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Novel coumarin glycoside and phenethyl vanillate from *Notopterygium forbesii* and their binding affinities for opioid and dopamine receptors

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Abstract—Bioactivity-guided fractionation of *Notopterygium forbesii* has resulted in the isolation of one new coumarin glycoside and one new phenethyl vanillate, together with seventeen known compounds. The structures of these compounds were characterized by spectroscopic methods. These compounds were evaluated for their binding affinities to the opioid and dopamine receptors, and falcarindiol showed weak binding affinities to opioid receptors and moderate affinity for D1 receptor ($K_i = 192 \pm 6$ nM).

1. Introduction

Alcohol and drug abuse pose serious medical, social, and economic problems in the United States and around the world.[1] A great deal of effort has been directed toward developing effective therapies, however, the complexity of drug dependence and the lack of effective remediation, especially for relapse, which is often precipitated by withdrawal and/or intense craving even after prolonged abstinence, pose a serious therapeutic challenge. In a consortium effort focusing on a systematic evaluation of Traditional Chinese Medicine for rescuing opium smoker has resulted in the isolation and identification of certain natural products with moderate to significant binding activities toward opioid receptors. *Notopterygium forbesii* is one of the five Chinese herbs in a formula (we call it as 'NPI-025') used clinically to treat heroin addiction in Hong Kong.[2,3] Heroin acts on opioid receptors to produce its pharmacological effects including mood changes, pain modulation, and addiction. Opioid receptors play important roles in many physiological functions.[4] The mu (μ) opioid receptor (MOR), delta (δ) opioid receptor (DOR), and kappa (κ) opioid receptor (KOR) are three main types of opioid receptors.[4] These receptors are differentiated by distinct genes and proteins, tissue expression patterns, functional properties, and pharmacological profiles of selective agonists and antagonists.[5] They are distributed throughout the central and peripheral nervous system and are involved in a variety of physiological processes, especially analgesia.[6,7]

Mesolimbic dopaminergic system has been shown to play a central role in reward system, which is usurped by drugs of abuse in drug addiction. Dopamine (DA) receptors belong to a family of large proteins characterized by having seven relatively hydrophobic segments that are assumed to be cell-membrane spanning.[8] There are five major DA receptor subtypes (D1–D5) with distinct differences in their gene and peptide composition, molecular functions, and neuropharmacology.[8] The D1 receptor is the most abundant DA receptor subtype in mammalian forebrain.[8] Compounds targeted to these DA receptor membrane proteins can activate or inhibit their biological functions as well as provide a rational treatment for drug abuse.[8]

Keywords: *Notopterygium forbesii*; Coumarin; Falcarindiol; Opioid receptor; Dopamine receptor.
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As part of our ongoing investigation of alternative therapies for substance addiction,[9] we initiated a comprehensive fractionation study guided by opioid receptor binding activity in an attempt to find new opioid receptor agonists or antagonists from *N. forbesii* Boiss (Apiaceae). The roots and rhizomes of *N. forbessi* have been used as a diaphoretic, an antifebrile, and an anodyne, and are officially listed in the Chinese Pharmacopoeia.[10–12] The major active constituents of this herb are coumarins, volatile oil, and organic acid.[12] Pharmacological studies demonstrated that coumarins including isoimperatorin, notopterol, and bergapten possess anti-inflammatory, analgesic, anti-cancer, and anti-coagulant activities.[13] Herein we report the isolation and characterization of a new coumarin glycoside (**4**) and a new phenethyl vanillate (**11**) together with 17 known compounds (**1**–**3**, **5**–**10**, **12**–**18**, and sucrose), and their opioid and dopamine receptor binding activities.

2. Results and discussion

The dried roots and rhizomes (400 g) of *N. forbesii* were sequentially extracted with hexane, ethyl acetate, acetone, methanol, and water. In our initial biological study as shown in Table 1, the hexane and acetone extracts showed potent binding activity to μ, δ, and κ opioid receptors and the ethyl acetate extract showed moderate binding activity to δ and κ opioid receptors, while the methanol extract only had moderate binding activity to δ opioid receptor.

