

RESEARCH ARTICLE

Design, synthesis, and biological evaluation of 3,4,5-trimethoxyphenyl acrylamides as antinarcotic agents

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Abstract

The synthesis and biological evaluation of 3,4,5-trimethoxyphenyl acrylamides **1a–f** as novel antinarcotic agents are described. The molecules were prepared by the Wittig reaction, followed by a coupling reaction between 3,4,5-trimethoxycinnamic acid (**9**) and aliphatic amines, which resulted in good yields. When tested for biological activity, compounds **1d–f** exhibited strong inhibitory effects on the morphine withdrawal syndrome in mice due to their high binding affinities with serotonergic 5-HT_{1A} receptors.

Keywords: 3,4,5-trimethoxyphenyl acrylamides; antinarcotic activity; morphine withdrawal syndrome; Wittig reaction; coupling synthesis

Introduction

Derivatives of cinnamic acid are common in plants. Methoxycinnamic acid and its derivatives have been shown to have interesting and important biological properties including analgesic¹, anti-arthritis², anti-inflammatory³, antipyretic⁴, antibacterial⁵, antiviral⁶, and anti-cancer⁷ effects. They are also useful key intermediates for many industrial products such as dyes⁸ and liquid crystals⁹. Hydroxylated (*p*-coumaric acid, **3**) or methoxylated (ferulic acid, **4** and sinapic acid, **5**) derivatives of methoxycinnamic acid have been used as antioxidant agents, as well as inhibitors of monocarboxylate transport across plasma membranes¹⁰. Recently, analogs of 3,4,5-trimethoxycinnamic acids were found to have antidepressant-like effects as well as sedative activity through suppression of norepinephrine in the locus coeruleus¹¹. A number of structurally diverse glucosidase inhibitors have exhibited potent anti-inflammatory and antiviral efficacy with little toxicity or few side effects in animal models (Figure 1)¹². The Hashimoto

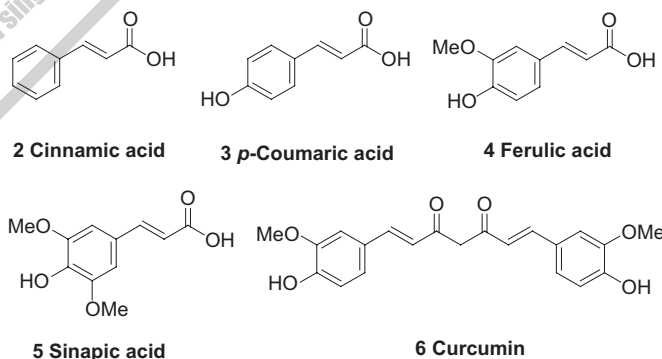


Figure 1. Chemical structures of cinnamic acid (**2**), *p*-coumaric acid (**3**), ferulic acid (**4**), sinapic acid (**5**), and curcumin (**6**).

group¹³ described the novel photoreactive cinnamic acid and its derivatives to elucidate phenylalanine ammonia-lyase. The Keung group¹⁴ demonstrated that aromatic-substituted cinnamic acid and its structural analogs inhibit monoamine oxidase A and exhibit antidepressant-like

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activity in mice. The Geraci group¹⁵ described the synthesis of hydroxycinnamic acid clustered by a calixarene and evaluated its radical scavenging and antioxidant activity by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH)-induced radicals and the azo-bis-isobutyronitrile (AIBN)-induced linoleic acid peroxidation test. Recently, 3,4,5-trimethoxycinnamic acid (TMCA) was identified as a component of Onji, a sedative treatment used in Japanese traditional Kampo medicine. The Kawashima group¹⁶ demonstrated that TMCA induces corticotropin-releasing hormone (CRH) and that TMCA has a sedative effect by suppressing norepinephrine (NE) content in the locus coeruleus (LC). Hyperactivity of LC neurons is an important symptom of opiate withdrawal. Opiate withdrawal syndrome is mediated by an excitatory amino acid input to the LC that causes hyperactivity in the LC region¹⁷. During naloxone-induced jumping behavior, there is a significant increase in extracellular levels of NE¹⁸. These studies suggest that TMCA may be active in opioid withdrawal by suppressing NE or by blocking excitatory amino acid input to the LC. We have synthesized 3,4,5-trimethoxyphenyl acrylamides, and determined their effect on morphine dependence in mice and their binding affinities on serotonergic receptors. We report herein the short synthesis protocol for novel 3,4,5-trimethoxyphenyl acrylamides **1a-f**, which we synthesized in high yields starting from commercially available 3,4,5-trihydroxybenzaldehyde (**7**) via Wittig and coupling reactions.

