Synthesis, Crystal Structure and Biological Activity of 1-(4-(4-Ethoxybenzyl)piperazin-1-yl)-2-(2-methylphenoxy)ethanone Postprint

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Abstract

One novel phenoxyacetamide derivative (C22H28N2O3, Mr = 368.47) was synthesized and characterized by NMR spectroscopy, high-resolution mass spectrometry, and single-crystal X-ray diffraction. The single crystal belongs to the monoclinic system, space group Cc with a = 14.910(3), b = 14.592(3), c = 38.683(8) Å, = 100.37(3)°, V = 8279(3) Å3, Z = 16, Dc = 1.183 g/cm3, F(000) = 3168, = 0.079 mm-1, MoK radiation (= 0.71073 Å), the final R = 0.0508 and wR = 0.0666 for 4120 observed reflections with I > 2 (I). There are four independent molecules in an asymmetric unit cell. The four symmetry-independent molecules exhibit a variety of different conformations, indicating considerable conformational freedom. The bioassay results indicated that the title compound displayed effective activity against glutamine-induced neurotoxicity in PC12 cells and significantly prolonged the survival time of mice subjected to acute cerebral ischemia.

Full Text

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ABSTRACT

A novel phenoxyacetamide derivative (C H N O , M = 368.47) has been synthesized and characterized by NMR spectroscopy, high-resolution mass spectrometry, and single-crystal X-ray diffraction. The single crystal belongs to the monoclinic system, space group Cc with a = 14.910(3), b = 14.592(3), c = 38.683(8) Å, = 100.37(3)°, V = 8279(3) Å³, Z = 16, Dc = 1.183 g/cm³, F(000) = 3168, = 0.079 mm ¹, MoK radiation (= 0.71073 Å), with final R = 0.0508 and wR = 0.0666 for 4120 observed reflections with I > 2 (I). There are four independent molecules in the asymmetric unit cell. These four symmetry-independent molecules exhibit a variety of different conformations, indicating considerable conformational freedom. Bioassay results demonstrated that the title compound displays effective activity against glutamine-induced neurotoxicity in PC12 cells and significantly prolongs the survival time of mice subjected to acute cerebral ischemia.

Keywords: phenoxyacetamide; crystal structure; synthesis; anti-ischemic activity

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1. INTRODUCTION

Acute ischemic stroke (AIS) is a disease characterized by neuronal dysfunction and apoptosis induced by interruption of blood supply resulting from vascular occlusion or rupture [**?**, **?**]. It represents the most common cause of death and a major cause of disability worldwide [**?**, **?**]. In China, treatment costs associated with ischemic stroke impose a substantial financial burden.

The past three decades have witnessed renewed surge in basic science investigations into the pathophysiological events following AIS. Although clinical trials have thus far repeatedly failed, neuroprotection remains a promising therapeutic option for AIS [**?**].

Our previous studies discovered that phenoxyacetyl diphenylmethylpiperazine analogs frequently exhibit neuroprotective activity in glutamate-induced PC12 cells [**?**]. Preliminary structure-activity relationship (SAR) studies indicated that replacing the diphenylmethylpiperazine moiety with a benzylpiperazine group likely improves neuroprotective efficacy. In the present investigation, we prepared a new phenoxyacetyl benzylpiperazine derivative, namely 1-(4-(4-ethoxybenzyl)piperazin-1-yl)-2-(2-methylphenoxy)ethanone (Scheme 1), and determined its crystal structure by X-ray diffraction. The neuroprotective properties of the title compound were also characterized.

2. EXPERIMENTAL

2.1 Materials and Apparatuses

All chemicals, reagents, and solvents were of analytical grade and used without further purification. Melting points were determined using an electrothermal digital apparatus model WRR-401 (Shanghai, China) without correction. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker ACF-500 instrument (Bruker) using CDCl as solvent and TMS as internal standard (chemical shifts expressed as values, J in hertz). High-resolution mass spectra (HRMS) were recorded on a MALDI Micro MX instrument (Waters). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). Horse serum (HS), penicillin, and streptomycin were obtained from Gibco BRL (Div. of Invitrogen, Gaithersburg, MD, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Synthesis