The hexane, ethyl acetate, acetone, and methanol extracts were chromatographed on silica gel columns to give a new coumarin glycoside (**4**) and a new phenethyl vanillate (**11**), together with 17 known compounds, diversoside (**1**),[14] anisocoumarin H (**2**),[15] 7-[(*E*)-7′-hydroxy-3′,7′-dimethylocta-2′,5′-dienyloxy]-coumarin (**3**),[16]

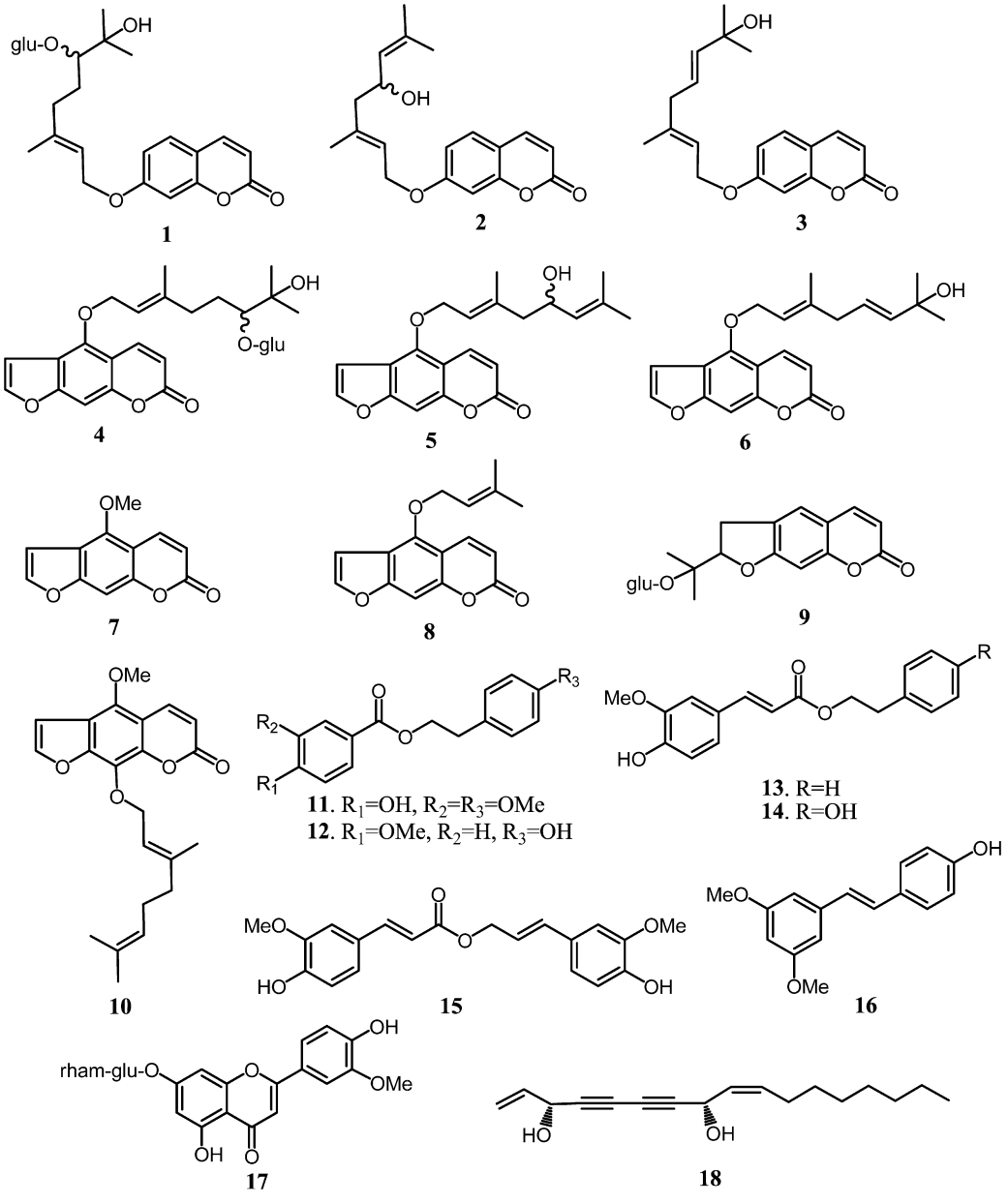


Table 1. Binding activities of crude extracts for opioid receptors

Sample ID	Concentration (mg/ml)	[^{3}H]DIP rMOR (% of control)	[^{3}H]DIP mDOR (% of control)	[^{3}H]DIP hKOR (% of control)
Hexane extract	50	−5 ± 1	−1.8 ± 0.2	−0.9 ± 1.1
AcOEt extract	50	56 ± 35	34 ± 18	39 ± 15
Acetone extract	50	10 ± 10	5 ± 4	15 ± 12
MeOH extract	50	61 ± 2	27 ± 3	53 ± 4
Water extract	50	NA	NA	NA

NA, no activity.

notopterol (**5**),[17] notoptol (**6**),[18] bergapten (**7**),[19] isoimperatorin (**8**),[20] nodakenin (**9**),[21] 8-geranyl-5-methoxypsoralen (**10**),[22] *p*-hydroxyphenethyl anisate (**12**),[23] phenethyl *trans*-ferulate (**13**),[24] *p*-hydroxyphenethyl *trans*-ferulate (**14**),[25] coniferyl ferulate (**15**),[26] pterostilbene (**16**),[27] chrysoeriol 7-rutinoside (**17**),[28] falcarindiol (**18**),[29] and sucrose. The known compounds were identified by comparison with their published data or standard compounds.