Materials and methods

General procedures

All other commercial reagents and solvents were used as received without further purification. Reaction solvents were distilled from calcium hydride for dichloromethane and from sodium metal and benzophenone for tetrahydrofuran. The reactions were monitored and the R_f (retention factor) values determined using analytical thin layer chromatography (TLC) with Merck silica gel 60, F-254 precoated plates (0.25 mm thickness). Spots on the TLC plates were visualized using ultraviolet light (254 nm) and a cerium sulfate/ammonium dimolybdate/sulfuric acid solution, followed by heating on a hotplate. Flash column chromatography was performed with Merck silica gel 60 (230–400 mesh) to purify the reaction mixture which was recrystallized by diethyl ether in dichloromethane. ¹H nuclear magnetic resonance (NMR) and ¹³C NMR spectra were recorded on a Bruker DRX 250 spectrometer at 250 MHz and 63 MHz, or a DRX 400 at 400 MHz and 100 MHz, respectively. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS; δ 0.00 ppm) or with the solvent reference relative to TMS employed as the internal standard (CDCl₃, δ 7.26 ppm). Data are reported as follows: chemical shift {multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration}. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard

(CDCl₃, 77.16 ppm). Infrared (IR) spectra were recorded on a Jasco FT/IR-430 spectrometer. Data are reported in wave numbers (cm⁻¹). Electrospray ionization-liquid chromatography-mass spectrometry (ESI-LC-MS) was done on a Waters ZQ 4000 LC-MS spectrometer. Melting points were determined on a Bibby Stuart Scientific melting point apparatus SMP3. Elemental analyses were performed on a CE instrument EA1110.

General procedure for the preparation of amides **1a-f**

To a stirred solution of 3,4,5-trimethoxycinnamic acid (**9**, 1.0 g, 4.2 mmol) in dry dichloromethane (30 mL) was added 1-ethyl(dimethylaminopropyl)carbodiimide (EDCI, 0.97 g, 5.0 mmol), 1-hydroxybenzotriazole (HOBT, 0.68 g, 5.0 mmol), and triethylamine (TEA, 0.70 mL, 5.0 mmol), and then the reaction mixture was stirred at room temperature for 30 min. The several amines were then added to the reaction mixture and the resulting mixture was stirred at room temperature for 24 h. The reaction mixture was washed with brine (20 mL) and water (20 mL). The organic layer was separated, dried over anhydrous MgSO₄, and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate/hexanes = 2:1, v/v).

(*E*)-*N*-Methyl-3-(3,4,5-trimethoxyphenyl) acrylamide (**1a**)
Yield: 81%, white solid. mp 123–124°C; R_f = 0.2 (ethyl acetate/hexanes = 3:1, v/v); IR ν_{\max} (CHCl₃, KBr) 3430 2936 2343 1659 1622 1556 1506 1422 1325 1285 1126 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 7.53 (d, 1H, J = 15.6), 6.70 (s, 2H), 6.62 (s, 1H), 6.46 (d, 1H, J = 15.6), 3.85 (s, 6H), 3.82 (s, 3H), 2.94 (d, 3H, J = 4.7); ¹³C NMR (63 MHz, CDCl₃) δ 166.8 153.3 140.3 139.3 130.5 120.3 104.8 60.9 56.0 26.5; LC-MS (ESI+) m/z 274.02 [M + Na].

