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# Dual-acting agents that possess free radical scavenging and antithrombotic activities: Design, synthesis, and evaluation of phenolic tetrahydro-β-carboline RGD peptide conjugates

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**Abstract**—A new approach to construct a single dual-acting agent is described. Compounds **6a**–**c** are potent free radical scavengers as demonstrated by the $EC_{50}$ values in PC12 cell survival assay in term of NO, $H_2O_2$, and ·OH scavenging activity. The Ach-induced vaso-relaxation assay further confirms the potent NO scavenging activity of compounds **6a**–**c**. In addition, **6a**–**c** are efficacious in a rat arterial thrombosis, and are active in ADP- or PAF-induced in vitro platelet aggregation assay, suggesting that compounds **6a**–**c** also possess anti-thrombotic activities. Since both free radical and thrombogenesis are important risk factors in myocardial ischemic/reperfusion injuries, these dual-acting agents having both free radical scavenging and antithrombolic activities may potentially be beneficial toward their treatment.


Cardiovascular disease is a major cause of disability and mortality in the developed countries of the world. Prolonged reduction in coronary blood flow due to atherosclerotic plaques or vasospasm can result in severe damage to the myocardium, thus leading to cellular injury and eventually cellular death due to apoptosis and/or necrosis. Re-instatement of blood flow, that is, reperfusion, is not without significant negative consequences and cellular damage. When oxygenated blood re-enters previously hypoxic cardiac tissues, it initiates a cascade of events that, paradoxically, results in further cellular and tissue damages. This tissue destruction is referred to as reperfusion injury. Reperfusion injury is hypothesized to be associated with the production of toxic free radical species after the re-supply of blood (or oxygen) to previously ischemic (or hypoxic) tissues.[1–3] Therefore, enhancing the organ antioxidant capacity may be viewed as a promising therapeutic strategy to attenuate I/R injury. It has been reported that a variety of free radical scavengers and antioxidants are capable of ameliorating ischemia-reperfusion injury.[4–10]

Tetrahydro-β-carbolines are naturally occurring substances found in food, alcoholic and nonalcoholic drinks, and fruit-derived products. It has been reported tetrahydro-β-carbolines are potent antioxidants and may thus be useful for the prevention of diseases associated with oxidative damage.[11–15] Furthermore, it is well recognized that phenolic compounds in foods possess several interesting biological and chemical properties, such as antioxidant activity and the ability to scavenge reactive oxygen species.[16,17] The phenolic compounds may hence prevent various diseases associated with oxidative stress, for example, cancers, cardiovascular diseases, and inflammation. In addition, phenolic tetrahydro-β-carbolines have been identified as effective free radical scavenger and antioxidants.[13]

Since acute myocardial ischemia is usually due to a thromboembolic event in the coronary arteries, causal treatment and secondary prevention always include one or more forms of anti-thrombotic therapies, such