Compound **4** was isolated as white solid with $[\alpha]_D^{23}$ −3.3° (*c* 0.12, MeOH). Its molecular formula was determined to be $C_{27}H_{34}O_{11}$ by HR ESI-MS. The ESI mass spectrum of **4** showed adduct molecular ion peaks at *m*/*z* 552 $[M+NH_4]^+$ and 535 $[M+H]^+$, and a fragment at *m*/*z* 373 $[M-162+H]^+$ representing $[aglycone+H]^+$. The ^{1}H NMR spectrum revealed the presence of furancoumarin, *O*- geranyl, and monosaccharide moieties. The ^{1}H NMR data of furancoumarin system were closely similar to those of **5**, **6**, **7**, and **8**, which included two doublets at δ 6.30 and 8.27 (J = 9.6 Hz) attributed to the pyran ring protons H-3 and H-4, two other doublets at δ 7.19 and 7.80 (J = 2.4 Hz) corresponding to the furan ring protons H-10 and H-9, and one olefinic proton at δ 7.20 (s) for H-8. The ^{1}H and ^{13}C NMR data of *O*-geranyl and monosaccharide moieties were almost super-imposable on those of **1**. Assignments of the ^{1}H and ^{13}C signals of **4** by 2D-NMR spectra showed that **4** was an analogue of 6′,7′-dihydroxybergamottin,[30] in which the glucose unit was attached at C-6′. This was further evidenced by ^{13}C–^{1}H long-range correlation in the HMBC spectrum (Fig. 1), in which the correlations [H-1″ (δ 4.27)/C-6′ (δ 89.5) and H-6′ (δ 3.39)/C-1″ (δ 105.4)] were clearly established. In addition, the HMBC correlation between H-1′ (δ 5.06) and C-5 (δ 150.3) confirmed that the *O*-geranyl moiety was linked to C-5. On the basis of these data, the structure of **4** was elucidated as 6′-*O*-β-D-glucosyl-7′-hydroxybergamottin.

