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Synthesis and in vitro evaluation of ambrisentan analogues as potential endothelin receptor antagonists

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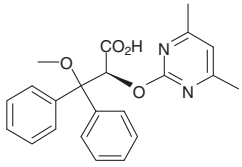
ABSTRACT

A series of novel 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3,3-diphenyl butyric acid derivatives were synthesized and evaluated for their antagonistic activity for endothelin-1-induced contraction in rabbit aorta. Within this series of compounds, 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-cyano-3,3-diphenylpropionic acid (**4**) displays comparable potency with ambrisentan (**1**), and warrants further investigation.

The endothelins consist of three subtypes: endothelin-1, 2 and 3 (ET-1, ET-2, ET-3). The endothelins function by binding to transmembrane G-proteincoupled receptors of which two major subtypes, ET_A and ET_B, have been identified.[1–3] Both receptor subtypes are found on smooth muscle cells and mediate the vasoconstrictor and pressor actions of endothelins. Elevated levels of endothelins have been associated with a number of physiological and pathological processes such as renal failure,[4] heart failure,[5] hypertension,[6] atherosclerosis,[7] acute myocardial infarction,[8] pulmonary arterial hypertension (PAH),[9] cerebral vasospasm,[10] subarachnoid hemorrhage[11] and metastatic prostate cancer.[12] Theoretically, selective ET_A-receptor antagonists should be more effective in achieving vasodilation than non-selective ET_A/ET_B receptor antagonists, given the role played by ET_B receptors in both vasodilation and ET-1 clearance.[13] In animal models of PAH, however, positive dilatory effects have been observed with both selective ET_A-receptor blockade and non-selective antagonism.[14] Hence, at the present time, there is no specific answer to the question that which class of antagonists is better.[14] In fact, bosentan, which is the first non-peptidic endothelin receptor antagonist approved for the treatment of PAH,[15] is a mixed ET_A/ET_B receptor antagonist. On the other hand, many research have been embarked on the discovery and development of selective ET_A receptor antagonists,[16–18] of which ambrisentan (**1**) has been approved for the treatment of PAH, and is regarded as 'the best-in-class' due to its lower hepatotoxicity and weaker drug interaction.[19] It was noticed that modifications at methoxy group in **1** led to analogues which retained ET_A affinity but exhibited ET_B affinity as well.[20] In this study, a series of ambrisentan analogues, 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3,3-diphenyl butyric acid derivatives, have been synthesized and evaluated for their in vitro antagonism of endothelin function in order to find more potent and safer endothelin receptor antagonists.



1 (Ambrisentan)

We envisioned that the methoxy group in **1** could be replaced by a hydroxymethyl group, resulting in compound **2**, an isomeride of ambrisentan (**1**). A series of ether and ester derivatives of **2** were further designed and synthesized to explore the structure–activity relationship. On the other hand, compound **4** with a cyano group in place of the methoxy group in **1** also attracted our attention. Moreover, the effect of introduction of sterically hindered and hydrophobic groups into **1** was studied through the design of compound **5**.