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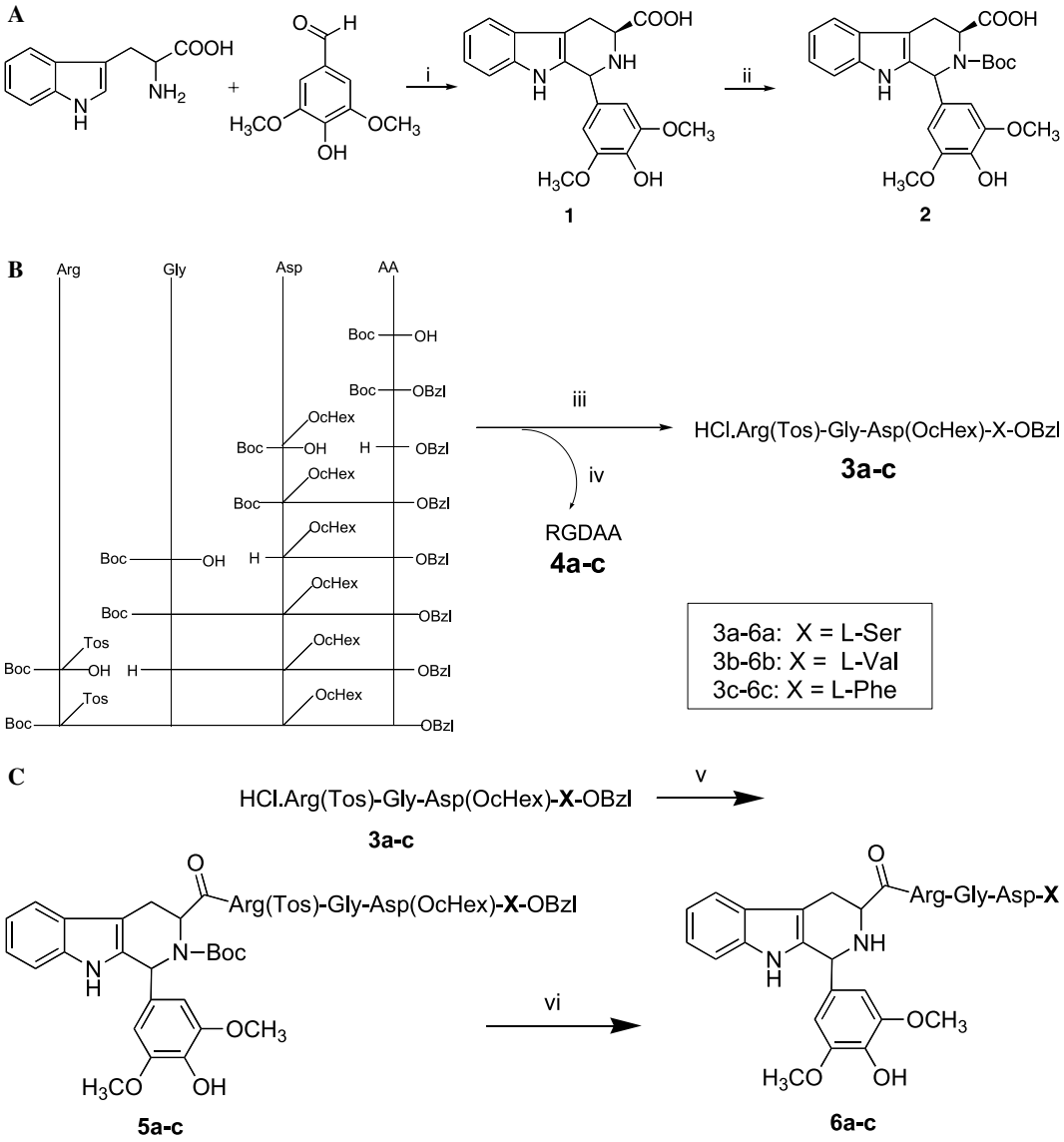
as anti-platelet agents, heparins, or fibrinolytic drugs. RGD (Arg-Gly-Asp) containing peptides have been investigated to develop therapeutic agents for treating thrombosis.[18–20] The mechanism of action of RGD peptides is inhibiting cell adhesion to extracellular matrix protein via binding of RGD peptide to the integrin receptors on the cell surface.[21] Several anti-thrombotic agents have been derived from RGD peptides.[18,22] For example, Aggrastat®, an RGD peptidomimetic, has been used clinically for treating patients with thrombosis.[23] In our previous studies, RGD-S, RGD-V, and RGD-F were used as the building block in the modification of the oligopeptides with anti-thrombotic and/or thrombolytic activity.[24–26] Considering the free radical scavenging properties possessed by phenolic tetrahydro-β-carboline and the anti-thrombic activity demonstrated by RGD peptides, we sought to link a phenolic tetrahydro-β-carboline moiety to the thrombolytic RGD peptides in hope that the resulting phenolic tetrahydro-β-carboline RGD peptide conjugates would exhibit both NO scavenging activity and thrombolytic activity.