Compound **11** was isolated as a colorless resin. Its molecular formula was established as $C_{17}H_{18}O_5$ by HR ESI-MS. The ^{1}H NMR spectrum displayed the signals due to a set of ABX type aromatic protons at δ 6.93 (1H, d, J = 8.1 Hz), 7.51 (1H, d, J = 2.1 Hz), and 7.61 (1H, dd, J = 2.1 and 8.1 Hz), a set of AB type aromatic protons at δ 6.86 and 7.20 (each 2H, d, J = 8.7 Hz), two methoxyl protons at δ 3.79 and 3.92 (each 3H, s), and aliphatic protons due to –CH_2CH_2O– moiety at δ 3.00 and 4.46 (each 2H, J = 7.2 Hz). The ^{13}C NMR spectral data of **11** were analyzed and ^{13}C–^{1}H connectivities in the molecule were confirmed with aid of the HMQC and HMBC spectra (Fig. 1). The above NMR data

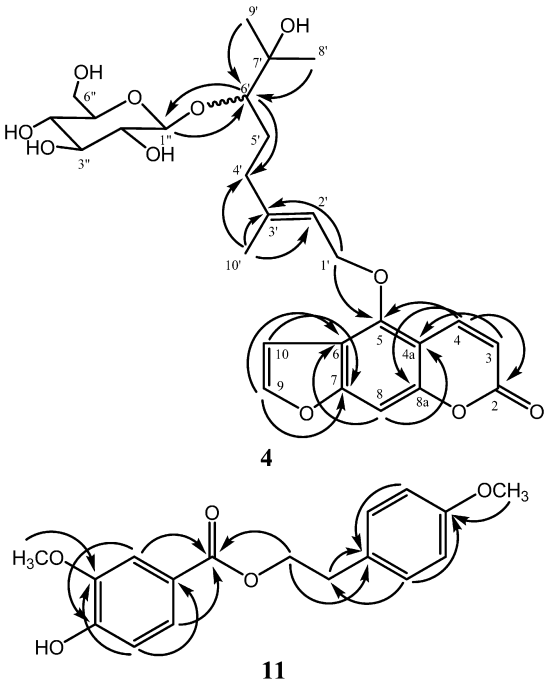
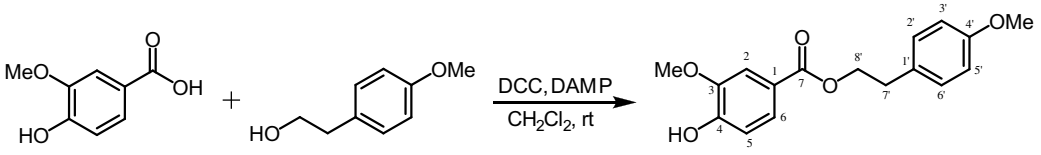


Figure 1. Selected HMBC correlations of **4** and **11**.

revealed the presence of 4-hydroxy-3-methoxybenzoic acid (vanillic acid) and 4-methoxyphenethyl alcohol moieties in the molecule. The structure of **11** was thus determined as 4′-methoxyphenethyl vanillate. The proposed structure was further confirmed by synthesis of **11** from vanillic acid and 4-methoxyphenethyl alcohol as shown in Scheme 1.

The affinities of the isolated compounds for the KOR, MOR, and DOR were determined by competitive inhibition of [^{3}H]diprenorphine binding to the human KOR (hKOR), the rat MOR (rMOR), and the mouse DOR (mDOR), stably expressed in Chinese hamster ovary (CHO) cells as reported previously.[31] All compounds were initially screened at 10 μM. Only compounds anisocoumarin H (**2**) and falcarindiol (**18**) at 10 μM inhibited [^{3}H]diprenorphine binding to the opioid receptors by more than 50%. Binding experiments were then carried out with a range of concentrations of the two compounds and the K_i value, a measure of binding affinity, of each compound for the rMOR, mDOR, and hKOR was determined (Table 2). Compound **18** showed weak affinities to rMOR (K_i = 1125 nM), mDOR (K_i = 1096 nM), and hKOR (K_i = 2515 nM), while **2** exhibited weaker affinity for



Scheme 1. Synthesis of **11**.