The title compound was conveniently synthesized as outlined in Scheme 1. Intermediates 2 and 3 were prepared according to our previous work [**?**]. The key intermediate 4-ethoxybenzyl bromide (6) was prepared by free-radical bromination of commercially available 4-ethoxytoluene (5) with a slight excess of N-bromosuccinimide (NBS) [**?**]. The title compound 1-(4-(4-ethoxybenzyl)piperazin-1-yl)-2-(2-methylphenoxy)ethanone (7) was obtained as follows: Under a nitrogen atmosphere, 2-methylphenoxyacetyl piperazine (3, 17.0 mmol), 4-ethoxybenzyl bromide (6, 17.0 mmol), and triethylamine (2.4 mL, 17.0 mmol) in acetonitrile (90 mL) were heated to reflux for 20 h. The reaction mixture was filtered and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (100–200 mesh) using a mixture of ethyl acetate and petroleum ether (V/V 2:1) as eluent to afford target compound 7 as a white solid (yield 67.8%). m.p.: 77.3–80.2 °C. ^{1}H NMR (CDCl , 500 MHz) : 7.19–7.13 (m, 2H, Ar-H), 7.14–7.11 (m, 2H, Ar-H), 6.89–6.81 (m, 4H, Ar-H), 4.67 (s, 2H, OCH CO), 4.03 (q, J = 7 Hz, 2H, PhOCH CH), 3.63–3.60 (m, 4H, CON(CH)), 3.43 (s, 2H, PhCH N), 2.41–2.39 (m, 4H, PhCH N(CH)), 2.23 (s, 3H, PhCH), 1.41 (t, J = 7 Hz, 3H, PhOCH CH). ^{13}C NMR (CDCl , 75 MHz) : 16.23, 42.24, 45.52, 51.96, 52.62, 53.10, 60.23, 67.96, 111.03, 121.27, 126.63, 126.95, 127.19, 129.84, 129.88, 130.90, 131.14, 131.48, 138.78, 155.95, 166.56. HRMS (ESI, m/z): Calcd. for C H N O [M + H] 369.2178, found 369.2176.

Single crystals of the title compound suitable for X-ray diffraction were grown by slow evaporation of acetonitrile at room temperature.

Scheme 1. Synthesis of the title phenoxyacetamide compound 7

2.3 X-ray Crystal Structure Determination

A colorless single crystal of the title compound with dimensions 0.30 mm × 0.20 mm × 0.10 mm was selected for X-ray diffraction studies. Data were collected on an Enraf-Nonius CAD4/PC four-circle diffractometer equipped with graphite-monochromated MoK radiation (= 0.71073 Å) at 293(2) K using an /2 scan mode in the range 1.07 25.37° (0 h 17, 0 k 17, -46 l 45). The structure was solved by direct methods using SHELXS-97 and refined against F^2 by full-matrix least-squares method on the positional and anisotropic temperature parameters of non-hydrogen atoms, corresponding to 973 crystallographic parameters, using SHELXL-97 [**?**]. All H atoms were positioned geometrically and treated using a riding model by fixing the bond lengths at 0.93 Å for C-H atoms. A total of 8127 reflections were collected, of which 7919 were independent (Rint = 0.0723) and 4120 were observed with I > 2 (I). The final refinement gave R = 0.0508, wR = 0.0666 (w = 1/[²(Fo^2) + (0.0200P)²], where P = (Fo^2 + 2Fc^2)/3), S = 1.008, (Δ)max = 0.140, (Δ)min = -0.120 e/Å³.

2.4 Biological Activity

To investigate the in vitro neuroprotective activity, the title compound 7 was screened in glutamate-induced PC12 cells [**?**, **?**]. Cellular viability was assessed by MTT assay [**?**, **?**].

PC12 cells were cultured in Dulbecco' s modified Eagle' s medium (DMEM) supplemented with 10% (v/v) FBS, 5% (v/v) HS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C in a humidified atmosphere of 5% carbon dioxide.

PC12 cells were inoculated in a 96-well microplate (10 cells/well in 100 µL medium) for 24 h. After washing with PBS, the PC12 cells were incubated with glutamate (10 mmol/L) or glutamate (10 mmol/L) with compound 7 (0.1, 1, 10 mol/L) or edaravone (90 mol/L) for 24 h, after which MTT (10 L, 5 mg/mL) was added to each culture well. Following incubation at 37 °C for an additional 4 h, the formazan crystals were dissolved by addition of 150 L dimethyl sulfoxide (DMSO), and the plates were shaken vigorously to ensure complete solubilization. Formazan absorbance was assessed at 490 nm using an ELISA plate reader [**?**, **?**].