For the synthesis of the phenolic tetrahydro-β-carboline RGD peptide conjugates, L-tryptophan was first reacted with phenolic aldehyde (Scheme 1A). Specifically, syringaldehyde in acidic-aqueous conditions affords **1** in 46% yield.[39] This reaction occurs through a Pictet–Spengler intramolecular cyclization of the Schiff base to afford the 1,3-disubstituted tetrahydro-β-carbolines. 1,3-Disubstituted tetrahydro-β-carbolines that arise from L-tryptophan and syringaldehyde provided two diastereoisomers (1*S*,3*S*-**1** and 1*R*,3*S*-**1**), which were then separated via RP-HPLC. Diastereoisomer assignments were based on acquired positive NOE spectra of H-1 and H-3 signals in the cis-isomer. H-1 signals corresponding to the cis-isomer appeared at higher field than in the trans-isomer, whereas C-1 and C-3 NMR signals corresponding to the trans-isomer appeared at higher field than those of the cis-isomer. This observation is




**Scheme 1.** Synthetic route for compounds **6a**–**c**. Reagents: (i) $H_2SO_4$; (ii) Boc-$N_3$; (iii) 4 N HCl/ethyl acetate; (iv) HF; (v) **2**, DCC; (vi) HF.

in agreement with the expected pattern for 1,3-disubstituted tetrahydro-β-carbolines. Subsequent treatment of **1** with Boc-$N_3$ gave the N-Boc protected **2** in 50% yield.

The protected tetrapeptide intermediates were prepared via the solution method according to the synthetic route shown in Scheme 1B.[40] The stepwise synthesis (C → N in 83–96% yield) was performed starting with benzyl ester of L-Ser (Bzl), L-Val, and L-Phe as the C-terminal residue, respectively. The tetrapeptide intermediates were then deprotected with HF to afford **4a–c** (**4a**, RGD-S, 63% yield; **4b**, RGD-V, 85% yield; **4c**, RGD-F, 83% yield). If the Boc groups of the protected tetrapeptide intermediates were removed, the corresponding N-terminal tetrapeptides **3a–c** were obtained in theoretical yield.

As shown in Scheme 1C, deprotected tetrapeptides **3a–c** were acylated with **2** to give **5a–c** in 50–56% yields. After removal of the protecting groups, the final desired products **6a–c** were obtained in ~60% yields.

The free radical scavenging activities of **6a–c** against NO, $H_2O_2$, and ·OH were evaluated in PC12 cells and compared with that of **1** (which has previously been identified as effective free radical scavenger and antioxidant[13]) using a published method.[27–29] The results are expressed as $EC_{50}$ (μM) values. As shown in Table 1, the $EC_{50}$ values of **6a–c** were found to be similar to that of **1**, suggesting that they were effective scavengers of NO, $H_2O_2$, and ·OH as **1**. The scavenging capacity ($EC_{50}$) among all of the tested compounds ranged from ~87 to 92 μM for NO, from ~30 to 67 μM for $H_2O_2$, and ~86 to 95 μM for ·OH.

Next, the NO scavenging abilities of **6a–c** were further evaluated in the acetylcholine (Ach)-evoked, endothelium-mediated relaxation assay (normal saline, NS, was used as a control).[29,30] The endothelium controls the tone of the underlying vascular smooth muscle through the production of vasodilator mediators.[31–33] In this experimental model, Ach acts on the endothelium to release nitric oxide (NO), a potent vasodilating mediator. The decreased relaxation response in rat arotic strip could be attributed to a reduction in NO (synthesized by the endothelium). Therefore, the present study was undertaken to assess the NO scavenging capability of these compounds. The results are expressed as a percentage inhibition of acetylcholine (Ach)-induced vaso-relaxation by test compounds and are summarized in Table 2. It was observed that Ach-induced relaxation was reversed significantly by compounds **1** and **6a–c**. At a concentration of $10^{-6}$ mol/L, compound **1** showed good inhibition ability with 79.9%, whereas compounds **6a–c** displayed higher inhibition activities at ~91–92%, respectively. We hypothesized that the NO scavenging activity of compounds **6a–c** led to attenuation of the NO concentration in vitro, which then led to the inhibition of Ach-induced vaso-relaxation.[34–36]

