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Design, synthesis, monoamine oxidase inhibition and docking studies of new dithiocarbamate derivatives bearing benzylamine moiety

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ABSTRACT

A new series of thirteen 2-[(4-fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl-1-substituted-carbodithioate derivatives (**4a-4m**) were synthesized and tested for their human monoamine oxidase A and B (*h*MAO-A and *h*MAO-B) inhibitory potential by an *in vitro* fluorometric method. Most of the compounds have found to be selective towards MAO-B than MAO-A. Compound **4j** that carrying 4-nitrophenyl piperazine moiety, was detected as the most active agent amongst all compounds with the IC_{50} value of 0.097 ± 0.003 μM for MAO-B while that of selegiline was 0.040 ± 0.002 μM. The enzyme kinetic study reported that compound **4j** is a reversible and non-competitive inhibitor. Interaction modes between the *h*MAO-B and compound **4j** were determined by docking studies. The study also revealed that compound **4j** has the highest binding scores. Besides, compound **4j** has not cytotoxicity at its effective concentration against *h*MAO-B.

1. Introduction

The decreased levels of monoaminergic neurotransmitters in brain are closely associated with depression and neurological disorders including Parkinson's and Alzheimer's diseases [1,2]. One of the reasonable approaches to tackle this issue is increasing their intracellular concentrations in synaptic gaps through suppressing monoamine oxidases (MAOs). Human MAOs (*h*MAO-A and *h*MAO-B) are flavin-linked enzymes bound to the surface membrane of mitochondria and encoded by separate genes [3]. Although their amino acid sequences are ~70% identical, they vary with substrate and inhibitor specificity and tissue distribution [4,5]. MAO-A predominantly deaminates serotonin, adrenaline, noradrenaline, and is selectively inhibited by clorgyline and moclobemide while MAO-B has a higher affinity for β-phenylethylamine and benzylamine, and is irreversibly inhibited by selegiline [6]. Crystallographic data of MAO enzymes revealed that two isoforms diverge depending on the shape and volume of their active sites. Both enzymes have quite hydrophobic substrate binding sites [7].

The roles of MAO enzymes in the catabolism of monoamine neurotransmitters make them vital medicinal targets in clinic: selective MAO-A inhibitors are often employed as antidepressants [8,9] whereas selective MAO-B inhibitors are used to cope with the symptoms related to Parkinson's and Alzheimer's diseases [10,11]. The prolonged use of these inhibitors generates some adverse effects due to their irreversible and nonselective mechanism of actions, which preclude their therapeutic use [12]. Researchers, therefore, focused on the discover of selective *h*MAO inhibitors with improved clinical practice considering the crystallographic structures of both isoforms.

Recently, there has been a widespread interest directed toward the introduction of more effective and selective MAO inhibitors. In this respect, arylalkyl amines are of importance in medicinal chemistry and commonly exist in many MAO inhibitors such as selegiline, pargyline, clorgyline, tranylcypromine and moclobemide [13]. The inhibitory potency of compounds bearing arylalkyl amine moiety is presumably thought to arise from the contest with endogenic amines due to their structural similarity. Structural variations on the aromatic ring of arylalkyl amine moiety may guide scientist to develop more potent and selective inhibitors [14]. Numerous studies have reported the MAO inhibitory activity of compounds bearing arylalkyl amine moiety and its variations [15–19].

Prompted from above observations, we have recently reported a study, including N-(3 or 4-nitrobenzyl)-N-(4-substitutedphenyl)-2-((5-substitutedbenzo[*d*]thiazol-2-yl)thio)acetamide derivatives as MAO inhibitors [13]. By the fact that one of the important

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parameters for the delivery a MAO inhibitor agent to the central nervous system (CNS) is ability to penetrate the blood-brain barrier (BBB) [20,21], in this study, we designed new compounds with similar chemical structures. Thus, we combined arylalkyl amine scaffold with dithiocarbamate moiety, including more heteroatoms to interact with enzyme active sites. As a result, taking into consideration our recent findings, we goaled to design and synthesize a potent and selective MAO inhibitor.

2. Materials and methods

2.1. Chemistry

2.1.1. General

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: ^{1}H NMR, Bruker DPX 300 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA); ^{13}C NMR Bruker DPX 75 NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, using TMS as internal standard; M + 1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan).