The in vivo anti-ischemic activity was tested using bilateral common carotid artery occlusion [**?**, **?**]. Kunming mice of both sexes were randomly divided into groups (10 mice per group). The title compound was dissolved in aqueous 0.5% sodium carboxymethyl cellulose (CMCNa) solution before use and administered intraperitoneally (i.p.). Nimodipine was administered i.p. at 80 mg/kg as a positive control. The negative control group received normal saline (NS) in the same volume as other groups. All groups received drugs twice daily for 3 days. Sixty minutes after the last administration, all mice were anesthetized with ether and underwent surgical ligation of the common carotid arteries and vagus nerves. The survival time of mice was then recorded.

3. RESULTS AND DISCUSSION

3.1 Crystal Structure of Compound 7

The ^{1}H NMR, ^{13}C NMR, HRMS, and X-ray diffraction data for the product are in good agreement with the structure of the title compound 7. Its molecular structure and packing diagram are shown in Figs. 1 and 2, respectively. Selected bond distances, bond angles, and torsion angles are listed in Table 1 , and the corresponding hydrogen bond lengths and angles are given in Table 2 .

Compound 7 crystallizes in the monoclinic Cc space group. There are four independent molecules in the asymmetric unit cell. For convenience, these molecules are designated as A, B, C, and D. The average bond lengths and bond angles of the benzene rings are normal. The C=O bond distances in all four molecules—C(14)=O(2) (1.234(5) Å), C(36)=O(5) (1.229(5) Å), C(58)=O(8) (1.209(5) Å), and C(80)=O(11) (1.241(5) Å)—are nearly equal to a typical C=O double bond (1.20 Å) [**?**]. The distances of N(2)-C(14), N(4)-C(36), N(6)-C(58), and N(8)-C(80) are 1.347(6), 1.326(6), 1.328(6), and 1.349(6) Å, respectively. These are remarkably shorter than the typical C(sp^2)-N bond (1.426 Å) but closer to the C=N double bond (1.33 Å) [**?**].

As shown in Fig. 1 [Figure 1: see original paper], the piperazine rings in all four molecules adopt a chair conformation with the N-COCH CO- and N-benzyl groups in equatorial positions. The C(sp^2)-N bonds lie in the planes of the carbonyl groups in all four molecules (N(2)-C(14)-C(15)-O(3) -175.8(4)°, N(4)-C(36)-C(37)-O(6) -171.4(4)°, N(6)-C(58)-C(69)-O(9) -179.3(5)°, and N(8)-C(80)-C(81)-O(12) 178.9(4)°). Each molecule contains two essentially planar phenyl rings that are not coplanar. In molecule A, the 4-ethoxyphenyl ring forms a dihedral angle of 57.7(3)° with the 2-methylphenyl ring. In molecule B, the corresponding angle is 59.5(3)°, while values of 64.6(3)° and 63.6(4)° are observed in molecules C and D, respectively. The bond angles of the amide groups in molecules A, B, C, and D are 122.7(5)°, 122.8(5)°, 123.3(5)°, and 122.0(5)°, respectively. The torsion angles of O(2)-C(14)-C(15)-O(3), O(5)-C(36)-C(37)-O(6), O(8)-C(58)-C(59)-O(9), and O(11)-C(80)-C(81)-O(12) are 5.7(7)°, 6.2(7)°, -0.8(7)°, and 1.4(7)°, respectively.

Molecules of 7 exhibit both intra- and intermolecular hydrogen bonding. Among the four molecules of 7, similar ends of pairs A, C and B, D form dimers via C–H $\cdots$ O interactions. Piperazine hydrogens (H(11A), H(33A), H(56A), and H(78B)) and phenoxyacetyl group hydrogens (H(15A), H(37A), H(59A), and H(81B)) are involved in C–H $\cdots$ O interactions. In the crystal, C–H $\cdots$ O hydrogen bonds link the molecules into two crystallographically independent chains, with each chain formed by two alternating independent molecules. These C–H $\cdots$ O hydrogen bonds and weak C–H $\cdots$ interactions strengthen the three-dimensional network integration. These interactions likely play a role in stabilizing the crystal structure.

