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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and biological evaluation of benzofuran derivatives as ET receptor antagonists

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Abstract A series of novel benzofuran carboxylic acid derivatives have been designed and synthesized, with their antagonism effect screened on ET-1-induced contraction in the rat thoracic aortic ring. Some target compounds demonstrated significant inhibitory activity, especially benzo[*c*]thiadiazole and benzo[*c*]oxadiazole compounds **29** and **30** showed potent inhibition percentage higher than the contrast compound **BQ123**. Further affinity and selectivity for ET binding assay showed that **29** demonstrated a dual ET_A/ET_B antagonism activity in nanomole level. Moreover, **30** was effective in relieving hypoxia-induced pulmonary arterial hypertension.

Keywords Benzofuran · Endothelin · Pulmonary arterial hypertension · SAR · Synthesis

Introduction

Endothelin (ET), a peptide of 21-amino acids, was initially identified from endothelial cells as a potent vasoconstrictor (Yanagisawa *et al.*, 1988). There are three isoforms (ET-1, ET-2, ET-3) (Inoue *et al.*, 1989). ET-1 is the predominant component of the three ET-isopeptides largely because of its ability to constrict vascular and nonvascular smooth muscle (Prasanna *et al.*, 2003). ET is a small peptide hormone that is believed to play a critical role in the control of blood flow and cell growth. Elevated ET blood levels are associated with several cardiovascular disease conditions, including systemic and pulmonary arterial hypertension (Stewart *et al.*, 1991), congestive heart failure (Van Beneden *et al.*, 2004), renal failure (Lariviere and Lebel, 2003), prostate cancer (Zhou *et al.*, 2007; Roh and Abdulkadir, 2010) and atherosclerosis (Minamino *et al.*, 1997; Skalska and Grodzicki, 2010). The ETs function by binding to transmembrane G-protein-coupled receptors of which two major subtypes, ET_A and ET_B, have been identified (Jacobs *et al.*, 2006; Masaki *et al.*, 1994). The ET_A subtype, which is selective for ET-1 and ET-2 over ET-3, is found principally in peripheral tissues such as vascular smooth muscle (Arai *et al.*, 1990). The ET_A receptor mediates vasoconstriction and vascular smooth muscle proliferation. However, the binding of ET to ET_B receptors located on the vascular endothelium causes vasodilation through the production of nitric oxide and prostacyclin (Seo *et al.*, 1994). Antagonists of ET receptors can be classified pharmacologically as selective and non-selective antagonists or chemically as peptide and non-peptide compounds. Bosentan (Tracleers®), a non-selective ET receptor antagonist, and Ambrisentan (letairise®), a selective ET_A receptor antagonist have been approved for the treatment of pulmonary arterial hypertension by the US Food and Drug Administration and they are non-peptide compounds with high bioavailability (Li *et al.*, 2008; Patel *et al.*, 2010).

Screening of the Parke–Davis compound library identified two benzofuran carboxylic acid derivatives **1** and **2** (Fig. 1) that showed potent selectivity for the ET_A receptor (IC_{50} = 11 μM for **1**, IC_{50} = 3.8 μM for **2**) (Kaltenbronn *et al.*, 1997). The previous report of structure and activity relationship of benzofuran carboxylic acid derivatives indicated that 3-methyl group and the carboxyl group were essential for ET_A binding affinity. Therefore, we introduced

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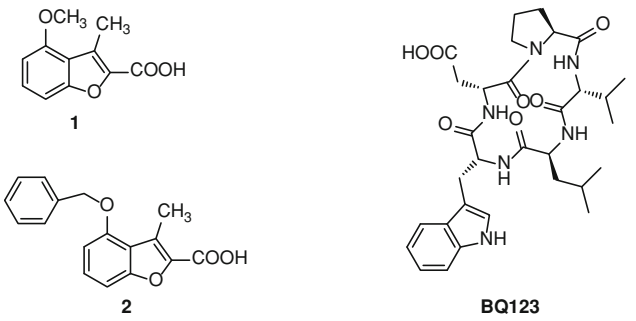


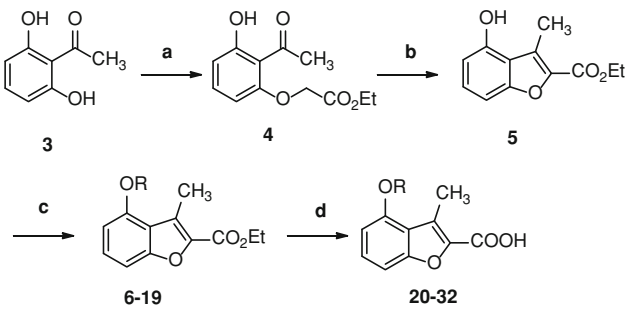
Fig. 1 Chemical structure of **1**, **2** and **BQ123**

some other substituents in 4-position, such as longer aliphatic chains, substituted benzene rings and benzothiadiazoles, which were pharmacophores of some known ET_A antagonists in former report (Yuan *et al.*, 2006). This paper described the synthesis and SAR of a series of derivatives where *R* function was varied sequentially.

Results and discussion

Chemistry

Compounds **20**–**32** were prepared as illustrated in Scheme 1. 1-(2,6-dihydroxyphenyl)-ethanone **3** as starting material was monoalkylated with ethyl 2-bromoacetate to give **4** in 61 % yield. Cyclization of **4** with NaOMe in MeOH afforded **5** in low yield due to partial hydrolysis of the ester under the alkali conditions in this step. Alkylation of 4-phenolic hydroxyl group permitted easy access to the intermediate ethers **6**–**19**, which was hydrolyzed to produce **2**, **20**–**32** in yields of 38–84 %. As summarized in Table 1, up to fourteen target compounds (**2**, **20**–**32**) were synthesized.