2.1.2. Synthesis of N-(4-fluorophenyl)-1-(4-nitrophenyl)methanimine (**1**)

Equivalent amounts of 4-nitrobenzaldeyde (53 mmol, 8 g) and 4-fluoroaniline (53 mmol, 5.88 g) were refluxed in ethanol (250 mL) with the presence of catalytic amount of glacial acetic acid (0.5 mL). After cooling, the precipitated product was filtered and dried.

2.1.3. Synthesis of 4-fluoro-N-(4-nitrobenzyl)aniline (**2**)

N-(4-fluorophenyl)-1-(4-nitrophenyl)methanimine (**1**) (41 mmol, 10 g) was dissolved in methanol (250 mL) and sodium borohydride (82 mmol, 3.10 g) was added in four equal portions while stirring at room temperature. After completion of the addition, the reaction mixture was stirred for an additional 1 h. The solvent was evaporated under reduced pressure, crude product was washed with water, dried and crystallized from ethanol.

2.1.4. Synthesis of 2-chloro-N-(4-fluorophenyl)-N-(4-nitrobenzyl) acetamide (**3**)

To a mixture of compound **2** (33 mmol, 8 g) and triethylamine (39.6 mmol, 5.5 mL) in tetrahydrofuran (250 mL), chloroacetyl chloride (39.6 mmol, 3.15 mL) was added dropwise in an ice bath while stirring. The mixture was stirred for an additional 1 h at room temperature. Solvent was evaporated, product was washed with water, dried and crystallized from ethanol.

2.1.5. Synthesis of 2-[(4-fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl-1-substituted-carbodithioate derivatives (**4a-4m**)

A mixture of 2-chloro-*N*-(4-fluorophenyl)-*N*-(4-nitrobenzyl) acetamide (**3**) (2 mmol) and appropriate dithiocarbamate potassium salt (2 mmol) were stirred in acetone (40 mL) at room temperature for 8 h. After completion of reaction, the mixture was poured into ice-water, the precipitated product was collected by filtration and the residue was purified by crystallization with ethanol.

*2.1.5.1. 2-[(4-Fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl pyrrolidine-1-carbodithioate (**4a**).* Yield %69; m.p. 172 °C. IR (KBr) ν_{max} (cm^{-1}): 3043 (aromatic C—H), 2872 (aliphatic C—H), 1680 (C=O), 1523 (C=C), 1504–1342 (NO_2), 1220 (C—N), 821 (C—H out of plane deformation). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 1.88–1.94 (2H, m, pyrrolidine-H), 2.00–2.07 (2H, m, pyrrolidine-H), 3.64 (2H, t, J = 6.8 Hz, pyrrolidine-H), 3.74 (2H, t, J = 6.4, pyrrolidine-H), 4.04 (2H, s, CO-CH_2), 5.00 (2H, s, N—CH_2), 7.27 (2H, t, J = 8.8 Hz, aromatic-H), 7.39–7.42 (2H, m, aromatic-H), 7.54 (2H, d, J = 8.4 Hz, aromatic-H), 8.16 (2H, d, J = 8.4 Hz, aromatic-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 23.72 (CH_2), 25.61 (CH_2), 40.20 (CH_2), 50.55 (CH_2), 52.25 (CH_2), 55.10 (CH_2), 116.39 (2CH, d, J = 22.1 Hz), 123.38 (2CH), 129.04 (2CH), 130.35 (2CH, d, J = 8.4 Hz), 137.67 (C), 145.11 (C), 146.70 (C), 161.30 (C, d, J = 243.9 Hz), 166.85 (C=O), 190.16 (C=S). MS (ESI) [M + 1]$^+$: m/z 434.

