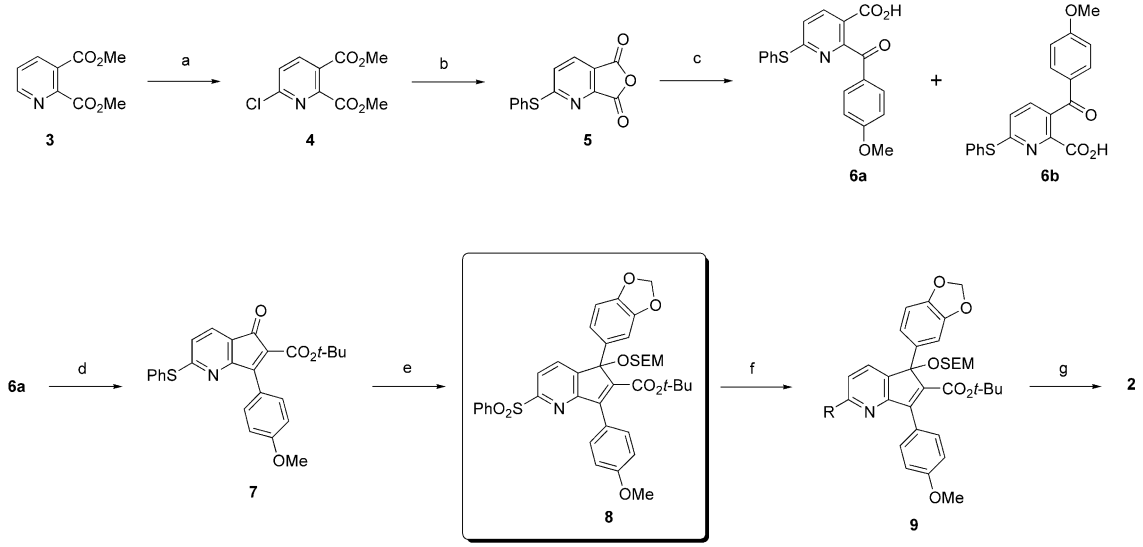
In the conventional method,[7] a substituent on the pyridine ring had to be installed during an early stage of the synthesis, which prevented us from efficiently synthesizing a number of compounds to elucidate the SARs of this series. Therefore, we had to develop a robust synthetic method for substituted 5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acid derivatives. It is known that phenylsulfonyl group at the 2- and 4-position of pyridine is substituted with alkylmetal reagents to provide 2- and 4-alkylpyridines, respectively.[8] We applied it to the cyclopenteno[1,2-*b*]pyridine system and investigated the synthesis of 2-substituted analogues. After extensive investigation, we developed a versatile method to synthesize a variety of 2-substituted cyclopenteno[1,2-*b*]pyridines via substitution reaction of a intermediate **8** with various nucleophiles. The synthetic procedure is shown in Scheme 1.

Synthesis of the key intermediate **8** was started from dimethyl 2,3-pyridinedicarboxylate **3**, which was converted to 2-phenylthiopyridine-5,6-dicarboxylic anhydride **5** by a five-step reaction sequence in 38% overall yield. The anhydride **5** was reacted with a 4-methoxyphenyl Grignard reagent to provide a mixture of regioisomers **6a** and **6b** (6:1) in 96% yield. Transformation of **6a** to an enone **7** was achieved in 45% yield by the following reaction steps:[7] (1) conversion of the carboxylic acid to the corresponding imidazolide; (2) condensation of the imidazolide with the enolate of *tert*-butyl acetate followed by cyclization; and (3) dehydration assisted by silica gel. Addition of a 3,4-methylenedioxyphenyl Grignard reagent to the enone **7** gave an allyl alcohol in 96% yield. Protection of the tertiary alcohol as a 2-(trimetylsilyl)ethoxymethyl (SEM) ether followed by oxidation of the phenyl sulfide to the corresponding sulfone with *m*-CPBA afforded the key intermediate **8** in 91% yield.

The phenylsulfonyl group was substituted for an alkyl group with an alkyllitium at −78 °C to give the adduct **9** in 50–60% yield.[8] A similar substitution reaction was achieved by treatment with a metal reagent such as lithium alkylamide, potassium alkoxide, or potassium alkylmercaptide to produce an alkylamino, alkoxy, or alkylthio derivative, respectively. After deprotection of the SEM ether under an acidic condition (HCl/MeOH), the allyl alcohol system was reduced with zinc in the presence of hydrogen chloride, which afforded the desired *cis–cis* isomer as a major product.[7] Epimerization and hydrolysis of the ester moiety were simultaneously accomplished under basic conditions (NaOH/dioxane) to give the final compound **2**. This method enabled us to efficiently synthesize a variety of compounds for investigation of SARs of this class of compounds.