Scheme 1 Synthetic route for the preparation of the target compounds **20**–**32**. *Reagents and conditions a* $BrCH_2CO_2Et$, K_2CO_3, acetone, 3 h, reflux, 61 %; *b* NaOEt, EtOH, 9 h, reflux, 36 %; *c* RX, KI, K_2CO_3, DMF, 1 h, 60 °C, 84 %; *d* NaOH, EtOH, H_2O, 1 h, reflux, 38–84 %

Biological evaluation

Functional assessment of antagonism on ET-1-induced contraction of rat thoracic aortic ring

The synthesized compounds were evaluated for their antagonism of ET-1-induced contraction in the rat thoracic aortic ring. **BQ123** was taken as a positive control and DMSO was taken as a blank control in the assays. The results reported in Table 2 showed that ethyl and propyl group substituted compounds **20** and **21** demonstrated significant inhibition activity, but with prolongation of the alkyl chain, the activity of **22** and **23** became lower. Among the alkylbenzol ethers **24**–**27**, substitution with either electron-donating or electron-withdrawing groups gave moderate inhibition, it was noticeable for compound **26** that percentage of ET-1 inhibition was −39 %, we speculated that **26** may show some ET_A agonist activity or it could potentiate effect of ET-1-induced contraction with an unknown mechanism. These results indicated that the increase of R groups volumes would not benefit for the inhibition activity of the compounds. However, it was worth pointing out that significant inhibition was achieved for benzo[*c*]thiadiazole and benzo[*c*]oxadiazole compounds **29** and **30** ($P < 0.01$) with potent inhibition percentage higher than the contrast compound **BQ123**, which confirmed our preliminary design idea.

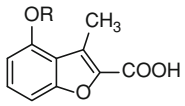
Selective potency of compounds on the ET_A and ET_B subtype of rat myocardium

Therefore, the further test of radio-receptor assay of compound **29** and **30** were done for purpose of quantitative evaluation for selectivity of effect on ET_A and ET_B (Table 3). Compared with **BQ123**, **29** demonstrated more potent binding affinity on ET_A in nanomole level (23 nM), and the ratio of ET_A/ET_B was 40. So we could infer that **29** was a non-selective dual antagonist of ET_A and ET_B, and had potential for further development in cardiovascular diseases. The next study would evaluate the ability of orally administered **29** to relieve hypoxia-induced pulmonary arterial hypertension in rats.

*Relief activity of compound **29** on pulmonary arterial hypertension*

After hypoxic exposure for 28 days, the right ventricular systolic pressure (RVSP) and central vein pressure (CVP) of rats were elevated by +118 % ($P < 0.01$) and +1,071 % ($P < 0.05$), respectively, relative to control, indicating hypoxic PAH was established. Nifedipine p.o. was used as a positive reference in this study due to its wide use in treating PAH. After treatment with compound **29** (40, 80, 160 mg/kg) and nifedipine (10 mg/kg), the elevated RVSP and

Table 1 Structure of compounds **20–32**



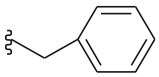
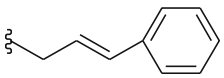
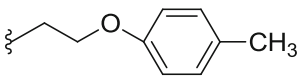
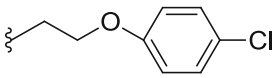
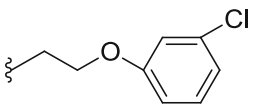
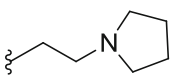
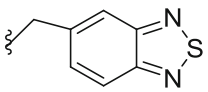
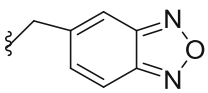
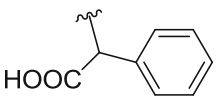
Compound	R	Yield (%)	Mp (°C)	Formula
20	$-CH_2CH_3$	52	226–228	$C_{12}H_{12}O_4$
21	$-(CH_2)_2CH_3$	78	188–190	$C_{13}H_{14}O_4$
22	$-(CH_2)_3CH_3$	63	182–183	$C_{14}H_{16}O_4$
23	$-(CH_2)_4CH_3$	81	186–188	$C_{15}H_{18}O_4$
2		62	183–184	$C_{17}H_{14}O_4$
24		67	191–193	$C_{19}H_{16}O_4$
25		60	239–241	$C_{19}H_{18}O_5$
26		38	250–252	$C_{18}H_{15}ClO_5$
27		63	208–210	$C_{18}H_{15}ClO_5$
28		75	217–218	$C_{16}H_{19}NO_4$
29		81	256–258	$C_{17}H_{12}N_2O_4S$
30		66	224–226	$C_{17}H_{12}N_2O_5$
31		84	213–214	$C_{18}H_{14}O_6$

Table 1 continued

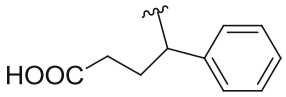
Compound	R	Yield (%)	Mp (°C)	Formula
32		79	230–232	$C_{20}H_{18}O_6$

Table 2 In vitro functional vascular ET-1 antagonism of test compounds in concentration 10 μM; inhibition of the ET-1 (2 nM)-induced contraction of rat thoracic aortic ring