Table 1. Selected Bond Lengths (Å) and Bond Angles (°)/Torsion Angles (°) of Compound 7

Parameter	Value
Bond Lengths	
O(2)-C(14)	1.234(5)
O(5)-C(36)	1.229(5)
O(8)-C(58)	1.209(5)
O(11)-C(80)	1.241(5)
N(2)-C(14)	1.347(6)
N(4)-C(36)	1.326(6)
N(6)-C(58)	1.328(6)
N(8)-C(80)	1.349(6)
O(3)-C(15)	1.427(6)
O(6)-C(37)	1.404(6)
O(9)-C(59)	1.449(6)
O(12)-C(81)	1.404(6)
Bond Angles	
O(2)-C(14)-N(2)	122.7(5)
O(5)-C(36)-N(4)	122.8(5)
O(8)-C(58)-N(6)	123.3(5)
O(11)-C(80)-N(8)	122.0(5)
Torsion Angles	
O(2)-C(14)-C(15)-O(3)	5.7(7)
O(5)-C(36)-C(37)-O(6)	6.2(7)
O(8)-C(58)-C(59)-O(9)	-0.8(7)
O(11)-C(80)-C(81)-O(12)	1.4(7)
N(2)-C(14)-C(15)-O(3)	-175.8(4)
N(4)-C(36)-C(37)-O(6)	-171.4(8)
N(6)-C(58)-C(59)-O(9)	-179.3(5)
N(8)-C(80)-C(81)-O(12)	178.9(4)

Table 2. Hydrogen Bond Lengths (Å) and Bond Angles (°)

D-H ··· A	H ··· A	D ··· A	D-H ··· A
C(11)-H(11A) ··· O(8)	2.58	3.341(7)	134
C(12)-H(12B) ··· O(2)	2.38	2.753(7)	104
C(15)-H(15A) ··· O(8)	2.58	3.351(6)	135
C(22)-H(22A) ··· O(3)	2.33	2.740(8)	106
C(33)-H(33A) ··· O(11)	2.54	3.292(7)	134
C(34)-H(34B) ··· O(5)	2.39	2.772(8)	105
C(37)-H(37A) ··· O(11)	2.53	3.319(6)	136
C(44)-H(44A) ··· O(6)	2.32	2.725(8)	106

D–H ··· A	H ··· A	D ··· A	D–H ··· A
C(55)–H(55B) ··· O(8)	2.38	2.744(8)	103
C(56)–H(56A) ··· O(2)	2.59	3.365(7)	135
C(59)–H(59A) ··· O(2)	2.52	3.253(7)	132
C(66)–H(66A) ··· O(9)	2.32	2.734(7)	105
C(77)–H(77A) ··· O(11)	2.38	2.760(7)	103
C(78)–H(78B) ··· O(5)	2.53	3.327(7)	135
C(81)–H(81B) ··· O(5)	2.51	3.231(6)	131
C(88)–H(88A) ··· O(12)	2.38	2.760(7)	103

Symmetry codes: (i) -1/2+x, 3/2-y, 1/2+z; (ii) x, 2-y, -1/2+z; (iii) 1/2+x, -1/2+y, z

Fig. 1. Molecular structure of compound 7

Fig. 2. Packing diagram of compound 7 [Figure 2: see original paper]

3.2 Biological Activity

The in vitro and in vivo biological activities of the title compound were evaluated. In vitro bioassay results showed that compound 7 effectively protects PC12 cells against glutamate-induced neuronal injury. As shown in Table 3 , cell protection at three test concentrations (0.1, 1.0, 10 μM) was 35.28%, 30.65%, and 28.93%, respectively. Notably, compound 7 demonstrated good neuroprotective activity at all three test concentrations (protection > 20%). The compound exhibited the highest protection at the lowest concentration of 0.1 μM and showed better neuroprotection than edaravone at this concentration.

Furthermore, compared with nimodipine, the title compound 7 significantly prolonged the survival time of mice subjected to acute cerebral ischemia and decreased the mortality rate at all doses tested (50–400 mg/kg) in vivo (Table 4). Compound 7 demonstrates effective neuroprotection and may serve as a lead compound for further discovery of neuroprotective agents for treating cerebral ischemic stroke.

Table 3. Neuroprotective Effects of Compound 7 against Glutamate-Induced Neurotoxicity in PC12 Cells

Compound	Protection (%)
	10 mol/L
The title compound	28.93*
Edaravone (90 mol/L)	33.0*

*$p < 0.05$ vs. glutamate-treated group

Table 4. Effects of the Title Compound 7 on Survival Time of Mice Subjected to Bilateral Common Carotid Artery Ligation

Compound	Survival time (min)
	400 mg/kg
Compound 7	8.48 ± 1.15*
Nimodipine	2.04 ± 0.61

- $p < 0.05$ vs. NS.

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Note: Figure translations are in progress. See original paper for figures.