*2.1.5.2. 2-[(4-Fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl piperidine-1-carbodithioate (**4b**).* Yield %70; m.p. 159 °C. IR (KBr) ν_{max} (cm^{-1}): 3010 (Aromatic C—H), 2933 (Aliphatic C—H), 1668 (C=O), 1504–1342 (NO_2), 1435 (C=C), 1247 (C—N), 840 (C—H out of plane deformation). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 1.57–1.65 (6H, m, piperidine-H), 3.91 (2H, br s, piperidine-H), 4.04 (2H, s, CO—CH_2), 4.18 (2H, br s, piperidine-H), 5.01 (2H, s, N—CH_2), 7.28 (2H, t, J = 9.2 Hz, aromatic-H), 7.41–7.45 (2H, m, aromatic-H), 7.55 (2H, d, J = 8.8 Hz, aromatic-H), 8.17 (2H, d, J = 8.8 Hz, aromatic-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 23.41 (CH_2), 25.09 (CH_2), 25.74 (CH_2), 40.32 (CH_2), 51.09 (CH_2), 52.18 (CH_2), 52.52 (CH_2), 116.40 (2CH, d, J = 22.8 Hz), 123.39 (CH), 123.48 (CH), 129.00 (2CH), 130.37 (2CH, d, J = 8.3 Hz), 137.65 (C), 145.16 (C), 146.64 (C), 161.30 (C, d, J = 243.8 Hz), 166.84 (C), 193.03 (C). MS (FAB) [M + 1]$^+$: m/z 448.

*2.1.5.3. 2-[(4-Fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl morpholine-4-carbodithioate (**4c**).* Yield %65; m.p. 167 °C. IR (KBr) ν_{max} (cm^{-1}): 3064 (aromatic C—H), 2841 (aliphatic C—H), 1668 (C=O), 1506–1344 (NO_2), 1471 (C=C), 1232 (C—N), 1112 (C=O), 856 (C—H out of plane deformation). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 3.68 (4H, t, J = 4.8 Hz, morpholine-H), 3.98–4.17 (6H, m, CO-CH_2, morpholine-H), 5.03 (2H, s, N—CH_2), 7.29 (2H, t, J = 8.8 Hz, aromatic-H), 7.43–7.46 (2H, m, aromatic-H), 7.56 (2H, d, J = 8.4 Hz, aromatic-H), 8.17 (2H, d, J = 8.8 Hz, aromatic-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 40.15 (CH_2), 50.39 (CH_2), 51.15 (CH_2), 52.20 (CH_2), 65.50 (2CH_2), 116.41 (2CH, d, J = 22.5 Hz), 123.37 (2CH), 129.00 (2CH), 130.36 (2CH, d, J = 8.9 Hz), 137.59 (C), 145.09 (C), 146.66 (C), 161.31 (C, d, J = 244.3 Hz), 166.66 (C), 195.00 (C). MS (ESI) [M + 1]$^+$: m/z 450.

*2.1.5.4. 2-[(4-Fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl thiomorpholine-4-carbodithioate (**4d**).* Yield%64; m.p. 180 °C. IR (KBr) ν_{max} (cm^{-1}): 3012 (Aromatic C—H), 2900 (Aliphatic C—H), 1668 (C=O), 1506–1344 (NO_2), 1471 (C=C), 1219 (C—N), 819 (C—H out of plane deformation). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 2.69 (4H, br s, thiomorpholine-H), 4.02 (2H, s, CO-CH_2), 4.22–4.33 (4H, m, thiomorpholine-H), 4.97 (2H, s, N—CH_2), 7.26 (2H, t, J = 8.8 Hz, aromatic-H), 7.38–7.41 (2H, m, aromatic-H), 7.51 (2H, d, J = 8.4 Hz, aromatic-H), 8.13 (2H, d, J = 8.4 Hz, aromatic-H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 26.62 (2CH_2), 40.36 (CH_2), 52.12 (CH_2), 52.99 (CH_2), 54.07 (CH_2), 116.40 (2CH, d, J = 22.8 Hz), 123.38 (2CH), 128.99 (2CH), 130.34 (2CH, d, J = 9.2 Hz), 137.55 (C), 145.08 (C), 146.63 (C), 161.28 (C, d, J = 244.6 Hz), 166.59 (C), 194.44 (C). MS (ESI) [M + 1]$^+$: m/z 466.

*2.1.5.5. 2-[(4-Fluorophenyl)(4-nitrobenzyl)amino]-2-oxoethyl 4-benzylpiperidine-1-carbodithioate (**4e**).* Yield %72; m.p. 147 °C. IR (KBr) ν_{max} (cm^{-1}): 3023 (Aromatic C—H), 2929 (Aliphatic C—H), 1664 (C=O), 1517–1344 (NO_2), 1487 (C=C), 1234 (C—N), 839 (C—H out of plane deformation). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 1.06–1.21 (2H, m, piperidine-H), 1.69 (2H, br s, piperidine-H),