Compound	Percentage of inhibition ± SD (%)[c]	P[d]	Cpd.	Percentage of inhibition ± SD (%)	P[d]
DMSO	29.9 ± 18.3 (8)	–	**25**	40.8 ± 37.7 (3)	0.5181
BQ123[a]	68.1 ± 31.3 (3)	0.02985*	**26**	−39 ± 26.0 (3)	0.00073**
20	61.0 ± 13.7 (3)	0.02715*	**27**	21.7 ± 22.2 (3)	0.54744
21	69.7 ± 26.4 (3)	0.01807*	**28**	34.1 ± 22.5 (3)	0.75313
22	56.7 ± 28.2 (4)	0.0723	**29**	82.2 ± 12.6 (3)	0.00141**
23	46.8 ± 38.4 (3)	0.33102	**30**	70.0 ± 15.5 (4)	0.0039**
2[b]	40.7 ± 24.2 (4)	0.40349	**31**	29.0 ± 26.8 (3)	0.95234
24	50 ± 44.1 (3)	0.28848	**32**	44.0 ± 42.6 (3)	0.43836

[a] **BQ123** (Petersen *et al.*, 2008): $IC_{50} = 37$ nM *for* hET_A

[b] Reported else where

[c] values are the means of the indicated number of experiments (n)

[d] P value of T test (* $P < 0.05$; ** $P < 0.01$)

Table 3 ET_A and ET_B receptor binding affinities for **29**, **30** and **BQ123**

Compound	IC_{50} (nM)		Selectivity for ET_A
	ET_A	ET_B	
29	23	930	40
30	39	1,070	27
BQ123	28	–	Specificity

CVP decreased individually ($P < 0.01$), respectively, compared with the untreated group (Fig. 2). The response to **29** was dose dependent with the lowest dose demonstrating potent effect on relieving hypoxia-induced pulmonary arterial hypertension.

Experimental section

General

All reagents were purchased from commercial sources and used without further purification. Melting points were measured in open capillaries and are uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker-ACF 300 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were obtained from Agilent 1100LC/MS Spectrometry Services. Elemental analyses were performed on Elementar Vario ELIII instrument. All compounds were routinely checked by TLC with silica gel GF-254 glass plates and viewed under UV light at 254 nm.

Synthesis

*Ethyl 2-(2-acetyl-3-hydroxyphenoxy)acetate (**4**)*

1-(2,6-dihydroxyphenyl)-ethanone **3** (15.2 g, 0.1 mol) and K_2CO_3 (41.4 g, 0.3 mol) were dissolved in acetone (200 mL). Ethyl 2-bromoacetate (20 g, 0.12 mol) was added to this solution. The mixture was heated to reflux for 3 h. After

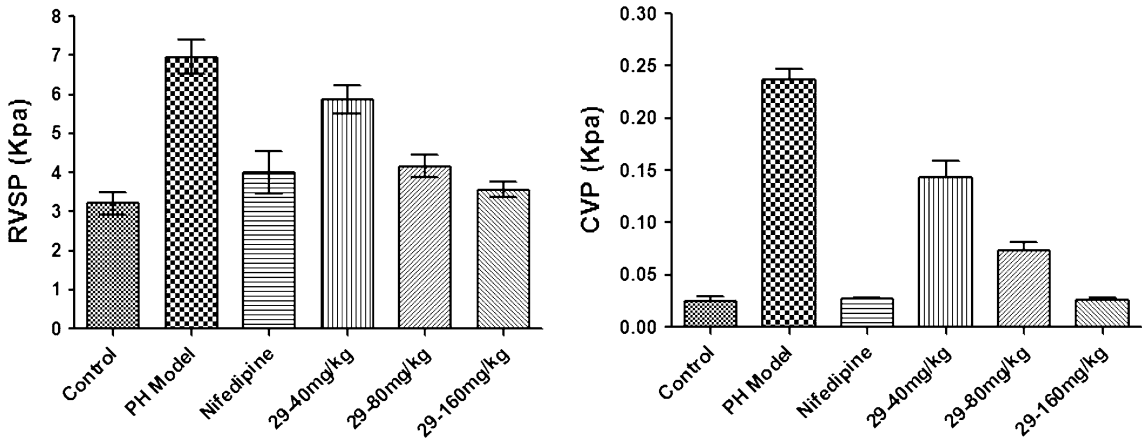


Fig. 2 Reversion of an elevation in RVSP and CVP in hypoxic pulmonary arterial hypertensive (PAH) rats by a dual ET receptor antagonist **29**. $N = 10$

cooling to room temperature, the precipitate was filtered and washed with acetone, about 2/3 of the filtrate was evaporated under reduced pressure. Cold water (300 mL) was added, and the precipitate obtained was filtered, washed with water, and dried to yield **4** as a white tabular crystal (14.6 g, 61 %). M.p. 80–82 °C. IR (KBr, cm^{-1}): 3320, 2918, 1708, 1509, 1443, 1276; ^{1}H-NMR($CDCl_3$), δ: 1.38 (t, $J = 7.0$ Hz, 3H, $CO_2CH_2C\underline{H}_3$), 2.63 (s, 3H, $-CH_3$), 4.35 (q, 2H, $J = 7.0$ Hz, $CO_2C\underline{H}_2CH_3$), 4.98 (s, 2H, $-CH_2$), 6.55 (d, 1H, $J = 7.5$ Hz, PhH), 7.04 (d, 1H, $J = 8.1$ Hz, PhH), 7.26 (t, 1H, $J = 8.0$ Hz, PhH); ^{13}C-NMR, δ: 13.9, 33.9, 61.5, 66.9, 106.7, 106.8, 113.1, 136.1, 160.5, 163.6, 170.3, 203.9; MS (EI) *m/e*: 238 (M^+); Anal. $C_{12}H_{14}O_5$. Calcd: C, 60.50; H, 5.92; Found: C, 60.63; H, 6.05.