**Table 1.** The free radical scavenging activities in PC12 cell survival assay

| Compound | $EC_{50}$ (μM) values ($\overline{X} \pm SD$) | | |
|---|---|---|---|
| | $EC_{50}$/NO | $EC_{50}/H_2O_2$ | $EC_{50}$/OH |
| **1** | 89.67 ± 2.45 | 43.66 ± 3.21 | 86.77 ± 2.61 |
| **6a** | 87.61 ± 3.22 | 30.63 ± 2.41 | 95.43 ± 2.34 |
| **6b** | 90.32 ± 2.12 | 33.49 ± 1.89 | 92.32 ± 2.01 |
| **6c** | 92.76 ± 3.56 | 67.92 ± 2.13 | 90.43 ± 1.95 |

**Table 2.** Inhibition of Ach-induced vaso-relaxation

| Compound | Inhibition percentage ($\overline{X} \pm SD\%$) | | |
|---|---|---|---|
| | $10^{-6}$ mol/L | $10^{-7}$ mol/L | $10^{-8}$ mol/L |
| NS | | 1.63 ± 1.45 | |
| **1** | 79.93.1[a,b] | 57.22.4[a,b] | 10.91.6 |
| **6a** | 91.92.9[a,b] | 60.73.5[a,b] | 12.82.8 |
| **6b** | 92.21.4[a,b] | 71.21.9[a,b] | 13.32.0 |
| **6c** | 90.90.7[a,b] | 73.31.8[a,b] | 25.42.1[a] |

[a] In comparison with normal saline, NS, $p < 0.001$, $n = 6$.
[b] In comparison with $10^{-8}$ mol/L of the same compound, $p < 0.001$.

In vitro anti-platelet aggregation activities of compounds **1**, **4a–c**, and **6a–c** were again assayed using our previously reported method.[26,37,38] Briefly, platelet-rich plasma was prepared by centrifugation of normal rabbit blood anti-coagulation with sodium citrate at a final concentration of 3.8%. The platelet counts were adjusted to $2 \times 10^5$/μL through addition of autologous plasma. Platelet aggregation studies were conducted in an aggregometer using the standard turbidimetric technique. The agonists used were platelet activating factor (PAF, final concentration $10^{-7}$ mol/L) and adenosine diphosphate (ADP, final concentration $10^{-5}$ mol/L). The effects of compounds **1**, **4a–c**, and **6a–c** on PAF- or ADP-induced platelet aggregation were then evaluated. The maximal rate of platelet aggregation (Am%) was represented by the peak height of aggregation curve. The obtained data are listed in Tables 3 and 4, respectively. It was observed that compounds **6a–c** displayed a remarkable dual-anti-platelet aggregation activity in both of ADP- and PAF-induced platelet aggregation assay. For the ADP-induced platelet assay, with **6a–c** at a concentration of $10^{-6}$ mol/L, the platelet aggregation rates were collectively decreased to ~26–28%. (NS: 56.40 ± 5.23; **1**: 46.65 ± 4.60, $n = 8$, $p < 0.001$). Similar observation was observed in PAF-induced platelet

**Table 3.** Effects of **1**, **4a–c**, and **6a–c** on ADP-induced platelet aggregation

| Compound | Am% ($\overline{X} \pm SD$) | | |
|---|---|---|---|
| | $10^{-7}$ mol/L | $10^{-6}$ mol/L | $10^{-5}$ mol/L |
| NS | | 56.40 ± 5.23 | |
| **1** | 54.88 ± 2.43 | 46.65 ± 4.60[a] | 27.33 ± 2.02[a] |
| **4a** | 52.50 ± 2.43 | 39.62 ± 3.00[a] | 16.60 ± 2.61[a] |
| **4b** | 51.78 ± 3.20[b] | 29.61 ± 2.63[a] | 11.65 ± 1.84[a] |
| **4c** | 34.99 ± 2.57[a] | 22.71 ± 2.64[a] | 7.87 ± 1.76[a] |
| **6a** | 49.37 ± 3.22[b] | 28.31 ± 2.58[a,c] | 11.33 ± 1.71[a,c] |
| **6b** | 50.20 ± 3.13[b] | 29.60 ± 3.03[a,c] | 14.38 ± 1.50[a,c] |
| **6c** | 38.00 ± 3.11[a,c] | 29.43 ± 3.14[a,c] | 13.11 ± 2.51[a,c] |

$N = 8$; NS, vehicle.
[a] Compared to NS, $p < 0.001$.
[b] Compared to NS, $p < 0.01$.
[c] Compared to **1**, $p < 0.001$.