(*E*)-*N*-Allyl-3-(3,4,5-trimethoxyphenyl) acrylamide (**1b**)
Yield: 81%, white solid. mp 148–149°C; R_f = 0.4 (ethyl acetate/hexanes = 2:1, v/v); IR ν_{\max} (CHCl₃, KBr) 3439 1634 1124 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 7.56 (d, 1H, J = 15.6), 6.72 (s, 2H), 6.42 (d, 1H, J = 15.5), 6.19 (t, 1H), 5.82–5.97 (m, 1H), 5.24 (dd, 1H, J = 1.4, 17.2), 5.16 (dd, 1H, J = 1.3, 10.2), 4.02 (t, 2H), 3.86 (s, 6H), 3.85 (s, 3H); ¹³C NMR (63 MHz, CDCl₃) δ 165.9 153.4 141.1 139.5 134.2 130.5 120.1 116.6 104.9 61.0 56.1 42.2; LC-MS (ESI+) m/z 300.12 [M + Na].

(*E*)-*N*-Butyl-3-(3,4,5-trimethoxyphenyl) acrylamide (**1c**)
Yield: 69%, white solid. mp 152–153°C; R_f = 0.3 (ethyl acetate/hexanes = 2:1, v/v); IR ν_{\max} (CHCl₃, KBr) 3272 2958 2933 1659 1621 1583 1552 1505 1463 1417 1323 1279 1241 1212 1126 1004 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 7.54 (d, 1H, J = 15.5), 6.67 (s, 2H), 6.36 (d, 1H, J = 15.5), 5.90 (t, 1H), 3.87 (s, 9H), 3.39 (q, 2H); 1.50–1.61 (m, 2H), 1.34–1.46 (m, 2H), 0.94 (t, 3H); ¹³C NMR (63 MHz, CDCl₃) δ 165.9 153.6 140.9 139.8 130.6 120.4 105.1 61.1 56.3 39.6 31.9 20.3 13.9; LC-MS (ESI+) m/z 316.29 [M + Na].

(*E*)-*N*-Hexyl-3-(3,4,5-trimethoxyphenyl) acrylamide (**1d**)
Yield: 67%, white solid. mp 127–128°C; R_f = 0.4 (ethyl acetate/hexanes = 2:1, v/v); IR ν_{\max} (CHCl₃, KBr) 3286 2932 2853 1659 1620 1584 1556 1505 1463 1418 1324 1278 1127 1005 cm⁻¹; ¹H NMR (250 MHz, CDCl₃) δ 7.54 (d, 1H, J = 15.5), 6.73 (s, 2H), 6.35 (d, 1H, J = 15.5), 5.82 (t, 1H), 3.78 (s, 9H); 3.38 (q, 2H),

1.51–1.59 (m, 2H), 1.31 (br s, 6H), 0.86 (br s, 3H); ^{13}C NMR (63 MHz, CDCl_3) δ 165.9 153.4 140.8 139.7 130.6 120.3 104.8 61.1 56.2 39.9 31.6 29.7 26.7 22.7 14.2; LC-MS (ESI+) m/z 344.35 [M + Na].

(E)-N-Octyl-3-(3,4,5-trimethoxyphenyl) acrylamide (1e)
Yield: 86%, white solid. mp 119–120°C; R_f =0.6 (ethyl acetate/hexanes=2:1, v/v); IR ν_{max} (CHCl_3 , KBr) 3291 2919 2852 1653 1619 1582 1454 1418 1384 1325 1282 1241 1128 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) δ 7.54 (d, 1H, J =15.5), 6.72 (s, 2H), 6.34 (d, 1H, J =15.5), 5.79 (t, 1H), 3.87 (s, 9H), 3.38 (q, 2H), 1.50–1.60 (m, 2H), 1.26 (br s, 10H), 0.87 (br s, 3H); ^{13}C NMR (63 MHz, CDCl_3) δ 165.9 153.4 140.9 139.3 130.6 120.2 104.7 61.1 56.1 39.9 31.9 29.8 29.4 29.3 27.1 22.8 14.2; LC-MS (ESI+) m/z 372.56 [M + Na]; Elemental analysis Calcd for $\text{C}_{20}\text{H}_{31}\text{NO}_4$: C, 68.74; H, 8.94; N, 4.01%. Obsd: C, 68.87; H, 8.96; N, 4.17%.