*Ethyl 4-hydroxy-3-methylbenzofuran-2-carboxylate (**5**)*

To absolute ethanol (180 mL) was added in portions (2.7 g, 0.118 mol) of Na. When all had reacted, **4** (13.6 g, 0.057 mol) was added, and the solution heated at reflux for 9 h. The solution was poured into the cold water and the pH adjusted to 3 with dilute HCl. The precipitate obtained was filtered, washed with water and dried. The crude product was purified by silica gel flash chromatography (AcOEt/pet = 1:2) to yield **5** as a white powder (4.5 g, 36 %). M.p. 158–159 °C. IR (KBr, cm^{-1}): 3328, 2925, 1715, 1521, 1442, 1279; ^{1}H-NMR ($CDCl_3$), δ: 1.43 (t, 3H, $J = 7.1$ Hz, $CO_2CH_2C\underline{H}_3$), 2.72 (s, 3H, 3-CH_3), 4.43 (q, 2H, $J = 7.1$ Hz, $CO_2C\underline{H}_2CH_3$), 6.57 (d, 1H, $J = 7.6$ Hz, 5-H), 7.08 (d, 1H, $J = 8.3$ Hz, 7-H), 7.27 (t, 1H, $J = 8.1$ Hz, 6-H); ^{13}C-NMR, δ: 10.0, 14.0, 61.8, 106.1, 113.2, 113.9, 126.1, 126.3, 142.5, 152.2, 159.9, 160.8; MS (EI) *m/e*: 220 (M^+); Anal. $C_{12}H_{12}O_4$. Calcd: C, 65.45; H, 5.49; Found: C, 65.57; H, 5.65.

*Alkylation of the 4-hydroxyl group to give **6–19**. A general procedure illustrated with the preparation of ethyl 4-ethoxy-3-methylbenzofuran-2-carboxylate (**6**)*

5 (0.9 g, 3.6 mmol) was dissolved in DMF (20 mL), K_2CO_3 (3.5 g), KI (0.5 g) and bromoethane (1 mL) were added to this solution. This mixture was heated to 60 °C for 2 h. After cooling to room temperature, the solution was poured into the cold water. The precipitate obtained was filtered and recrystallized by methanol to yield **6** as colorless granular crystal (0.76 g, 84 %). M.p. 94–95 °C. IR (KBr, cm^{-1}): 2992, 1675, 1587, 1447, 1268, 1165, 1083, 739; ^{1}H-NMR ($CDCl_3$), δ: 1.36–1.52 (m, 6H, $OCH_2C\underline{H}_3$), 2.70 (s, 3H, 3-CH_3), 4.06 (q, 2H, $J = 6.9$ Hz, $OC\underline{H}_2CH_3$), 4.41 (q, 2H, $J = 7.0$ Hz, $CO_2C\underline{H}_2CH_3$), 6.58 (d, 1H, $J = 8.0$ Hz, 5-H), 7.03 (d, 1H, $J = 8.0$ Hz, 7-H), 7.29 (t, 1H, $J = 7.9$ Hz, 6-H); ^{13}C-NMR, δ: 10.1, 14.1, 15.3, 61.5, 66.8, 103.5, 112.3, 113.9, 126.7, 128.5, 139.8, 145.7, 161.3, 167.9; MS (EI) *m/e*: 248 (M^+); Anal. $C_{14}H_{16}O_4$. Calcd: C, 67.73; H, 6.50; Found: C, 67.92; H, 6.63.

*Hydrolysis of **6–19** to give **2**, **20–32**. A general procedure illustrated with the preparation of 4-ethoxy-3-methylbenzofuran-2-carboxylic acid (**20**)*

6 (0.47 g, 3 mmol) was dissolved in ethanol (30 mL), 10 % NaOH (10 mL) was added to this solution. The mixture was heated to reflux for 1 h. After cooling to room temperature, the pH adjusted to 3 with dilute HCl. The precipitate obtained was filtered, washed with water, and dried to get crude product. Recrystallization from 75 % ethanol gave **20** as colorless needle crystal (0.38 g, 52 %). M.p. 226–228 °C. IR (KBr, cm^{-1}): 2980, 1677, 1582, 1442, 1273, 1163, 1087, 732; ^{1}H-NMR($CDCl_3$), δ: 1.45 (t, 3H, $J = 7.1$ Hz,

$OCH_2C\underline{H}_3$), 2.69 (s, 3H, 3-CH_3), 4.06 (q, 2H, J = 7.1 Hz, $OC\underline{H}_2CH_3$), 6.56 (d, 1H, J = 8.0 Hz, 5-H), 7.02 (d, 1H, J = 8.2 Hz, 7-H), 7.27 (t, 1H, J = 8.0 Hz, 6-H), 10.37 (1H, br, 2-CO_2H); ^{13}C-NMR, δ: 10.2, 15.2, 66.5, 103.8, 112.5, 113.7, 126.3, 128.1, 139.4, 145.3, 161.6, 167.8; MS (EI) *m/e*: 220 (M^+); Anal. $C_{12}H_{12}O_4$. Calcd: C, 65.45; H, 5.49; Found: C, 65.36; H, 5.59.