**Table 4.** Effects of **1**, **4a–c**, and **6a–c** on PAF-induced platelet aggregation

| Compound | Am% ($\overline{X} \pm$ SD) | | |
|---|---|---|---|
| | $10^{-7}$ mol/L | $10^{-6}$ mol/L | $10^{-5}$ mol/L |
| NS | | 58.70 ± 4.11 | |
| **1** | 55.80 ± 3.21 | 51.42 ± 4.01 | 36.34 ± 3.89[a] |
| **4a** | 56.69 ± 3.10 | 36.47 ± 3.00[a,d] | 22.03 ± 2.31[a] |
| **4b** | 55.07 ± 3.27 | 34.84 ± 2.51[a,d] | 20.01 ± 2.12[a] |
| **4c** | 40.39 ± 2.74[a] | 24.02 ± 2.20[a,d] | 7.73 ± 2.20[a] |
| **6a** | 55.62 ± 3.20 | 34.59 ± 2.19[a,d] | 28.61 ± 2.14[a,c] |
| **6b** | 54.06 ± 3.22[b] | 35.80 ± 2.70[a,d] | 25.88 ± 2.21[a,c] |
| **6c** | 36.59 ± 3.11[a,c] | 25.33 ± 2.22[a,d] | 21.52 ± 2.19[a, c] |

$N = 8$; NS, vehicle.
[a] Compared to NS, $P < 0.001$.
[b] Compared to NS, $p < 0.01$.
[c] Compared to **1**, $p < 0.001$.

aggregation assay. Thus, the in vitro assay indicated that the new class of phenolic tetrahydro-β-carboline RGD peptides conjugates was very likely to be potent platelet aggregation inhibitors.

The anti-thrombotic effects of compounds **6a–c** were evaluated again using our previously reported in vivo rat model method,[26,37,38] and were expressed as the reduction of thrombus mass. The obtained results are summarized in Table 5. The thrombus weight after the treatment with **6a–c** was 5.00 ± 1.04, 4.85 ± 1.20, and 2.68 ± 0.45 mg, respectively. (NS: 9.05 ± 1.01, $p < 0.01$). Thus, the anti-thrombotic activities of **6a–c** were in fact higher than that of RGD-S, RGD-V, and RGD-F at a dosage of 5 μmol/kg.

In summary, compounds **6a–c** were observed to demonstrate good free radical scavenging activity as that of parental compound **1** as demonstrated by the $EC_{50}$ values in PC12 cell survival assay. The Ach-induced vaso-relaxation test further confirmed the potent NO scavenging activity of compounds **6a–c**. In addition, **6a–c** were efficacious in a rat arterial thrombosis, and were active in ADP- or PAF-induced in vitro platelet aggregation assay, which suggests that compounds **6a–c** also possess anti-thrombotic activity. Taken together, a novel class of potential dual-action agents which possess both anti-thrombotic and free radical scavenging capabilities is described. Such composite dual-acting agent should constitute a promising approach in the development for new protective therapy against ischemia-reperfusion related injuries.