Table 2. K_i values of the isolated compounds for opioid and dopamine receptors

Compound	K_i (nM) for [^{3}H]DIP rMOR	K_i (nM) for [^{3}H]DIP mDOR	K_i (nM) for [^{3}H]DIP hKOR	K_i (nM) for [^{3}H]SCH rD1R
2	>10,000	>10,000	2600 ± 450	>10,000
5	>10,000	>10,000	>10,000	1422 ± 68
18	1125 ± 51	1096 ± 35	2515 ± 54	192 ± 6

the hKOR (K_i = 2600 nM). Furthermore, the isolated compounds (**1**–**18**) were screened for inhibition of binding to rat DA 1 receptors. Compounds **5** and**18** inhibited [^{3}H]SCH binding to rat D1 receptors by more than 50% at a concentration of 10 μM. The binding affinities of **5** and **18** were also studied. Compound **5** exhibited low affinity (K_i = 1422 nM), while **18** showed moderate affinity to D1 receptor (K_i = 192 nM).

Falcarindiol (**18**) was isolated with a yield of 1.3% (5.2 g from 400 g raw material), and it should be the principal constituent for the binding activities to μ, δ, and κ opioid receptors and D1 receptor. Compound **18** belongs to aliphatic C17-polyacetylenes, and is widely distributed in the Apiaceae and Araliaceae,[32,33] and exhibited significant cytotoxic, antimicrobial, antibacterial, antifungal, antimutagenic, antiproliferative, and hepatoprotective activities.[34–40] Recently, Kim et al. reported that **18** dose-dependently inhibited the iNOS-mediated nitric oxide (NO) production in LPS-activated BV-2 and microglia, and inhibited the NO-mediated neuronal death by reducing excessive NO production in the LPS-treated organotypic hippocampal cultures.[41] Kim's results suggest that **18** is a useful inhibitor for NO-mediated neuronal death.[41] In addition, **18** can elevate the neurotransmitter GABA levels in the central nervous system by an inhibitory action on the GABA degradative enzyme GABA transaminase.[42] Furthermore, it has been reported compounds **14** and **18** exhibited inhibitory effects on [^{3}H]LSD binding to the serotonin (5-HT_7) receptors.[43]

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with an AUTOPOL III digital polarimeter. NMR spectra were recorded on a Varian VXR300 spectrometer with TMS as the internal standard. EI-MS spectra were obtained on a HP5972 Series Mass spectrometer. ESI-TOFMS spectra were measured on a Micromass Plateform II mass spectrometer. HPLC was carried out on a Varian Prostar 210 Solvent Delivery Module (California, USA) equipped with a Varian ProStar 320 UV/Vis Detector at a wavelength of 254 nm. Silica Gel (Fisher Scientific, USA) was used for column chromatography. TLC was performed on precoated silica gel 60 F254 plates (Merck, Germany).

3.2. Plant material

The dried roots and rhizomes of *N. forbesii* were purchased in April, 2006 from downtown of Boston, MA. Its botanical identification was confirmed by Dr. S.L. Chen at IMPLAD in China. A voucher specimen (MCL-2006-01) has been deposited in the Bio-Organic and Natural Products Laboratory, McLean Hospital, Belmont, MA.