(E)-N-Decyl-3-(3,4,5-trimethoxyphenyl) acrylamide (1f)
Yield: 78%, white solid. mp 117–120°C; R_f =0.7 (ethyl acetate/hexanes=2:1, v/v); IR ν_{max} (CHCl_3 , KBr) 3439 3276 2919 2849 1655 1615 1582 1541 1505 1454 1413 1321 1271 1239 1129 cm^{-1} ; ^1H NMR (250 MHz, CDCl_3) δ 7.53 (d, 1H, J =15.5), 6.71 (s, 2H), 6.37 (d, 1H, J =15.5), 6.00 (s, 1H), 3.86 (s, 6H), 3.85 (s, 3H), 3.38 (q, 2H), 1.56 (m, 2H), 1.23 (br s, 14H), 0.88 (t, 3H); ^{13}C NMR (63 MHz, CDCl_3) δ 165.9 153.4 140.7 139.4 130.6 120.4 104.9 61.0 56.1 39.9 32.0 31.8 29.8 29.6 29.4 29.4 27.1 26.7 22.7 14.2; LC-MS (ESI+) m/z 400.04 [M + Na]; Elemental analysis Calcd for $\text{C}_{22}\text{H}_{35}\text{NO}_4$: C, 69.99; H, 9.34; N, 3.71%. Obsd: C, 69.90; H, 9.43; N, 3.78%.

Animals and drug administration

C57BL/6 mice were obtained from Daehan Biolink (Eumsung, Korea). They were housed in a 12 h light–dark cycle with temperature maintained at $24 \pm 3^\circ\text{C}$. All animal procedures were in accordance with the Institutional Animal Care and Use Committee of Ewha Womans University. The mice (male, 20 ± 2 g) were randomly divided into 10 groups and were given saline, morphine, or both morphine and 3,4,5-trimethoxyphenyl acrylamides. Morphine chloride (10 mg/kg/day; Myungmun Pharm., Seoul) was dissolved in saline, and 3,4,5-trimethoxyphenyl acrylamides (5 mg/kg/day) were dissolved in 10% Cremophor solution containing 2% dimethyl sulfoxide. Morphine and 3,4,5-trimethoxyphenyl acrylamides were administered daily for 7 days intraperitoneally. 3,4,5-Trimethoxyphenyl acrylamides were administered 30 min prior to the injection of morphine. Naloxone hydrochloride (5 mg/kg, i.p.) was injected 6 h after the final morphine injection for the induction of morphine withdrawal syndrome in mice.

Measurement of morphine withdrawal syndrome

Morphine withdrawal syndrome was induced by the injection of naloxone (5 mg/kg), which is a competitive antagonist with high opioid receptor affinity. Immediately after the naloxone injection, mice were placed into individual observation cylinders (24 cm in diameter and 50 cm in height), and the frequency of jumps of each mouse was observed for 30 min.

