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Carbazolothiophene-2-carboxylic acid derivatives as endothelin receptor antagonists

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Abstract—The SmI_2-promoted three-component coupling reaction of thiophene-2-carboxylate, indole-2-carbaldehyde and acetophenone provides an expedient route to a series of tetracyclic carbazolothiophene compounds bearing the indole and thiophene rings. Among these samples, 9-benzyl-4-methyl-4-(4-hydroxyphenyl)-10-oxo-4,10-dihydrocarbazolo[2,3-*b*]thiophene-2-carboxylic acid (**18**) shows the most potent inhibition against the endothelin-1 induced increase of intracellular calcium ion concentration.

Human endothelin-1 (ET-1) is a 21 amino acid peptide that exhibits a potent vasoconstrictor activity, conceivably through its selective interaction with specific receptor subtypes (ET_A, ET_B and ET_C).[1] ET-1 contains six highly conserved amino acid residues (His^{16}-Trp^{21}) at the C-terminus, and this hydrophobic C-terminal hexapeptide alone shows some affinity for ET_A receptor. Several antagonists including BQ123 [cyclo(L-Leu-D-Val-L-Pro-D-Asp-D-Trp)][2] are designed on the basis of this peptide structures that incorporate indole moieties. Some non-peptide endothelin antagonists also consist of indole scaffolds such as the indole-2-carboxylic acids PD159433[3] and SB209598[4] (Fig. 1). On the other hand, the molecular modeling indicates that an indan derivative SB209670[5] possesses two phenyl substituents to mimic the amino acid residues of Try-13 and Phe-14 in ET-1. The two carboxylic groups in SB209670 also mimic the Asp-18 residue and the C-terminus of ET-1, which ligate Zn^{2+} ion on binding with endothelin receptor.[1] We speculated that a new class of carbazolothiophene derivatives (e.g., **7**–**22**) bearing appropriate substituents might serve as endothelin receptor antagonists. Indeed, 5-benzyloxy-3-isopropoxy-benzothiophene-2-carboxylic acid[3,6] has been utilized as a lead compound for development of endothelin antagonists. A thiophene-3-sulfonamide TBC11251[7] is also known as an ET_A-selective antagonist, in which the sulfonamide moiety is considered an isostere of carboxylic acid. We are thus interested in applying our established method of three-component coupling reactions of thiophene-2-carboxylate[8] to synthesize carbazolothiophene-2-

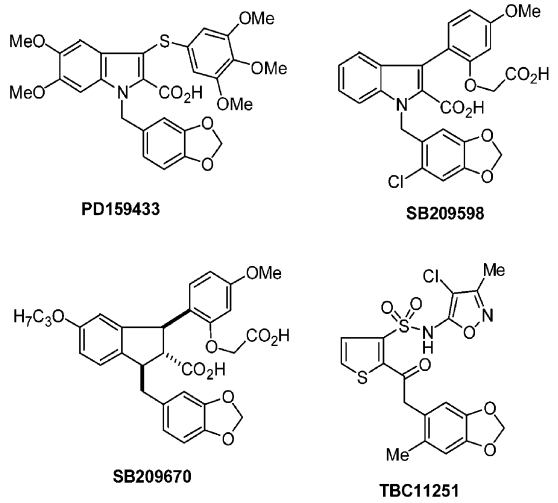

Figure 1. Some representative endothelin receptor antagonists constructed by the indole, indan and thiophene scaffolds.