3.3. Extraction and isolation

The dried roots and rhizomes of *N. forbesii* (400 g) were powdered and extracted with sonication three times with hexane (1.6 L each time) at room temperature (25 °C) for 30 min, and the hexane solution was evaporated in vacuo to give a residue (21 g). The ethyl acetate, acetone, methanol, and water extracts (32, 7, 30, and 26 g) were obtained by the same procedure. The hexane extract (20 g) was chromatographed over silica gel (200 g) using hexane with increasing amounts of ethyl acetate (20:1, 10:1, 5:1, 2:1, and 1:1) to give five fractions A–E. Fraction B was further purified by silica gel column with a gradient of methylene chloride and ethyl acetate to afford **8** (430 mg) and **18** (3.0 g). Fraction C was chromatographed over silica gel column with a gradient of methylene chloride and ethyl acetate to afford **10** (3 mg), **11** (3 mg), **13** (140 mg), and **16** (2 mg). Fraction D was purified by silica gel column with a gradient of hexane and ethyl acetate to afford **2** (15 mg), **3** (10 mg), **5** (30 mg), **6** (10 mg), and **12** (330 mg). Fraction E was chromatographed over silica gel column with a gradient of methylene chloride and ethyl acetate to afford **7** (3 mg). The ethyl acetate extract (30 g) was separated by normal phase silica gel column chromatography to obtain **8** (450 mg), **9** (15 mg), **12** (4.5 g), **14** (3 mg), **15** (5 mg), and **18** (2.0 g). The acetone extract (6 g) was separated by a combination of normal phase silica gel column chromatography and HPLC (ODS column, 10 × 250 mm, 60% methanol, 2 ml/min) to obtain **1** (160 mg), **4** (5 mg), **8** (10 mg), **9** (80 mg), **12** (100 mg), and **18** (250 mg). In a similar manner, **1** (50 mg), **9** (20 mg), **17** (15 mg), and sucrose (8.0 g) were isolated from the methanol extract (28 g).

3.4. 6′-*O*-β-D-Glucosyl-7′-hydroxybergamottin (4)

White powder; $[\alpha]_D^{23}$ −3.3° (*c* 0.12, MeOH); ^{1}H NMR data (300 MHz, CD_3OD): δ 8.27 (1H, d, *J* = 9.6 Hz, H-4), 7.80 (1H, d, *J* = 2.4 Hz, H-9), 7.20 (1H, s, H-8), 7.19 (1H, d, *J* = 2.4 Hz, H-10), 6.30 (1H, d, *J* = 9.6 Hz, H-3), 5.66 (1H, t, *J* = 6.6 Hz, H-2′), 5.06 (1H, d, *J* = 6.6 Hz, H-1′), 4.27 (1H, d, *J* = 7.8 Hz, H-1″), 3.84 (1H, dd, *J* = 2.4 and 12.0 Hz, H-6″), 3.64 (1H, dd, *J* = 5.4 and 12.0 Hz, H-6″), 3.39 (1H, dd, *J* = 2.4 and 9.6 Hz, H-6′), 3.16–3.33 (4H, m, H-2″, H-3″, H-4″ and H-5″), 2.45 (1H, m, H-4′), 2.27 (1H, m, H-4′), 1.70 (1H, s, H-10′), 1.48-1.70 (2H, m, H-5′), 1.45 (1H, s, H-8′), and 1.10 (1H, s, H-9′); ^{13}C NMR data (75 MHz, CD_3OD): δ 163.2 (C-2), 159.7 (C-7), 153.8 (C-8a), 150.3 (C-5), 146.9 (C-9), 144.4 (C-3′), 141.5 (C-4), 121.0 (C-2′), 115.9 (C-6), 113.1 (C-3), 108.8 (C-4a), 106.3 (C-10), 105.4 (C-1″), 94.8 (C-8), 89.5 (C-6′), 78.1 (C-3″), 78.0 (C-5″), 75.3 (C-2″), 73.4 (C-7′), 71.4 (C-4″), 70.7 (C-1′), 62.5 (C-6″), 36.8 (C-4′), 31.0 (C-5′), 26.5 (C-9′), 24.5 (C-8′), 16.6 (C-10′); ESI-MS *m/z*: 552 $[M+NH_4]^+$, 535 $[M+H]^+$, 373 $[M-162+H]^+$; HR ESI-MS: *m/z* 552.2452, $[(M+NH_4)^+$, calcd for $C_{27}H_{38}O_{11}N$ 552.2445].