**Table 5.** Effects of the compounds **1**, **4a–c**, and **6a–c** on the thrombus weight ($\overline{X} \pm$ SD)

| Compound | Dosage | Wet thrombus (mg) | Dry thrombus (mg) |
|---|---|---|---|
| NS | 3 ml/kg | 45.89 ± 3.60 | 9.05 ± 1.01 |
| **1** | 5 μmol/kg | 43.76 ± 3.18 | 7.27 ± 0.79[a] |
| **4a** | 5 μmol/kg | 42.90 ± 3.18 | 7.62 ± 1.10 |
| **4b** | 5 μmol/kg | 35.60 ± 3.20[b] | 6.43 ± 1.06[b] |
| **4c** | 5 μmol/kg | 26.68 ± 3.15[a] | 4.05 ± 0.98[a] |
| **6a** | 5 μmol/kg | 28.01 ± 4.00[a,c] | 5.00 ± 1.04[b,d] |
| **6b** | 5 μmol/kg | 29.05 ± 2.94[b,e] | 4.85 ± 1.20[b,e] |
| **6c** | 5 μmol/kg | 22.09 ± 3.12[a,f] | 2.68 ± 0.45[a,g] |

$N = 8$.
[a] In comparison with normal saline (NS), $P < 0.001$.
[b] In comparison with NS, $P < 0.01$.
[c] In comparison with **4a**, $p < 0.01$.
[d] In comparison with **4a**, $p < 0.05$.
[e] In comparison with **4b**, $p < 0.05$.
[f] In comparison with **4c**, $p < 0.001$.
[g] In comparison with **4c**, $p < 0.01$.

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## References and notes

1. Cannon, R. O. *Nat. Clin. Pract. Cardiovasc. Med.* **2005**, *2*, 88.
2. Neuzil, J.; Rayner, B. S.; Lowe, H. C.; Witting, P. K. *Redox Rep.* **2005**, *10*, 187.
3. Saeed, S. A.; Waqar, M. A.; Zubairi, A. J.; Bhurgri, H.; Khan, A.; Gowani, S. A.; Waqar, S. N.; Choudary, M. I.; Jalil, S.; Zaidi, A. H.; Ara, I. *J. Coll. Physician Surg. Pak.* **2005**, *15*, 507.
4. Parlakpinar, H.; Sahna, E.; Acet, A.; Mizrak, B.; Polat, A. *Toxicology* **2005**, *209*, 1.
5. Parlakpinar, H.; Ozer, M. K.; Acet, A. *Mol. Cell. Biochem.* **2005**, *277*, 137.
6. Esrefoglu, M.; Gul, M.; Parlakpinar, H.; Acet, A. *Fundam. Clin. Pharmacol.* **2005**, *19*, 365.
7. Jin, Z. Q.; Zhou, H. Z.; Cecchini, G.; Gray, M. O.; Karliner, J. S. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *288*, 2986.
8. Sahna, E.; Acet, A.; Ozer, M. K.; Olmez, E. *J. Pineal. Res.* **2002**, *33*, 234.
9. Lee, Y. M.; Chen, H. R.; Sheu, J. R.; Wang, J. J.; Yen, M. H. *J. Pineal. Res.* **2002**, *33*, 72.
10. Sahna, E.; Olmez, E.; Acete, A. *J. Pineal. Res.* **2002**, *32*, 194.
11. Herraiz, T.; Galisteo, J. *J. Agric. Food Chem.* **2003**, *51*, 7156.
12. Ichikawa, M.; Ryu, K.; Yoshida, J.; Ide, N.; Yoshida, S.; Sasaoka, T.; Sumi, S. *Biofactors* **2002**, *16*, 57.
13. Herraiz, T.; Galisteo, J.; Chamorro, C. *J. Agric. Food Chem.* **2003**, *51*, 2168.
14. Herraiz, T.; Galisteo, J. *Free Radic. Res.* **2002**, *36*, 923.
15. Pless, G.; Frederiksen, T. J.; Garcia, J. J.; Reiter, R. J. *J. Pineal Res.* **1999**, *26*, 236.
16. Hsu, L. Y.; Lin, C. F.; Hsu, W. C.; Hsu, W. L.; Chang, T. C. *Biol. Pharm. Bull.* **2005**, *28*, 1211.
17. Yilmaz, Y.; Toledo, R. T. *Trends Food Sci. Technol.* **2004**, *15*, 422.
18. Nicholson, N. S.; Abood, N. A.; Panzer-Knodle, S. G.; Frederick, L. G.; Page, J. D.; Salyers, A. K.; Suleymanov, O. D.; Szalony, J. A.; Taite, B. B.; Anders, R. J. *Med. Res. Rev.* **2001**, *21*, 211.