[^3H]8-OH-DPAT binding to serotonin 5-HT_{1A} receptor

Membranes from a stable CHO-K1 cell line expressing the human recombinant 5-HT_{1A} serotonin receptor (PerkinElmer Life and Analytical Sciences, MA, USA) were used. For the binding assay, aliquots of receptor membranes, 0.25 nM [^3H]8-OH-DPAT (8-hydroxy-2-(di-*N*-propylamino)tetralin, 154.3 Ci/mM; New England Nuclear, Boston, MA) and appropriate concentrations of test compounds were added to 0.25 mL of 50 mM Tris-HCl (pH 7.4) buffer containing 10 mM MgSO_4 , 0.5 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% ascorbic acid. Incubations were carried out for 60 min at 27°C , and these were terminated by rapid filtration using a MicroBeta FilterMate-96 harvester (PerkinElmer) through a Filtermat A glass fiber filter presoaked in 0.3% polyethylenimine (PEI). The filter was washed with ice-cold 50 mM Tris-HCl buffer solution (pH 7.4), and was then covered with MeltiLex, sealed in a sample bag, and dried in an oven. The radioactivity retained in the filter was finally counted using MicroBeta Plus (Wallac, Finland). Nonspecific binding was defined with 0.5 μM of methiothepin. The binding affinity (IC_{50}) of a compound was calculated by computerized nonlinear regression analysis (GraphPad Prism Software; GraphPad, San Diego, USA) using 7–8 varied concentrations of the compound run in duplicate tubes.

[^3H]Ketanserin binding to serotonin 5-HT_{2A} receptor

For serotonin 5-HT_{2A} binding, an aliquot of frozen membrane from a CHO-K1 cell line expressing the human recombinant 5-HT_{2A} receptor (PerkinElmer) and [^3H]ketanserin (1 nM; PerkinElmer) were used in the presence of mianserin (20 μM) as nonspecific. The reaction mixture was incubated for 60 min at 27°C using 50 mM Tris-HCl (pH 7.4) buffer containing 4 mM CaCl_2 and 0.1% ascorbic acid, and harvested through a Filtermat A glass fiber filter presoaked in 0.5% PEI.

[^3H]Mesulergine binding to serotonin 5-HT_{2C} receptor

Frozen membranes from a stable CHO-K1 cell line expressing the human recombinant 5-HT_{2C} receptor (PerkinElmer) were used. For the binding assay, [^3H]mesulergine (1 nM; Amersham Biosciences, Buckinghamshire, UK), receptor membrane, and test compounds were added into 50 mM Tris-HCl (pH 7.4) buffer containing 0.1% ascorbic acid and 4 mM CaCl_2 . Nonspecific binding was determined using 100 μM mianserin. The incubations were performed for 60 min at 27°C , and these were terminated by rapid filtration through a Filtermat A glass fiber filter presoaked in 0.5% PEI.

[^3H]LSD binding to serotonin 5-HT₆ and 5-HT₇ receptors

For receptor binding assays, human 5-HT₆ and 5-HT₇ serotonin receptors expressed in CHO-K1 cells (PerkinElmer) were used. For 5-HT₆ receptor binding, frozen membrane, 1 nM [^3H]LSD (lysergic acid diethylamide; PerkinElmer), and appropriate concentrations of test compounds were added to 0.25 mL of 50 mM Tris-HCl (pH 7.4) buffer containing 0.1% ascorbic acid and 4 mM CaCl_2 . Nonspecific binding was determined using 10 μM methiothepin. The incubations were performed for 60 min at 27°C , and these were

terminated by rapid filtration through a Filtermat A glass fiber filter presoaked in 0.5% PEI. For the 5-HT₇ receptor binding assay, cell membrane, 3 nM [³H]LSD, and appropriate concentrations of test compounds were added to 0.25 mL of 50 mM Tris-HCl (pH 7.4) buffer containing 10 mM MgSO₄ and 0.5 mM EDTA. The mixture was incubated for 120 min at 27°C, and the reaction was terminated by rapid filtration through a Filtermat A glass fiber filter presoaked in 0.3% PEI. Nonspecific binding was determined using 10 μM methiothepin.

[³H]Imipramine binding to serotonin transporter

For serotonin transporter binding studies, frozen membrane from a stable HEK-293 cell line expressing the human recombinant 5-HT transporter (PerkinElmer), 3 nM [³H]imipramine (PerkinElmer), and appropriate concentrations of test compounds were added to 0.25 mL of 50 mM Tris-HCl (pH 7.4) buffer containing 120 mM NaCl and 5 mM KCl. Nonspecific binding was determined using 200 μM imipramine. The incubations were performed for 30 min at 27°C, and these were terminated by rapid filtration through a Filtermat A glass fiber filter presoaked in 0.5% PEI.