Keywords: Coupling reactions; Endothelin; Samarium diiodide; Thiophene; Indole.
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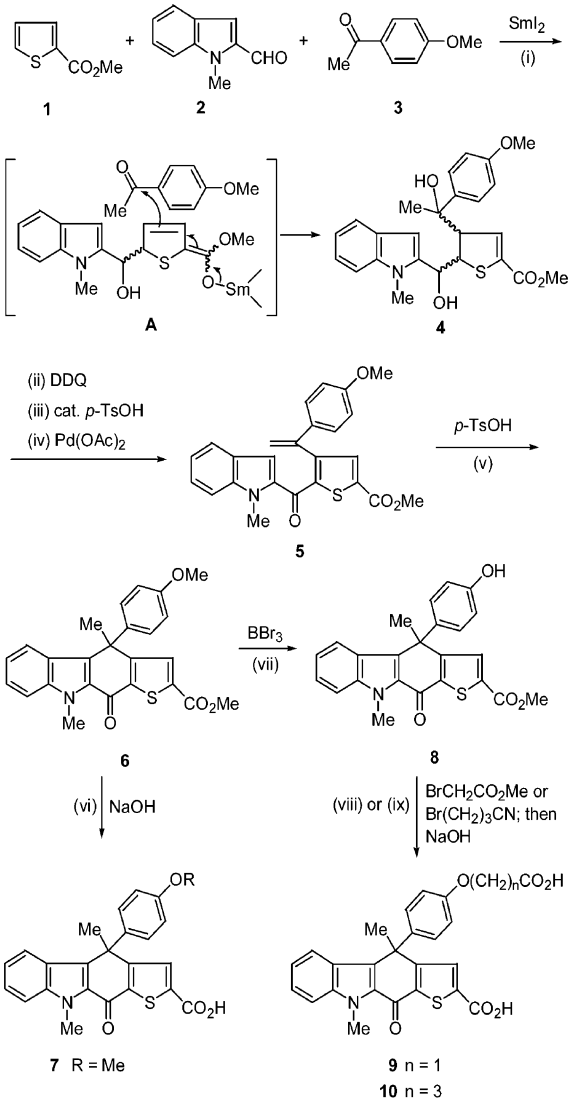
carboxylate derivatives **7**–**22**, and examined their antagonism against the binding of ET-1 with ET_A receptor.

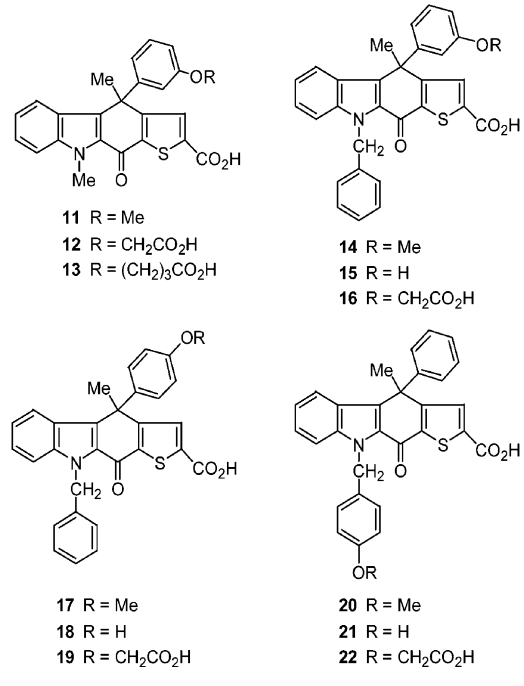
By the promotion of samarium diiodide, the three-component coupling reaction of methyl thiophene-2-carboxylate (**1**) with *N*-methylindole-2-carbaldehyde (**2**) and 4-methoxyacetophenone (**3**) occurred smoothly to afford a 77% yield of **4** (Scheme 1).[9,10] This one-pot operation presumably proceeded by an initial coupling of ester **1** with aldehyde **2** to give a dienolate intermediate **A**,[8,11] which was then trapped by ketone **3**. Although diol **4** existed as a mixture of diastereomers, the subsequent oxidation and dehydration would yield a single product. Conversion of **4** to **5** was achieved by a three-step sequence: an oxidation with DDQ to give the corresponding ketone, an acid-catalyzed dehydration to eliminate a water molecule, and an oxidative aromatization by using $Pd(OAc)_2$ to afford the thiophene product **5**. The subsequent intramolecular Friedel–Crafts alkylation thus furnished the tetracyclic skeleton, giving carbazolothiophene-2-carboxylate **6** as a pivotal compound for the synthesis of other derivatives **7**–**10**. Saponification of **6** afforded acid **7**, whereas demethylation of **6** with BBr_3 gave phenol **8**. Alkylation of phenol **8** with methyl 2-bromoacetate or 4-bromobutanenitrile, followed by hydrolysis in alkaline conditions, gave diacids **9** and **10** in high yields.