19. Scarborough, R. M.; Gretler, D. D. *J. Med. Chem.* **2000**, *43*, 3453.
20. Zablocki, J. A.; Rico, J. G.; Garland, R. B.; Rogers, T. E.; Williams, K.; Schretzman, L. A.; Rao, S. A.; Bovy, P. R.; Tjoeng, F. S.; Lindmark, R. J., et al. *J. Med. Chem.* **1995**, *38*, 2378.
21. Ruoslahti, E. Integrins as Receptors for Extracellular Matrix. In *Cell Biology of Extracellular Matrix*; Hay, E. D., Ed.; Plenum Press: New York, NY, 1991.
22. Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Pierschbacher, M. D. *J. Med. Chem.* **1994**, *37*, 1.
23. Batchelor, W. B.; Tolleson, T. R.; Huang, Y.; Larsen, R. L.; Mantell, R. M.; Dillard, P.; Davidian, M.; Zhang, D.; Cantor, W. J.; Sketch, M. H., Jr.; Ohman, E. M.; Zidar, J. P.; Gretler, D.; DiBattiste, P. M.; Tcheng, J. E.; Califf, R. M.; Harrington, R. A. *Circulation* **2002**, *106*, 1470.
24. Zhao, M.; Peng, S. *J. Prakt. Chem.* **1999**, *341*, 668.
25. Zhao, M.; Wang, C.; Yang, J.; Peng, S. *Prep. Biochem. Biotechnol.* **2000**, *30*, 247.
26. Lin, N.; Zhao, M.; Wang, C.; Peng, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 585.
27. Desole, M. S.; Sciola, L.; Sircana, S.; Godani, C.; Migheli, R.; Delogu, M. R.; Piras, G.; De, N. G.; Miele, E. *Neurosci. Lett.* **1998**, *247*, 1.
28. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
29. Wu, Y.; Bi, L.; Bi, W.; Zhao, M.; Li, Z.; Ju, J.; Peng, S. *Bioorg. Med. Chem.*, in press.
30. Cowart, M.; Kowaluk, E. A.; Daanen, J. F.; Kohlhaas, K. L.; Alexander, K. M.; Wagenaar, F. L.; Kerwin, J. F. *J. Med. Chem.* **1998**, *41*, 2636.
31. Komori, K.; Vanhoutte, P. M. *Blood Vessels* **1990**, *27*, 238–245.
32. Moncada, S.; Palmer, R. M. J.; Higgs, E. A. *Pharmacol. Rev.* **1991**, *43*, 109.
33. Calles-Escandon, J.; Cippola, M. *Endocr. Rev.* **2001**, *22*, 36.
34. Chalupsky, K.; Lobysheva, I.; Nepveu, F.; Gadea, I.; Beranova, P.; Entlicher, G.; Stoclet, J.; Muller, B. *Biochem. Pharmacol.* **2004**, *67*, 1203.
35. Beranova, P.; chalupsky, K.; Kleschyov, A.; Schott, C.; Boucher, J.; Mansuy, D.; Munzel, T.; Muller, B.; Stoclet, J. *Eur. J. Pharmacol.* **2005**, *516*, 260.
36. Nakae, I.; Matsumoto, T.; Omura, T.; Takashima, H.; Ohira, N.; Tarutani, Y.; Yasuda, Y.; Matsuo, S.; Koh, T.; Nakamura, Y.; Kinoshitaa, M.; Horie, M. *Life Sci.* **2003**, *73*, 3083.