Primary cortical culture

Cortical cell culture was prepared from embryos of C57BL/6 mice at the gestational age of 15 days. The brain was dissected and kept in an ice-cold solution. The cortical tissues were dissociated to single cells by a gentle suspension. The cell suspension was centrifuged at 1000 rpm for 5 min, and the resulting pellets were suspended in minimum essential medium (MEM; Gibco-BRL, Rockville, MD, USA) supplemented with 5% heat-inactivated fetal calf serum and house serum (Gibco) and dispensed on a plate coated with poly-D-lysine (Sigma, St. Louis, MO, USA). The cells were cultured in a CO₂ incubator (5% (v/v), 37°C). Seven days after plating, the cells were treated with 10 μM cytosine arabinofuranoside (Ara C) to reduce the growth of contaminating non-neuronal cells. The cortical neuronal cells were cultured in MEM supplemented with 10% horse serum, but without glutamine.

Western immunoblot analysis

The 3,4,5-trimethoxyphenyl acrylamides (10 μM) were treated with or without WAY 100635 (1 μM), a selective antagonist of 5-HT_{1A} receptor, for 30 min in cortical neuronal cells.

The cortical neuronal cell samples were extracted with lysis buffer (150 mM NaCl, 50 mM Tris-HCl pH 8, 1 mM EDTA, 1% Triton-X 100, 1 mM PMSF (phenylmethylsulfonyl fluoride), 1 mM Na₃VO₄) and centrifuged at 13,500 rpm for 15 min at 4°C. Protein concentrations were determined using a protein assay kit (Pierce Biotechnology). Sample buffer (×4) was added to extract and boiled for 4 min at 100°C. Equal amounts of cellular protein were separated by 10% polyacrylamide gels and were electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes. Transfer blots were blocked in 3% skim milk in TBST (Tris-buffered saline-Tween 20) 0.1% for 1 h and incubated with antibodies specific to pERK1/2 (1:1000; Santa Cruz Biotechnology, CA, USA) or GAPDH (glyceraldehyde 3-phosphate dehydrogenase, 1:1000; Cell Signaling), at 4°C overnight. Blots were washed three times for 30 min in 0.1% TBST, and incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (Zymed Laboratories). Bound antibodies were visualized following chemiluminescence detection (Amersham Pharmacia Biotechnology, CA, USA).

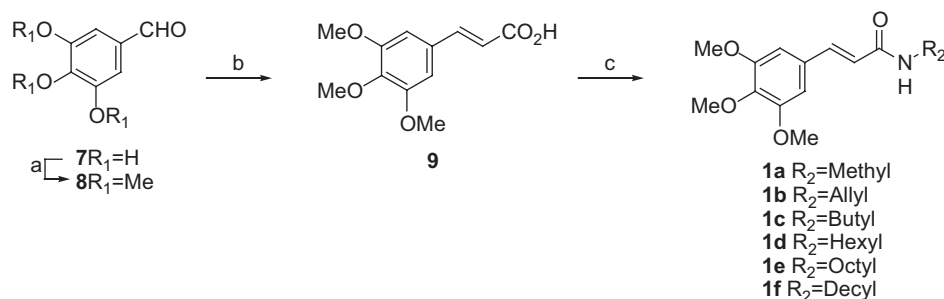
Statistical analysis

All values are expressed as mean ± standard error (SE). The results were subjected to analysis of variance (ANOVA) using the Newman-Keuls multiple comparison test.