*3-Methyl-4-propoxybenzofuran-2-carboxylic acid (**21**)*

Yield: 78 %. M.p. 188–190 °C. IR (KBr, cm^{-1}): 2965, 1674, 1582, 1451, 1357, 1266, 1162, 1091, 740; ^{1}H-NMR ($CDCl_3$), δ: 1.08 (t, 3H, J = 7.4 Hz, 4-$OCH_2CH_2C\underline{H}_3$), 1.81–1.89 (m, 2H, 4-$OCH_2C\underline{H}_2CH_3$), 2.69 (s, 3H, 3-$CH_3$), 4.07 (t, 2H, J = 6.5 Hz, 4-$OC\underline{H}_2CH_2CH_3$), 6.64 (d, 1H, J = 7.7 Hz, 5-H), 7.06 (d, 1H, J = 7.7 Hz, 7-H), 7.31 (t, 1H, J = 7.8 Hz, 6-H); ^{13}C-NMR, δ: 10.0, 10.5, 22.3, 69.3, 103.6, 112.3, 113.9, 126.0, 127.9, 140.1, 145.7, 161.9, 167.4; MS (EI) *m/e*: 234 (M^+); Anal. $C_{13}H_{14}O_4$. Calcd: C, 66.67; H, 5.98; Found: C, 66.65; H, 6.01.

*4-Butoxy-3-methylbenzofuran-2-carboxylic acid (**22**)*

Yield: 63 %. M.p. 182–183 °C. IR (KBr, cm^{-1}): 2960, 1671, 1581, 1499, 1449, 1359, 1259, 1162, 1090, 784, 740; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 0.99 (t, 3H, J = 6.9 Hz, 4-$O(CH_2)_3C\underline{H}_3$), 1.33–1.90 (m, 4H, 4-$OCH_2(C\underline{H}_2)_2CH_3$), 2.69 (s, 3H, 3-$CH_3$), 4.05 (t, 2H, J = 6.9 Hz, 4-$OC\underline{H}_2(CH_2)_2CH_3$), 6.64 (d, 1H, J = 7.3 Hz, 5-H), 7.04 (d, 1H, J = 7.5 Hz, 7-H), 7.30 (t, 1H, J = 7.8 Hz, 6-H); ^{13}C-NMR, δ: 10.1, 15.2, 19.9, 33.5, 69.1, 103.5, 112.7, 113.5, 126.1, 127.6, 140.5, 145.8, 161.7, 167.9; MS (EI) *m/e*: 248 (M^+); Anal. $C_{14}H_{16}O_4$. Calcd: C, 67.74; H, 6.52; Found: C, 67.66; H, 6.47.

*3-Methyl-4-(pentyloxy)benzofuran-2-carboxylic acid (**23**)*

Yield: 81 %. M.p. 186–188 °C. IR (KBr, cm^{-1}): 2987, 1671, 1581, 1501, 1452, 1350, 1259, 1162, 1090, 785, 741; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 0.94 (t, 3H, J = 7.1 Hz, 4-$O(CH_2)_4C\underline{H}_3$), 1.20–2.10 (m, 6H, 4-$OCH_2(C\underline{H}_2)_3CH_3$), 2.72 (s, 3H, 3-$CH_3$), 4.06 (t, 2H, J = 6.7 Hz, 4-$OC\underline{H}_2(CH_2)_3CH_3$), 6.62 (d, 1H, J = 7.8 Hz, 5-H), 7.04 (d, 1H, J = 7.8 Hz, 7-H), 7.30 (t, 1H, J = 8.0 Hz, 6-H); ^{13}C-NMR, δ: 10.0, 14.7, 22.8, 28.3, 30.3, 69.5, 103.1, 112.8, 113.3, 126.4, 127.8, 140.3, 145.9, 161.6, 167.5; MS (EI) *m/e*: 262 (M^+); Anal. $C_{15}H_{18}O_4$. Calcd: C, 68.70; H, 6.87; Found: C, 68.50; H, 6.87.

*4-(Benzyloxy)-3-methylbenzofuran-2-carboxylic acid (**2**)*

Yield: 62 %. M.p. 183–184 °C. [(Kaltenbronn *et al.*, 1997) M.p. 183–185 °C]. IR (KBr, cm^{-1}): 2991, 1682, 1584, 1500, 1446, 1358, 1258, 1163, 1090, 739; ^{1}H-NMR ($CDCl_3$), δ: 2.78 (s, 3H, 3-CH_3), 5.17 (s, 2H, 4-$OC\underline{H}_2$Ph), 6.69 (d, 1H, J = 7.9 Hz, 5-H), 7.08–7.49 (m, 7H, PhH and 6-H, 7-H), 11.55 (br, 1H, 2-CO_2H); ^{13}C-NMR, δ: 10.1, 72.3, 104.2, 112.1, 112.9, 126.3, 127.3, 127.4, 127.8, 128.0, 129.1, 129.2, 137.4, 140.7, 150.2, 162.3, 166.3; MS (EI) *m/e*: 282 (M^+); Anal. $C_{17}H_{14}O_4$. Calcd: C, 72.34; H, 4.96. Found: C, 72.38; H, 4.95.