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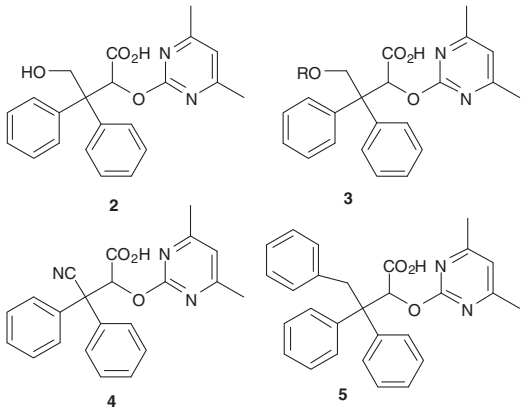
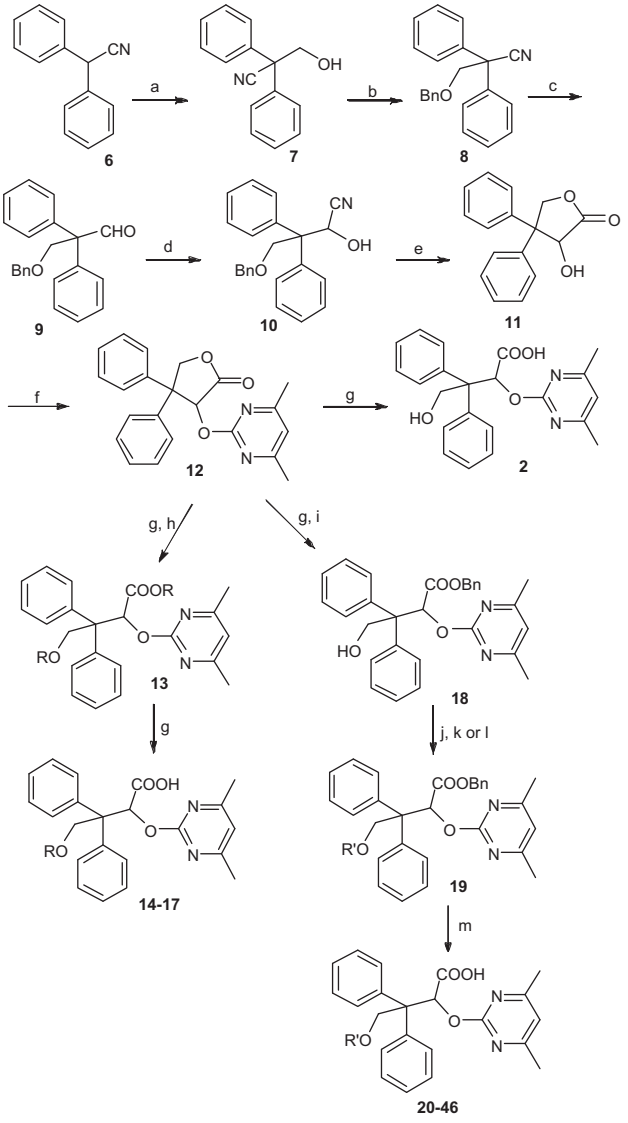


The synthesis of **2** and its derivatives **14–17** and **20–46** is outlined in Scheme 1. Reaction of diphenylacetonitrile **6** with paraformaldehyde gave alcohol **7**.[21] Benzylation of **7** with benzyl trichloroacetimidate afforded benzyl ether **8** (yield 71% from **6**). Reduction of **8** with DIBAl-H gave aldehyde **9**. Treatment of **9** with NaCN in the presence of $NaHSO_3$ provided cyanohydrine **10** (yield 85% from **8**). Unexpectedly, acidic hydrolysis of **10** gave lactone **11** as the major product instead of the corresponding α-hydroxy acid (yield 72%). Reaction of **11** with 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine in the presence of K_2CO_3 gave pyrimidine derivative **12** (yield 73%). Hydrolysis of **12** yielded compound **2** which could be cyclized again under acidic conditions to give **12**, and thus the acidification during work-up should be very careful, maintaining pH value around 7 (yield 95%). Hydrolysis of **12** and subsequent complete alkylation of the resulting hydroxyl and carboxylic groups with reactive alkyl halides (e.g., methyl iodide, ethyl iodide, allyl bromide and benzyl bromide) in the presence of K_2CO_3 and NaOH afforded the corresponding esters **13**, which were hydrolyzed to give the corresponding carboxylic acids **14**–**17** (yield 80–88%). When using less reactive alkyl halides such as butyl bromide, the above reactions did not work well. Therefore, we developed another approach to derivatization of **2**. Hydrolysis of **12** and subsequent selective benzylation of the resulting carboxylic group with benzyl bromide in the presence of water afforded benzyl ester **18**. Alkylation or acylation of **18**, followed by debenzylation by hydrogenolysis with Pd/C provided compounds **20–46** (yield 70–91%).