Results and discussion

Synthesis of 3,4,5-trimethoxyphenyl acrylamides

The synthesis of 3,4,5-trimethoxyphenyl acrylamides is outlined in Scheme 1. 3,4,5-Trimethoxy benzaldehyde (**8**) was prepared by modification of a previously reported procedure¹⁹, where commercially available 3,4,5-trihydroxy benzaldehyde (**7**) was treated with dimethylsulfate in the presence of KOH to generate aldehyde **8** in 60% yield. Compound **8** was produced by the Wittig reaction by using freshly prepared Wittig salts (triphenyl phosphine and ethyl 2-bromoacetate in toluene) and KOH in dimethylsulfoxide (DMSO) to give 3,4,5-trimethoxycinnamic acid (TMCA) (**9**) in 52% yield²⁰. Acid **9** was coupled with various amines (methylamine, allyl amine, *n*-butyl amine, *n*-hexyl amine, *n*-octyl amine, *n*-decyl amine) in the presence of 1-ethyl(dimethylaminopropyl) carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), and triethylamine (TEA)



Scheme 1. (a) (CH₃)₂SO₄, KOH, 10°C, 1 h, and then reflux 5 h (60%); (b) (Ph₃P⁺=CHCO₂C₂H₅)Br⁻, KOH, DMSO, 16 h (52%); (c) amines, HOBt, EDCI, TEA, CH₂Cl₂, room temp., 2 h (67–86%).

in dichloromethane to give 3,4,5-trimethoxyphenyl acrylamides **1a-f** in 67% to 86% yields (Scheme 1).

Antinarcotic properties of 3,4,5-trimethoxyphenyl acrylamides

Mice received morphine (10 mg/kg/day, i.p.) for 7 days to develop a dependence. The effects of 3,4,5-trimethoxyphenyl acrylamides (5 mg/kg/day, i.p.) were examined on naloxone-induced jumping behavior in morphine-dependent mice. As shown in Figure 2, the frequency of jumping, which is an indicator of morphine withdrawal symptoms, was decreased by treatment with 3,4,5-trimethoxyphenyl acrylamides. Interestingly, each of the 3,4,5-trimethoxyphenyl acrylamides were found to inhibit jumping frequency in morphine dependent mice: **1a** (13%), **1b** (27%), **1c** (57%), **1d** (78%), **1e** (88%), and **1f** (80%). Furthermore, compound **1e** displayed the greatest degree of attenuation, and 5 mg/kg of this compound administered intraperitoneally is as effective as the 5-HT_{1A} receptor agonist (+)8-OH-DPAT (0.5 mg/kg, i.p.).

Receptor binding affinity analysis of 3,4,5-trimethoxyphenyl acrylamides

To elucidate the mechanistic basis for the antinarcotic effect of 3,4,5-trimethoxyphenyl acrylamides, their ability to bind

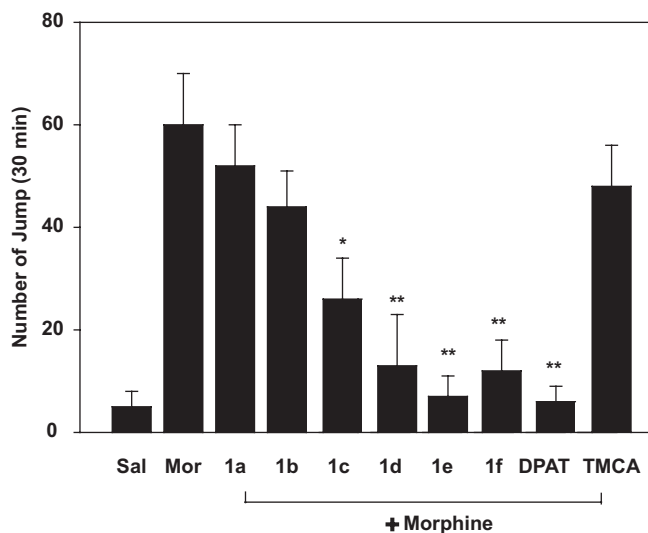


Figure 2. Effects of 3,4,5-trimethoxyphenyl acrylamides on naloxone-induced jumping behavior in morphine-dependent mice. TMCA and 3,4,5-trimethoxyphenyl acrylamides (5 mg/kg, i.p.) and (+)8-OH-DPAT (0.5 mg/kg, i.p.) were injected 30 min before morphine injection (10 mg/kg, i.p.) for 7 days. On the 7th day, naloxone (5 mg/kg, i.p.) was injected 6 h after final drug administration. The number of jumps in a 30 min interval was counted after naloxone injection. * $p < 0.05$, ** $p < 0.01$ in comparison with morphine only group.