Biological properties

Compounds that were synthesized via the above-described method were evaluated in the binding assay (inhib-



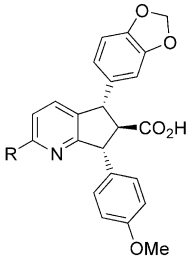
Scheme 1. Synthesis of 2-substituted 5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acids. Reagents and conditions: (a) (1) H_2O_2, Na_2WO_4, AcOH, 80 °C, 94%; (2) $POCl_3$, 130 °C, 55%; (b) (1) PhSH, K_2CO_3, DMF, 80 °C, 80%; (2) NaOH, MeOH, 60 °C, quant; (3) Ac_2O, 110 °C, 91%; (c) (1) 4-methoxyphenylmagnesium bromide, THF, −78 °C, 96%; (d) (1) CDI, DMF; (2) LDA, CH_3CO_2t-Bu, THF, −78 °C; (3) SiO_2, CH_2Cl_2, 45%; (e) (1) 3,4-methylenedioxyphenylmagnesium bromide, THF, −78 °C, 96%; (2) SEMCl, EtN(*i*-Pr)$_2$, CH_2Cl_2, 40 °C, 93%; (3) *m*CPBA, $CHCl_3$, 98%; (f) RLi, THF, −78 °C; (g) (1) HCl–MeOH, 0 °C; (2) Zn, HCl, THF–EtOH, 0 °C; (3) NaOH, dioxane–H_2O, 120 °C.

itory activity against ^{125}I-labled ET-1 binding to both human ET_A and ET_B receptors),[9] and the data were compared with those of the lead compound **1**. Table 1 shows the binding affinities of 2-alkylsubstituted analogues (**2a**–**2i**). With respect to the binding affinity to ET_A receptors, incorporation of a methyl (**2a**) or ethyl group (**2b**) afforded approximately a 2-fold reduction in potency. On the other hand, incorporation of a propyl (**2c**) or a butyl group (**2d**) resulted in approximately a 10-fold increase in potency. Introduction of a longer straight alkyl chain, pentyl group (**2e**), brought a considerable decrease in the binding affinity in comparison with that of **2c** or **2d**. 3-Butenyl analogue **2g** showed comparable binding affinity to that of the butyl analogue **2d**. Among branched alkyl analogues, a isobutyl analogue **2f** had the affinity between the propyl (**2c**) and the butyl (**2d**) analogue, and a cyclopropyl analogue **2h** had the affinity between the ethyl (**2b**) and the propyl (**2c**) analogue. These results suggest that the binding affinity to ET_A receptors is affected by the length of the alkyl chain, and a 4-carbon unit such as a butyl or 3-butenyl group is optimal. In regard to the binding affinity to ET_B receptors, the incorporation of a alkyl group enhanced the binding affinity, and the potency increased in proportion to the length of the alkyl chain, which indicates that 2-alkyl analogues are less ET_A selective than **1**. Introduction of phenyl group (**2i**) resulted in a significant decrease in binding affinity to ET_A receptors, and increased binding affinity to ET_B receptors. As a result, incorporation of alkyl groups at the 2-position led to identification of highly potent ET_A/ET_B mixed receptor antagonists such as **2d** ($IC_{50} = 0.21$ nM, 52-fold selectivity) and **2f** ($IC_{50} = 0.32$ nM, 26-fold selectivity).

The binding affinities of analogues with a heteroatom-containing substituent (**2j**–**2s**) are summarized in Table 2. Replacement of a carbon atom in the butyl group of **2d** with oxygen atom(s) gave interesting results. A 3-hydroxypropyl (**2j**), an ethoxymethyl (**2l**), or a propoxy (**2m**) analogue showed sub-nano molar binding affinity to ET_A receptors, while a 2-carboxyethyl analogue **2k** showed a drastic decrease in potency. These results suggest that a hydroxy and an alkoxy group at the 2-position are tolerated, while an acidic functional group is not tolerated. On the other hand, incorporation of an oxygen atom into the butyl group of **2d**, in particular at the β- and δ-position, resulted in a drastic reduction of the binding affinity to ET_B receptors, which led to identification of potent and ET_A selective antagonists such as **2j** ($IC_{50} = 0.55$ nM, 350-fold selectivity) and **2l** ($IC_{50} = 0.51$ nM, 270-fold selectivity). Introduction of a sulfur atom resulted in 40-fold decrease in the ET_A binding affinity and 5-fold decrease in the ET_B binding affinity in comparison with that of **2d**, which led to a ET_A/ET_B mixed antagonist **2n** (7-fold selectivity) though the binding affinity to ET_A receptors was lower than that of **1**.