Scheme 1. Reagents and conditions: (i) **1** (1 mmol), **2** (1 mmol), SmI_2 (3.6 mmol), THF, HMPA, 0 °C to rt, 1.5 h; then **3** (1.2 mmol), 0 °C to rt, 10 h; 77% yield of **4**. (ii) DDQ, PhH, rt, 4 h; 88%. (iii) cat. *p*-TsOH, PhH, reflux, 10 h; 95%. (iv) $Pd(OAc)_2$ (5 equiv), K_2CO_3, CH_3CN, rt, 12 h; 93%. (v) *p*-TsOH, CH_2Cl_2, rt, 2 h; 98%. (vi) aq NaOH (0.5%), THF, 0 °C to rt, 2.5 h; 99%. (vii) BBr_3, CH_2Cl_2, −78 °C; 96%. (viii) $BrCH_2CO_2Me$, K_2CO_3, CH_3CN, 80 °C, 15 h; aq NaOH (0.5%), THF, 0 °C to rt, 2.5 h; 95% yield of **9**. (ix) $Br(CH_2)_3CN$, K_2CO_3, CH_3CN, 80 °C, 48 h; aq NaOH (40%), MeOH, reflux, 1 h; 84% yield of **10**.

A series of carbazolothiophenes **11**–**22** were similarly prepared, initially by the SmI_2-promoted three-component coupling reactions with appropriate partner substrates. For example, the SmI_2-promoted coupling product of thiophene ester **1**, indole aldehyde **2** and 3-methoxyacetophenone was further elaborated, according to the procedure similar to that shown in Scheme 1, to give compounds **11**–**13** bearing MeO, CH_2CO_2H or $(CH_2)_3CO_2H$ substituents on the *meta* positions of the phenyl rings as the '*meta*-analogues' of **7**, **9** and **10**. Compounds **14**–**19** with the (substituted)benzyl groups on the nitrogen atoms were also prepared in high yields by elaboration of the coupling products obtained from ester **1**, 1-benzylindole-2-carbaldehydes and appropriate ketones. Compounds **20**–**22** are analogues of **17**–**19** having the substituents switched over. It was noted that the benzyl protons in compounds **14**–**22** occurred at low fields (ca. δ 6.1) presumably due to the deshielding effect of the adjacent carbonyl group.

The interaction of ET-1 with endothelin receptor is known to trigger an increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) as the consequence of multistep biological events initiated by G-protein.[12] To evaluate the potency of an endothelin receptor antagonist, one can measure its inhibitory ability against the ET-1



induced $[Ca^{2+}]_i$ change. According to the reported experimental protocol,[13] Chinese hamster ovary (CHO-K1) cells were transfected with the rat ET_A-expression plasmid DNA using lipofectin reagent (Life Technologies Inc., USA). The ET_A overexpression CHO-K1 cells were prior incubated with calcium chelating agent fura-2 applied as its penta(acetoxymethyl) ester,[14] and then treated with ET-1 in 10^{-7} M. The $[Ca^{2+}]_i$ increase was monitored at 510-nm fluorescence emission by a ratiometric method using dual excitations at 340 and 380 nm wavelengths.[13] This increment of functional assay was taken as the standard value (100%) to assess the inhibitory potency of compounds **7**–**22** against the ET-1 binding with receptor. On addition of the test sample **7** (10^{-6} M) along with ET-1 (10^{-7} M), only $30\pm5\%$ increment of $[Ca^{2+}]_i$ was observed (a mean value of three measurements), equivalent to ~70% inhibition. By comparisons with the known ET_A antagonists, 10^{-6} M of SB209670 completely inhibited the ET-1 induced $[Ca^{2+}]_i$, whereas BQ123 showed ~60% inhibition under our assay conditions. Accordingly, compounds **9**, **10**, **12**, **13** and **18** showed high inhibition (>75%) at 10^{-6} M. Compounds **7**, **11**, **15** and **19** showed medium inhibition (50–70%), whereas compounds **14**, **16**, **17** and **20**–**22** showed low inhibition. Among our examined samples, compound **18** appeared to be the best ET-1 antagonist with IC_{50} ~10 nM. In comparison, SB209670 is an even more potent antagonist showing ~85% inhibition at 10 nM.