*4-(Cinnamyloxy)-3-methylbenzofuran-2-carboxylic acid (**24**)*

Yield: 67 %. M.p. 191–193 °C. IR (KBr, cm^{-1}): 2862, 1681, 1582, 1497, 1446, 1358, 1254, 1166, 1071, 964, 784, 744, 717; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 2.74 (s, 3H, 3-CH_3), 4.81 (d, 2H, J = 4.9 Hz, 4-$OC\underline{H}_2$–), 6.47 (dt, 1H, J = 4.9 Hz and 16.0 Hz, 4-$OCH_2C\underline{H}$=CH–), 6.82 (d, 1H, J = 16.0 Hz, 4-OCH_2CH=C$\underline{H}$–), 6.85 (d, 1H, J = 8.0 Hz, 5-H), 7.05–7.42 (m, 7H, PhH and 6-H, 7-H); ^{13}C-NMR, δ: 10.0, 63.2, 103.9, 112.0, 112.8, 123.9, 125.6, 126.9, 127.5, 128.0, 128.5, 128.6, 128.8, 130.0, 137.1, 140.5, 150.1, 162.1, 166.3; MS (EI) *m/e*: 308 (M^+); Anal. $C_{19}H_{16}O_4$. Calcd: C, 74.51; H, 5.23. Found: C, 74.81; H, 5.36.

*3-Methyl-4-(2-(p-tolyloxy)ethoxy)benzofuran-2-carboxylic acid (**25**)*

Yield: 60 %. M.p. 239–241 °C. IR (KBr, cm^{-1}): 2928, 1674, 1585, 1513, 1455, 1356, 1274, 1239, 1163, 1100, 933, 800, 741; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 2.03 (s, 3H, Ph–$C\underline{H}_3$), 2.66 (s, 3H, 3-$C\underline{H}_3$), 4.37 (s, 4H, 4-$OC\underline{H}_2$-$C\underline{H}_2O$–), 6.65–7.23 (m, 6H, PhH and 5-H, 7-H), 7.32 (t, J = 7.9 Hz, 1H, 6-H); ^{13}C-NMR, δ: 10.2, 22.5, 68.8, 69.1, 103.8, 111.9, 112.7, 114.5, 114.6, 125.5, 127.5, 129.6, 129.8, 130.1, 140.5, 146.8, 156.5, 161.9, 166.2; MS (EI) *m/e*: 326 (M^+); Anal. $C_{19}H_{18}O_5$. Calcd: C, 69.94; H, 5.52. Found: C, 70.10; H, 5.74.

*4-(2-(4-Chlorophenoxy)ethoxy)-3-methylbenzofuran-2-carboxylic acid (**26**)*

Yield: 38 %. M.p. 250–252 °C. IR (KBr, cm^{-1}): 2935, 1678, 1581, 1494, 1454, 1358, 1270, 1242, 1162, 1101, 932, 823, 786; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 2.67 (s, 3H, 3-$C\underline{H}_3$), 4.41 (s, 4H, 4-$OC\underline{H}_2C\underline{H}_2O$–), 6.71 (d, 1H, J = 7.7 Hz, 5-H), 7.10 (d, 1H, J = 8.1 Hz, 7-H), 6.86–7.25 (m, 4H, PhH), 7.28 (t, 1H, J = 7.8 Hz, 6-H); ^{13}C-NMR, δ: 10.1, 68.9, 69.4, 103.7, 111.8, 112.6, 117.7, 117.8, 125.8, 126.0, 127.5, 130.6, 130.8, 140.6, 146.8, 157.5, 161.8, 166.3; MS (EI) *m/e*: 346 (M^+); Anal. $C_{18}H_{15}ClO_5$. Calcd: C, 62.34; H, 4.33. Found: C, 62.40; H, 4.53.

*4-(2-(3-Chlorophenoxy)ethoxy)-3-methylbenzofuran-2-carboxylic acid (**27**)*

Yield: 63 %. M.p. 208–210 °C. IR (KBr, cm^{-1}): 2930, 1680, 1595, 1452, 1357, 1271, 1161, 1101, 952, 786; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 2.66 (s, 3H, 3-C$\underline{H}_3$), 4.34–4.51 (m, 4H, 4-OC$\underline{H}_2$C$\underline{H}_2$O–), 6.70 (d, 1H, J = 7.6 Hz, 5-H), 6.65–7.23 (m, 6H, PhH and 6-H, 7-H); ^{13}C-NMR, δ: 10.0, 68.8, 69.4, 103.6, 111.7, 112.4, 112.5, 115.3, 120.5, 125.7, 127.3, 130.7, 136.5, 140.5, 146.8, 158.7, 161.7, 166.2; MS (EI) *m/e*: 346 (M^+); Anal. $C_{18}H_{15}ClO_5$. Calcd: C, 62.34; H, 4.33. Found: C, 62.17; H, 4.40.

*3-Methyl-4-(2-(pyrrolidin-1-yl)ethoxy)benzofuran-2-carboxylic acid (**28**)*

Yield: 75 %. M.p. 217–218 °C. IR (KBr, cm^{-1}): 3487, 3393, 2415, 1614, 1572, 1390, 1256, 1094, 786, 728; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6), δ: 2.03–2.29 (m, 4H, –NCH_2(C$\underline{H}_2$)$_2$–), 2.58 (s, 3H, 3-CH_3), 3.31–3.69 (m, 6H, N(C$\underline{H}_2$)$_3$), 4.42 (t, 2H, J = 7.1 Hz, 4-OCH_2–), 6.60 (d, 1H, J = 6.8 Hz, 5-H), 6.88 (d, 1H, J = 7.0 Hz, 7-H), 7.07 (t, 1H, J = 6.8 Hz, 6-H); ^{13}C-NMR, δ: 10.1, 23.8, 23.9, 56.7, 56.8, 57.1, 67.5, 103.4, 111.5, 112.5, 125.6, 127.4, 140.6, 146.9, 161.5, 166.3; MS (EI) *m/e*: 346 (M^+); MS (EI) *m/e*: 289 (M^+); Anal. $C_{16}H_{19}NO_4{\cdot}2H_2O$. Calcd: C, 59.08; H, 7.08; N, 4.31. Found: C, 59.55; H, 7.23; N, 4.44.