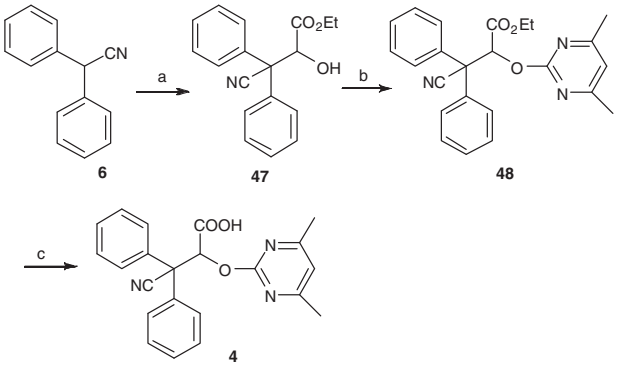
The synthesis of **4** is shown in Scheme 2. Reaction of diphenylacetonitrile **6** with ethyl glyoxalate afforded α-hydroxy ester **47** in 87% yield. Reaction of **47** with 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine in the presence of NaH at low temperature provided pyrimidine derivative **48** in 74% yield. Hydrolysis of **48** produced carboxylic acid **4** in 99% yield.

The synthesis of **5** is shown in Scheme 3. Reaction of diphenylacetic acid (**49**) with benzyl bromide in the presence of K_2CO_3 afforded benzyl ester **50**. Alkylation of **50** with benzyl bromide in the presence of NaH produced compound **51**. Reduction of **51** with $LiAlH_4$ yielded alcohol **52**, which was oxidized with PCC to give aldehyde **53**. Treatment of **53** with NaCN in the presence of $NaHSO_3$ furnished cyanohydrine **54** (yield 81% from **49**). Hydrolysis of **54** afforded α-hydroxy acid **55** smoothly, which reacted with 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine in the presence of K_2CO_3 to yield compound **5** (yield 80% from **54**).

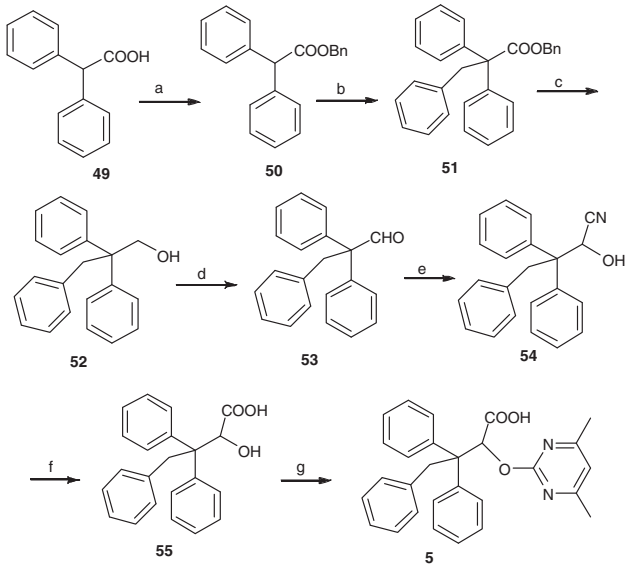
The functional inhibitory potency of the synthesized compounds against the contraction induced by endothelin-1 on rabbit aortic rings was assessed following the literature procedures[22–25] (for details see Supplementary data). The assay results are summarized in Table 1. As shown in Table 1, some test compounds (e.g., **2**, **4**, **15**, **16**, **23**, **30**, **43**) exhibited potent functional inhibitory activity against endothelin-1-induced vascular contraction. In this