In summary, a series of tetracyclic compounds **7**–**22** bearing the indole and thiophene rings were prepared in an expedient fashion. The functional assay indicated that one of these samples (compound **18**) can serve as a lead compound for future exploration of potent endothelin receptor antagonists. The structure–activity relationship also awaits further investigation.

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9. Representative procedure for the SmI_2 promoted three-component coupling reactions: Under an atmosphere of argon, a deep blue SmI_2 solution (0.1 M) was prepared by treatment of Sm (661 mg, 4.4 mmol) with 1,2-diiodoethane (1.01 g, 3.6 mmol) in HMPA[10] (2.8 mL, 16 mmol) and anhydrous THF (32 mL) for 1.5 h at room temperature. To the SmI_2 solution (cooled in an ice bath) were added a THF solution (3 mL) of methyl thiophene-2-carboxylate (142 mg, 1 mmol) and *N*-methylindole-2-carbaldehyde (159 mg, 1 mmol). The reaction mixture was stirred at 0 °C for 45 min, and then at room temperature (27 °C) for 45 min. A THF solution (2 mL) of 4-methoxyacetophenone (180 mg, 1.2 mmol) was added at 0 °C, and the mixture was stirred at 0–27 °C for additional 10 h. The reaction was quenched by addition of saturated aqueous NH_4Cl solution (0.1 mL). The mixture was passed through a short silica gel column by rinse with EtOAc/hexane (1:1). The filtrate was concentrated, and chromatographed on a silica gel column by elution with EtOAc/hexane (3:7) to give the desired three-component coupling product **4** (349 mg, 77%) as a mixture of isomers as shown by the ^1H NMR analysis. Compounds **5**–**22** were fully characterized by spectroscopic methods (IR, MS, HRMS, ^1H and ^{13}C NMR) and elemental analyses. Some pertinent data are listed. **5**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.59 (d, 1H, $J=8.0$ Hz), 7.35 (t, 1H, $J=8.0$ Hz), 7.24 (d, 1H, $J=8.0$ Hz), 7.11 (t, 1H, $J=8.0$ Hz), 6.99 (s, 1H), 6.82 (d, 2H, $J=8.5$ Hz), 6.59 (d, 2H, $J=8.5$ Hz), 5.34 (s, 1H), 5.33 (s, 1H), 3.92 (s, 3H), 3.70 (s, 3H), 3.57 (s, 3H). **6**: mp 113–114 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.42 (m, 2H), 7.25 (m, 3H), 7.01 (m, 1H), 6.79 (dd, 2H, $J=6.8$, 2.0 Hz), 4.28 (s, 3H), 3.86 (s, 3H), 3.74 (s, 3H), 2.09 (s, 3H). **7**: mp >300 °C. **8**: mp 251–252 °C. **9**: mp 211–212 °C. **10**: mp 261–262 °C. **11**: mp 241–242 °C. **12**: mp 259–260 °C. **13**: mp 271–272 °C. **14**: mp 263–264 °C. **15**: mp 162–163 °C. **16**: mp 149–150 °C. **17**: mp 286–288 °C. **18**: mp 307–308 °C; ^1H NMR (400 MHz, CD_3COCD_3) δ 7.61 (s, 1H), 7.58 (dd, 1H, $J=8.4$, 1.2 Hz), 7.35–7.18 (m, 9H), 7.02 (dt, 1H, $J=7.6$,

0.8 Hz), 6.78 (td, 2H, $J = 8.8$, 3.2 Hz), 6.16 (d, 1H, $J = 16.0$ Hz), 6.11 (d, 1H, $J = 16.0$ Hz), 2.19 (s, 3H). **19**: mp 244–245 °C. **20**: mp 217–218 °C. **21**: mp 291–292 °C. **22**: mp 150–151 °C.

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