Figure 3. WAY 100635 inhibits 3,4,5-trimethoxyphenyl acrylamide-induced pERK expression. Cultured neuronal cells were treated with 3,4,5-trimethoxyphenyl acrylamides (10 μ M) and/or WAY 100635 (1 μ M) for 30 min. The expression of pERK was examined by Western blot analysis.

several serotonergic receptors including 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₆, 5-HT₇, and the 5-HT transporter was evaluated. Interestingly, all of the 3,4,5-trimethoxyphenyl acrylamides showed a high binding affinity to the 5-HT_{1A} receptor. Similarly, the basal form of 3,4,5-trimethoxycinnamic acid (TMCA) also had a high affinity for the 5-HT_{1A} receptor (Table 1). Among the six 3,4,5-trimethoxyphenyl acrylamides **1a-f**, compound **1e** showed the highest binding affinity to the 5-HT_{1A} receptor. Interestingly, compound **1f** exhibited a significant binding affinity to several serotonergic receptors including 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₆, 5-HT₇, and the 5-HT transporter.

Effect of 5-HT_{1A} receptor-specific antagonist on 3,4,5-trimethoxyphenyl acrylamide-induced pERK expression in cultured cortical neurons

The ability of all the newly synthesized 3,4,5-trimethoxyphenyl acrylamides to act as 5-HT_{1A} receptor agonists was tested by monitoring the activation of ERK1/2 expression in the presence of WAY 100635, a selective 5-HT_{1A} receptor antagonist. As shown in Figure 3, when cortical neuronal cells were treated with 3,4,5-trimethoxyphenyl acrylamides **1a-f** (10 μ M) for 30 min, pERK expression was elevated. However, this induction of pERK was suppressed upon treatment with WAY 100635 (1 μ M). Of the compounds tested, **1a** and **1c** were the most potent activators of pERK expression in the absence of WAY 100635.

Conclusions

The 3,4,5-trimethoxyphenyl acrylamides **1a-f** were synthesized and tested for their antinarcotic properties in naloxone-induced morphine withdrawal syndrome in mice and receptor binding affinity on serotonergic receptors.

Table 1. Serotonin (5-HT) receptor and transporter binding affinities of 3,4,5-trimethoxyphenyl acrylamides.

Compound	Receptor binding affinity (IC ₅₀ , μ M) ^a					5-HT transporter
	5-HT _{1A}	5-HT _{2A}	5-HT _{2C}	5-HT ₆	5-HT ₇	
1a	5.6	>10	>10	>10	>10	>10
1b	5.9	>10	>10	>10	>10	>10
1c	4.9	>10	>10	8.8	>10	>10
1d	3.8	>10	>10	9.4	>10	>10
1e	1.2	>10	8.8	>10	>10	>10
1f	2.1	6.8	4.5	5.0	5.6	6.7
TMCA ^b	7.6	>10	2.5	>10	>10	>10

^aAll compounds were tested at a maximum concentration of 10 μ M and their binding affinities were calculated as an IC₅₀ (μ M) value.

^b3,4,5-Trimethoxycinnamic acid

Four of the six 3,4,5-trimethoxyphenyl acrylamide compounds tested, **1c-f**, elicited significant inhibition of the morphine withdrawal syndrome in mice and showed strong binding affinities for serotonergic 5-HT_{1A} receptors. Of these analogs, compound **1e** exhibited the most potent inhibitory action against jumping, which is an indicator of morphine-induced withdrawal symptoms. These results suggest that 3,4,5-trimethoxyphenyl acrylamides could be useful for the development of antinarcotic agents.

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