Scheme 1. Reagents and conditions: (a) HCHO, CaO/THF, H_2O, 30 °C; (b) benzyl trichloroacetimidate, TfOH/cyclohexane, CH_2Cl_2, rt; (c) Dibal-H, toluene, 0 °C to rt; (d) NaCN, $NaHSO_3$, THF, H_2O, rt; (e) AcOH, 67% H_2SO_4, 100–110 °C, reflux; (f) 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine, K_2CO_3, DMF, 90 °C; (g) 10%NaOH, MeOH, reflux; (h) RX, K_2CO_3, NaOH, DMF; (i) BnBr, K_2CO_3, H_2O, DMF; (j) R′X, NaH/THF; (k) $(R'CO)_2O$ or R′COCl/Pyr; (l) R′COCl, NaH/THF; (m) H_2,10% Pd–C, THF.

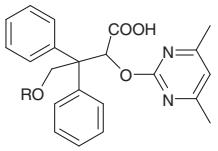


Scheme 2. Reagents and conditions: (a) HCOCOOEt, NaH/THF, −20 °C; (b) 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine, NaH, THF, −20 °C; (c) 10%NaOH, MeOH, reflux.

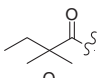
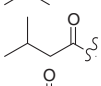
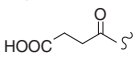
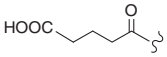
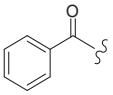
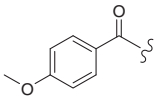
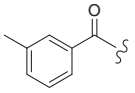
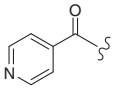
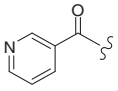
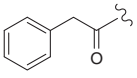
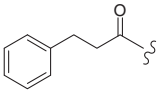
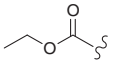
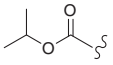
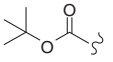
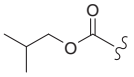


Scheme 3. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt; (b) BnBr, NaH, THF, 0 °C; (c) K_2CO_3, THF, 0 °C; (d) PCC/CH_2Cl_2, 0 °C to rt; (e) NaCN, $NaHSO_3$, THF, H_2O, rt; (f) AcOH, 67% H_2SO_4, 100–110 °C; (g) 4,6-dimethyl-2-methylsulfonyl-1,3-pyrimidine, $LiNH_2$, DMF, 50 °C.

Table 1
Inhibition of endothelin-induced contractions on isolated rabbit aortic rings



2, 14-17, 20-46

Compd	R	Inhibition rate (%)
Ambrisentan	—	43.23
Bosentan	—	22.54
2	H	23.19
4	—	42.20
5	—	11.77
12	—	14.32
14	Methyl	NA
15	Ethyl	20.64
16	Allyl	23.44
17	Benzyl	NA
18	—	NA
20	*n*-Propyl	NA
21	*n*-Butyl	15.11
22	Acetyl	9.64
23	Propionyl	21.68
24	Butyryl	1.11
25	Valeryl	5.88
26	Caproyl	NA
27	Capryloyl	5.61
28		NA
29		NA
30		21.80
31		NA
32		NA
33		3.47
34		8.09
35		NA
36		NA
37		13.16
38		7.82
39		NA
40		NA
41		3.40
42		15.52
43		22.02
44		NA
45		13.65
46	Ts	NA

(ET-1: 10^{-8} M; test compounds: 10^{-6} M).

functional assay, **2**, **15**, **16**, **23**, **30**, and **43** displayed comparable potency with bosentan, whereas **4** (42.2% inhibition) was much more potent than bosentan (22.54% inhibition) and equally potent with ambrisentan (43.23% inhibition). We further determined the effects of **4** and ambrisentan on the concentration–contractile response curve to ET-1 in isolated rabbit aorta (Figs. 1 and 2). The results again confirmed that **4** was a very potent antagonist against endothelin-1-induced vascular contraction.