37. Zhao, M.; Bi, L.; Bi, W.; Wang, C.; Yang, Z.; Ju, J.; Peng, S. *Bioorg. Med. Chem.* **2006**, *14*, 4761.
38. Liu, J.; Zhao, M.; Wang, C.; Peng, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4065.
39. Synthesis of (1*S*,3*S*)- and (1*R*,3*S*)-1-(4′-hydroxy-3′,5′-dimethoxyphenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*cis*-**1** and *trans*-**1**): L-tryptophan (306 mg, 1.5 mmol) dissolved in 0.05 N $H_2SO_4$ (13 mL) was stirred with the syringaldehyde (1.65 mmol) (Aldrich). The reaction mixture was allowed to react at 70 °C for 6 days, and a precipitate of **1** was obtained (1.1:1) (254 mg, 46%). *cis*-**1**: $^1$H NMR ($CD_3OD$ + TFA) 3.77 (m, 2H, H-4), 3.97–4.02 [2s, 6H, $(OCH_3)_2$], 4.80 (dd, 1H, H-3, $J_{3,4a}$ = 12.1 Hz, $J_{3,4b}$ = 5.3 Hz), 6.01 (s, 1H, H-1), 6.85, 6.98 (2s, 2H, Ph), 7.2–7.65 (m, 4H, indol); $^{13}$C NMR ($CD_3OD$ + TFA) 23.73 (C-4), 56.91 ($OCH_3$), 57.69 (C-3), 60.58 (C-1), 108.30 (C-4a), 108.47 (Ph), 112.56 (C-8), 119.25 (C-5), 120.84 (C-6), 124.04 (C-7), 124.45 (Ph), 127.16 (C-4b), 129.43 (C-9a), 138.78 (C-8a), 149.73, 158.84, 160.24 (Ph), 171.06 (COOH); RP-HPLC-ESI-MS ($t_R$ = 15.95 min) $(M+H)^+$ 369, (M+H−73) 296. *trans*-**1**: $^1$H NMR ($CD_3OD$ + TFA) 4.72 (m, 1H, H-3), 6.22 (s, 1H, H-1); $^{13}$C NMR ($CD_3OD$ + TFA) 53.60 (C-3), 57.37 (C-1); other signals as *cis*-**1**: RP-HPLC-ESI-MS ($t_R$ = 16.8 min) $(M+H)^+$ 369, (M+H−73) 296.
40. Boc-Arg(Tos)-Gly-Asp(OcHex)-Ser(Bzl)-OBzl, mp 73–75 °C, $[\alpha]_D^{20}$ −34 (*c* 0.4, $CHCl_3$), FAB-MS (*m*/*e*): 950 $[M+H]^+$; BocArg-(Tos)-Gly-Asp(OcHex)-Val-OBzl, mp 79–81 °C, $[\alpha]_D^{20}$ −24 (*c* 0.4, $CHCl_3$), FAB-MS (*m*/*e*): 872 $[M+H]^+$; Boc-Arg(Tos)-Gly-Asp(OcHex)-Phe-OBzl, mp 165–170 °C, $[\alpha]_D^{20}$ −25 (*c* 0.4, $CHCl_3$), FAB-MS (*m*/*e*): 920 $[M+H]^+$; RGD-S, mp 110–111 °C, $[\alpha]_D^{20}$ −20 (*c* 0.2, 6 N HCl), FAB-MS (*m*/*e*): 434 $[M+H]^+$; RGD-V, mp 120–121 °C, $[\alpha]_D^{20}$ −20 (*c* 0.3, 6 N HCl), FAB-MS (*m*/*e*): 446 $[M+H]^+$; RGD-F, mp 125–126 °C, $[\alpha]_D^{20}$ −40 (*c* 0.2, 6 N HCl), FAB-MS (*m*/*e*): 494 $[M+H]^+$; compound **6a**: $[\alpha]_D^{20}$ 38 (*c* 1, $H_2O$), FAB-MS (*m*/*e*): 784 $[M+H]^+$; compound **6b**: $[\alpha]_D^{20}$ 18 (*c* 1, $H_2O$), FAB-MS (*m*/*e*): 796 $[M+H]^+$; compound **6c**: $[\alpha]_D^{20}$ 22 (*c* 1, $H_2O$), FAB-MS (*m*/*e*): 844 $[M+H]^+$.