3.5. 4′-Methoxyphenethyl vanillate (11)

Colorless resin; ^{1}H NMR data (300 MHz, $CDCl_3$: δ 7.61 (1H, dd, *J* = 2.1 and 8.1 Hz, H-6), 7.51 (1H, d, *J* = 2.1 Hz, H-2), 7.20 (2H, d, *J* = 8.7 Hz, H-2′ and H-6′), 6.93 (1H, d, *J* = 8.1 Hz, H-5), 6.86 (2H, d, *J* = 8.7 Hz, H-3′ and H-5′), 4.46 (2H, t, *J* = 7.2 Hz, H-8′), 3.92 (3H, s, C-4-OMe), 3.79 (3H, s, C-4′-OMe), 3.00 (2H, t, *J* = 7.2 Hz, H-8′); ^{13}C NMR data (75 MHz, $CDCl_3$): δ 166.3 (C-7), 158.3 (C-4′), 153.8 (C-8a), 150.0 (C-4), 146.1 (C-3), 130.0 (C-1′), 129.9 (C-2′ and C-6′), 124.1 (C-6), 122.4 (C-1), 114.0 (C-5), 113.9 (C-3′ and C-5′), 111.7 (C-2), 65.5 (C-8′g286), 56.0 (C-4-OMe), 55.2 (C-4′-OMe), and 34.4 (C-7′); ESI-MS *m/z*: 320 $[M+NH_4]^+$, 303 $[M+H]^+$; HR ESI-MS: *m/z* 320.1495 $[(M+NH_4)^+$, calcd for $C_{17}H_{22}O_5N$ 320.1498].

3.6. Synthesis of 4′-methoxyphenethyl vanillate (11)

To a stirred methylene chloride solution of vanillic acid (152 mg, 1.00 mmol), 4-methoxyphenethyl alcohol (200 mg, 1.19 mmol), and DMAP (catalytic amount) was added DCC (216 mg, 1.05 mmol). The reaction mixture was stirred at room temperature for 20 h. The solvent was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (methylene chloride/ethyl acetate, 20:1) to give **11** (52 mg).

3.7. Competitive inhibition of [^{3}H]diprenorphine binding to opioid receptors

The assay was carried out as described previously.[31] The extracts showing more than 50% displacement of [^{3}H]diprenorphine at 50 mg/ml were defined as active and subjected to further fractionation and purification. The isolated compounds showing more than 50% displacement of [^{3}H]diprenorphine at 10 μM were further tested for the affinity (K_i). The K_i value of each compound was calculated from the competitive inhibition curves using the Prism 3.0 program (GraphPad Software Inc., San Diego, CA).

3.8. Competitive inhibition of [^{3}H]SCH23390 binding to dopamine receptor

Chinese hamster ovary cells (CHO) stably transfected with HA-tagged rat D1 dopamine receptor (rD1R) were grown in 100-mm culture dishes in Dulbecco's modified Eagle's medium F12 HAM supplemented with 10% fetal calf serum, 0.1 mg/ml hygromycin B, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere consisting of 5% CO_2 and 95% air at 37 °C. Membranes were prepared from the CHO cells stably transfected with rD1R as described previously.[44] Binding to membranes prepared from CHO cells stably transfected with the rD1R was performed with [^{3}H]SCH23390 (0.2 nM) in 50 mM Tris–HCl buffer containing 1 mM EGTA (pH 7.4) (TE buffer) at room temperature for 1 h in duplicate. Nonspecific binding was defined as binding in the presence of Fluphenazine (10 μM). The purified compounds showing more than 50% displacement of [^{3}H]SCH23390 at 10 μM were further tested for the affinity (K_i) by using GRAPHPAD PRISM.

Acknowledgments

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