SAR analysis showed that replacement of the methoxy group in **1** with a hydroxymethyl group retained the activity, albeit resulting in a decrease in potency (23.19% inhibition for **2** vs 43.23% inhibition for **1**). Alkylation or acylation of the hydroxyl group in **2** led to mixed results: in some cases the activity remained (e.g., **15**, **16**, **23**, **30**, **43**), and in other cases a loss in potency was observed (e.g., **14**, **17**, **20**, **28**). The pattern of substitution appeared to be less crucial, but the size and length seemed to have an impact on the potency. Moderate substitutions were beneficial to potency (e.g., **16**, **23**, **30**, **43**), while large (e.g., **17, 25**, **26**, **27**, **28, 29, 39, 44**) or small (e.g., **14**, **22**, **41**) substituents led to significant decreases in potency. Replacement of the methoxy group in **1** with hydrophobic groups resulted in a decrease in potency (e.g., **5**). It was interesting to note that introduction a cyano group to replace the methoxy group in **1** resulted in a very potent compound (**4**).

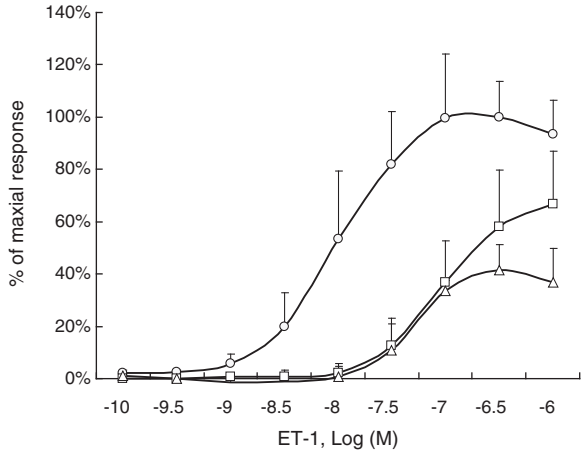


Figure 1. Effects of compound **4** and ambrisentan on the concentration–contractile response curve to ET-1 in isolated rabbit aorta: vehicle (open circle), compound **4**: 10^{-6} M (open squares), ambrisentan: 10^{-6} M (open triangles). The date points represent mean value ± SEM (n = 6–7).

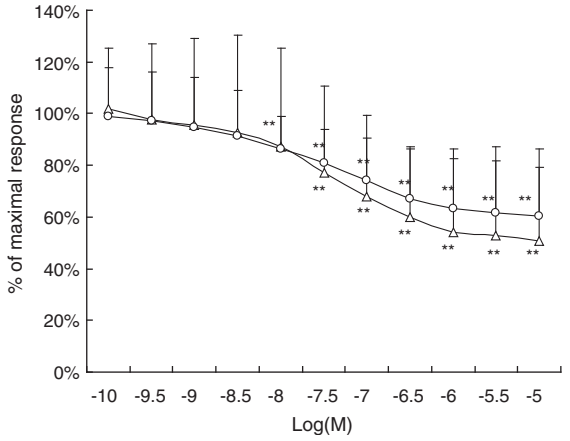


Figure 2. Effects of ET-1 on the concentration–contractile response cure to compound **4** (open circle) and ambrisentan (open triangles). The data points represent the mean percentage of the maximal response ± s (n = 6), $^{**}p$ <0.01 versus control (ET-1: 2×10^{-8} M).

In summary, a series of novel 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3,3-diphenyl butyric acid derivatives were synthesized and evaluated for their antagonistic activity for endothelin-1-induced contraction in rabbit aorta. Within this series of compounds, **2**, **4**, **15**, **16**, **23**, **30**, and **43** exhibited potent inhibitory activity against endothelin-1-induced vascular contraction. Particularly, 2-[(4,6-dimethylpyrimidin-2-yl)oxy]-3-cyano-3,3-diphenylpropionic acid (**4**) was comparable with ambrisentan (**1**) in potency. Since **4** is a racemic compound while ambrisentan is an optically active enantiomer, optically pure enantiomers of **4** warrants further investigation.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.034.

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