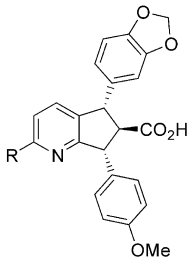
The effects of introducing a nitrogen atom into the butyl group of **2d** on the binding affinity to ET_A and ET_B receptors were also investigated. A propylamino analogue **2p** showed 2-fold higher ET_A binding affinity than **2d**, while an ethylaminomethyl analogue **2o** was 33-fold less active against ET_A receptors and 85-fold less active against ET_B receptors. These results suggest that incorporation of a nitrogen atom into the α-position of the butyl group is crucial to the binding affinities. On the other hand, ET_B binding affinity of **2p** decreased 6-fold in comparison with that of **2d**, which indicates that **2p** is the most ET_A selective antagonist with more than 500-fold selectivity for ET_A over ET_B receptors. An isopropylamino analogue **2q** showed similar binding affinities and selectivity to **2p**. It is interesting to note that *N*-methylation of **2q** resulted in a 32-fold reduction in the ET_A binding affinity and a 3-fold increase in ET_B

Table 1. In vitro potency of 2-alkylsubstituted derivatives



Compd	R	IC_{50} (nM) (*n*)		Selectivity
		ET_A	ET_B	ET_B/ET_A
1	H	2.4±0.5 (11)	410±110 (3)	170
2a	Methyl	5.7 (1)	100 (1)	18
2b	Ethyl	4.5 (1)	87 (1)	19
2c	Propyl	0.37±0.01 (2)	30±1 (2)	81
2d	Butyl	0.21±0.02 (5)	11±2 (5)	52
2e	Pentyl	1.9±0.5 (3)	9.1±1.4 (3)	4.8
2f	Isobutyl	0.32±0.04 (3)	8.4±1.0 (3)	26
2g	3-Butenyl	0.20±0.06 (3)	14±1 (2)	70
2h	Cyclopropyl	1.6±0.2 (2)	63±9 (2)	39
2i	Phenyl	15 (1)	130 (1)	8.7

Table 2. In vitro potency of derivatives with heteroatom-containing substituent at the 2-position



Compd	R	IC_{50} (nM) (*n*)		Selectivity
		ET_A	ET_B	ET_B/ET_A
2j	3-Hydroxypropyl	0.55±0.06 (2)	190±51 (2)	350
2k	2-Carboxyethyl	150±21 (2)	530±150 (2)	3.5
2l	Ethoxymethyl	0.51±0.03 (2)	140±16 (2)	270
2m	Propoxy	0.92±0.17 (6)	65±7 (6)	71
2n	Propylthio	8.2 (1)	53 (1)	6.5
2o	Ethylaminomethyl	7.0±0.6 (2)	940±540 (2)	130
2p	Propylamino	0.12±0.02 (2)	63±0.3 (2)	520
2q	Isopropylamino	0.10±0.03 (3)	42±19 (3)	420
2r	*N*-Methylisopropylamino	3.2 (1)	14 (1)	4.4
2s	1-Pyrrolidinyl	2.2±0.5 (2)	21±3 (2)	9.5

binding affinity compared with that of **2q**, which indicates that **2r** is the least selective antagonist with 4-fold selectivity. **2r** is considered to be an ideal ET_A/ET_B mixed antagonist though its binding affinity to ET_A receptors was comparable to that of **1**. A pyrrolidinyl analogue **2s** also showed an ET_A/ET_B mixed antagonistic character. These results suggest that the α-branch of the substituent at the 2-position also played a key role in the ET_A binding affinity. As a result, incorporation of a nitrogen atom into the butyl group led to the identification of highly potent ET_A selective antagonists such as **2p** ($IC_{50}=0.12$ nM, 520-fold selectivity) and **2q** ($IC_{50}=0.10$ nM, 420-fold selectivity) and of an ET_A/ET_B mixed antagonist such as **2r** ($IC_{50}=3.2$ nM, 4-fold selectivity).

Conclusion

In summary, we developed a versatile method to synthesize 2-substituted 5,7-diarylcyclopenteno[1,2-*b*]pyridine-6-carboxylic acids. Through the derivatization via the method, we found that an alkyl substituent such as a butyl or isobutyl group at the 2-position of **1** was effective in enhancing the binding affinities for both ET_A and ET_B receptors, and we identified new ET_A/ET_B mixed antagonists, **2d** ($IC_{50}=0.21$ nM, 52-fold selectivity) and **2f** ($IC_{50}=0.32$ nM, 26-fold selectivity). In contrast, introduction of a propylamino or isopropylamino group at the 2-position led to the identification of more potent and ET_A selective antagonists, **2p** ($IC_{50}=0.12$ nM, 520-fold selectivity) and **2q** ($IC_{50}=0.10$ nM, 420-fold selectivity). Further derivatization of the analogues **2d** and **2p** directed toward an orally active ET_A/ET_B mixed and ET_A selective antagonist will be described in the near future.

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