*4-(Benzo[c][1,2,5]thiadiazol-5-ylmethoxy)-3-methylbenzofuran-2-carboxylic acid (**29**)*

Yield: 81 %. M.p. 256–258 °C. IR (KBr, cm^{-1}): 2932, 1710, 1600, 1497, 1358, 1257, 1153, 1097, 867, 809, 776; ^{1}H-NMR ($CDCl_3$), δ: 2.79 (s, 3H, 3-CH_3), 5.41 (s, 2H, 4-OC$\underline{H}_2$–), 6.80 (d, 1H, J = 8.1 Hz, 5-H), 7.14 (d, 1H, J = 8.2 Hz, 7-H), 7.34 (t, 1H, J = 8.2 Hz, 6-H), 7.75 (d, 1H, J = 9.0 Hz, Ar-6′-H), 8.06 (d, 1H, J = 8.9 Hz, Ar-7′-H), 8.12 (s, 1H, Ar-4′-H); ^{13}C-NMR, δ: 10.1, 71.8, 103.5, 111.4, 112.3, 120.5, 122.6, 125.9, 127.7, 127.9, 140.5, 141.3, 150.2, 154.4, 155.6, 161.4, 166.2; MS (EI) *m/e*: 340 (M^+); Anal.$C_{17}H_{12}N_2O_4S$. Calcd: C, 60.00; H, 3.53; N, 8.24. Found: C, 59.85; H, 3.72; N, 8.16.

*4-(Benzo[c][1,2,5]oxadiazol-5-ylmethoxy)-3-methylbenzofuran-2-carboxylic acid (**30**)*

Yield: 66 %. M.p. 224–226 °C. IR (KBr, cm^{-1}): 3117, 1717, 1601, 1499, 1359, 1238, 1147, 1096, 778; ^{1}H-NMR ($CDCl_3$), δ: 2.75 (s, 3H, 3-CH_3), 5.35 (s, 2H, 4-OC$\underline{H}_2$–), 6.85 (d, 1H, J = 8.0 Hz, 5-H), 7.14 (d, 1H, J = 8.0 Hz, 7-H), 7.36 (t, 1H, J = 8.1 Hz, 6-H), 7.64 (d, 1H, J = 9.3 Hz, Ar-6′-H), 7.99 (d, 1H, J = 9.4 Hz, Ar-7′-H), 8.03 (s, 1H, Ar-4′-H); ^{13}C-NMR, δ: 10.1, 71.7, 103.6, 110.2, 111.5, 112.4, 116.5, 125.8, 126.3, 127.6, 140.1, 140.5, 147.5, 148.9, 150.2, 161.3, 166.1; MS (EI) *m/e*: 324 (M^+); Anal. $C_{17}H_{12}N_2O_5$. Calcd: C, 62.96; H, 3.70; N, 8.64. Found: C, 62.98; H, 4.04; N, 8.41.

*4-(Carboxy(phenyl)methoxy)-3-methylbenzofuran-2-carboxylic acid (**31**)*

Yield: 84 %. M.p. 213–214 °C. IR (KBr, cm^{-1}): 3034, 1682, 1586, 1497, 1357, 1255, 1100, 781, 737; ^{1}H-NMR ($CDCl_3$), δ: 2.82 (s, 3H, 3-CH_3), 5.72 (s, 1H, 4-OCH–), 6.62 (d, 1H, J = 8.0 Hz, 5-H), 7.11 (d, 1H, J = 8.2 Hz, 7-H), 7.27 (t, 1H, J = 8.2 Hz, 6-H), 7.36–7.67 (m, 5H, 4-OCHPh$\underline{H}$); ^{13}C-NMR, δ: 10.2, 93.6, 103.8, 111.2, 112.1, 125.8, 127.5, 127.6, 129.3, 129.4, 129.8, 129.9, 135.8, 140.4, 150.1, 161.3, 166.2, 178.5; MS (EI) *m/e*: 326 (M^+); Anal. $C_{18}H_{14}O_6{\cdot}0.2H_2O$. Calcd: C, 65.53; H, 4.37. Found: C, 65.77; H, 4.67.

*4-(3-Carboxy-1-phenylpropoxy)-3-methylbenzofuran-2-carboxylic acid (**32**)*

Yield: 79 %. M.p. 230–232 °C. IR (KBr, cm^{-1}): 3028, 2928, 1674, 1587, 1445, 1353, 1270, 1178, 1089, 738; ^{1}H-NMR ($CDCl_3$), δ: 2.21–2.43 (m, 2H, 4-OCHC$\underline{H}_2$–), 2.47–2.60 (m, 2H, –C$\underline{H}_2$CO_2H), 2.87 (s, 3H, 3-CH_3), 5.41 (t, 1H, J = 6.9 Hz, 4-OCH–), 6.45 (d, 1H, J = 7.9 Hz, 5-H), 7.01 (d, 1H, J = 8.1 Hz, 7-H), 7.12 (t, 1H, J = 8.1 Hz, 6-H), 7.24–7.40 (m, 5H, 4-OCHPh$\underline{H}$); ^{13}C-NMR, δ: 10.1, 30.1, 31.6, 84,5, 103.6, 111.3, 112.3, 125.7, 126.3, 127.3, 128.2, 128.3, 130.0, 130.1, 140.3, 141.8, 146.9, 161.2, 166.3, 178.3; MS (EI) *m/e*: 354 (M^+); Anal. $C_{20}H_{18}O_6$. Calcd: C, 67.80; H, 5.08. Found: C, 67.93; H, 5.07.

Biology

SD rats (200–220 g) were used through the studies. They were housed in room temperature of 25 ± 2 °C and humidity of 60 ± 5 %. Animals were free access to feed and water ad libitum during the experimental period. The animal experiments were carried out according to (the Committee for the purpose of Control and Supervision of Experimentation on Animals guideline) the Regulation of Experimental Animal Administration issued by State Committee of Science and Technology of PR China on 14 November, 1988 and Institutional Animal Ethics Committee approved all the procedures for the investigating experimental pain in conscious animals.

Inhibition of the ET-1 induced contraction of rat thoracic aortic ring

>Male rats were killed by decapitation and the thoracic aorta was quickly removed and placed in a Krebs–Henseleit buffer (120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 25 mM NaHCO3, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 11 mM glucose). The endothelium was removed by gentle rubbing of the intimal surface using a small cotton ball. The aorta was cut into rings of 2.5 mm in length and each ring was immersed in a 15 mL organ chamber filled with 37 °C Krebs–Henseleit buffer and constantly gassed with 95 % O_2 and 5 % CO_2. Vessel segments were attached to an isometric force transducer linked to a physiographic recorder for monitoring tension change. The ring was stretched by a resting force of 1 g and allowed to be equilibrated for 1 h. During this period, the ring was washed every 20 min with fresh 37 °C Krebs–Henseleit buffer. The tissues were contracted with phenylephrine (1 μM) followed by challenge with acetylcholine (1 μM). A negative relaxant response to acetylcholine confirmed the absence of endothelium. The rings were stimulated to contract with 60 mM KCl repeatedly until the contractile response to KCl became stable before starting the experiments. To examine the effect of test compound on ET-1-induced contraction, each individual ring was incubated with ET-1 (2 nM) for 20 min before exposure to test compound. Cumulative concentration–response curves to ET-1 were performed in the presence or absence of test compounds after a 30-min pretreatment period. Contractile responses were expressed as a percentage of the response (Li *et al.*, 2008).

Radio-receptor assay of endothelin receptors

Protein concentration of the pellet was determined by Lowry method and diluted to 1 mg/mL and reacted in a volume of 150 μL on 48 pore plate. [^{125}I] ET-1 was added to reach final concentration in 7 levels from 12.5–300 pmol/L. The nonspecific binding of the membrane preparation was determined in the presence of 1 μmol/L ET-1 with 0.2 mg/mL membrane protein and 50 μL [^{125}I] ET-1. The solution containing membrane protein was reacted for 1 h under incubation at 37 °C and rapid filtration proceeded in 12 pore cell collector though No. 49 glass fiber filter. The filters were rinsed with cold Tris–HCl for six times and calculated in automatic γ-calculator. The specific binding (SB) was obtained by total binding (TB) subtracting the nonspecific binding (NSB). The competitive receptor ligand binding test was conducted in triplicate. Scatchard analysis and Hill coefficient was calculated in assays.

Two kinds of radio-receptor assay were performed. A primary screening test of the target compounds were conducted to pick up the potent compound to inhibit the ET-1 binding sites on the rat myocardium. The IC_{50} of compounds was computed according to the linear equation individually. The second test of radio-receptor assay was done for purpose of quantitative evaluation for selectivity of effect on ET_A and ET_B in the presence of PD156707 and IC_{50} of each and the ratio of IC_{50} (ET_A)/IC_{50} ($ET_{B)}$ were calculated.

Hypoxic pulmonary arterial hypertension and treatment

Rats were divided into control, hypoxic PAH model (untreated), and hypoxic PAH, and orally medicated with **29** (40, 80, and 160 mg/kg) or nifedipine (10 mg/kg). Pulmonary arterial hypertension was induced by hypoxia as described (Pauvert *et al.*, 2004). In brief, the rats, except the control group, were housed in a hypoxic chamber for 8 h per day for 28 days. The oxygen percentage was monitored by a real-time oxygen detector and sustained at 10 ± 0.5 % by flowing N_2. Calcium chloride (30 g) and soda lime (20 g) were placed in the chamber to absorb CO_2 and moisture. Compound treatment started on the first day of hypoxic exposure and lasted for 28 days. All the chemicals were dissolved or suspended in 0.5 CMC (0.4 mL per rat) and the control group given vehicle only.

Hemodynamic measurements

On the 29th day, the rats were anesthetized with urethane (1.5 g/kg, i.p.) and subjected to surgical catheterization to measure hemodynamics. In brief, the jugular vein and carotid artery were exposed after a middle incision on the neck, from which polyethylene catheters connected with a pressure transducer (PE50, ID 0.58 mm, OD 0.965 mm) were inserted into the right jugular vein and then the vena cava and the right ventricle for measuring the CVP and RVSP, respectively. Signals were recorded by a real-time hemodynamic analyzer.

Conclusion

In conclusion, we have synthesized a series of novel benzofuran carboxylic acid derivatives and tested for their antagonism activity of ET-1 induced contraction in the rat thoracic aortic ring. Preliminary screening results showed that compounds **20**, **21**, **29**, and **30** demonstrated significant inhibition activities, especially **29** and **30** showed potent inhibition percentage higher than the contrast compound **BQ123**. Further affinity and selectivity for ET binding

assay showed that **29** demonstrated a dual ET_A/ET_B antagonism activity in nanomole level. Moreover, **29** was effective in relieving hypoxia-induced pulmonary arterial hypertension.

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