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RESEARCH ARTICLE

Synthesis of some novel 2-substituted benzothiazole derivatives containing benzylamine moiety as monoamine oxidase inhibitory agents

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Abstract

In the present work, 12 new 2-(5-substituted-benzothiazol-2-ylsulfanyl)-N-(substitutedbenzyl)-N-(4-substitutedphenyl) acetamide derivatives (**4a–l**) was designed and synthesized. The structures of the synthesized compounds were clarified using Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (¹H-NMR), carbon-13 nuclear magnetic resonance (¹³C-NMR) and high-resolution mass spectrometry (HRMS) spectral data. Purity of synthesized compounds was checked by high-performance liquid chromatography (HPLC) analyses and purity ratio was found between 96.5–99.9%. The inhibitory activity of the compounds against MAO-A and MAO-B enzymes was evaluated by using *in vitro* fluometric method in which kynuramine was used as a substrate. Most of the compounds exhibited more selective inhibitory activity towards monoamine oxidase B (MAO-B) than monoamine oxidase A (MAO-A). Compound **4h** was determined as the most potent compound against both enzyme types. The MAO-B enzyme kinetic of the compound **4h** was studied and nature of MAO-B inhibition, caused by this compound, was investigated. The graphical analysis of steady-state inhibition data indicated that compound **4h** is a mixed type inhibitor. Theoretical calculation of absorption, distribution, metabolism, excretion (ADME) properties for the synthesized compounds was also carried out and observed data supported the potential of compound **4h**.

Keywords

Benzothiazole, kynuramine, MAO-A, MAO-B

History

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Introduction

In the regulation of different biological processes, the monoamine oxidase (MAO) enzymes play a significant role, thereby crucial targets in drug design for the treatment of psychiatric and neurological disorders^{1,2}. MAOs are in charge of catalysing the oxidative deamination of neurotransmitters and intracellular amines and thus control their concentrations in brain and peripheral tissues^{3,4}. MAOs include the covalently linked cofactor flavinadenosine-dinucleotide (FAD) and exist in outer mitochondrial membrane^{5,6}. Relying on their affinities to inhibitors and specificities to substrates, two isoforms of MAO, namely MAO-A and MAO-B, have been identified in mammals^{7,8}. MAO-A predominantly metabolizes serotonin, adrenaline and noradrenaline, while MAO-B utilize phenylethylamine and benzylamine as substrate^{8,9}. On the other hand, tyramine and dopamine were metabolized by both forms of the enzyme¹⁰.

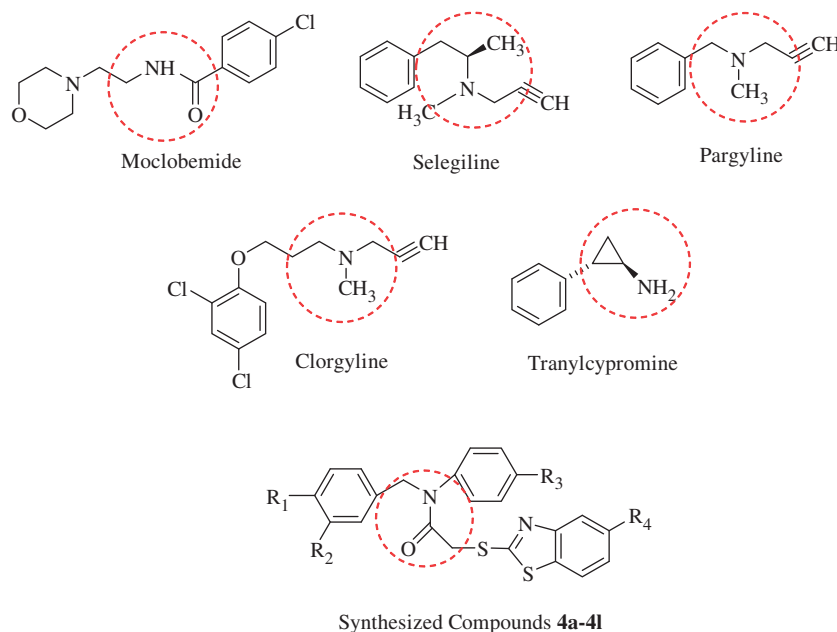
By means of their regulatory effect on the metabolism of neurotransmitters, MAOs are increasingly becoming an important area in medicinal chemistry. MAO-A inhibitors are clinically used mostly as antidepressants^{11,12}, whereas MAO-B inhibitors are

generally used in dealing with symptoms associated with Parkinson's and Alzheimer's diseases^{13,14}. Iproniazid is the first drug used in the therapy of depressive disorder during 1950s¹⁵. First-generation MAO inhibitors, such as iproniazid, imipramine and tranylcipromine, are irreversible and nonselective against MAO isoforms¹⁶. Despite their long clinical success, first-generation MAO inhibitors have a number of problems including interactions with other drugs and tyramine, which is a dietary amine and may cause a fatal hypertensive crisis^{17,18}. In view of these adverse actions and restrictions, the clinical use of first generation of MAO inhibitors have been decreased, whereas second-generation MAO inhibitors, such as selegiline and moclobemide, have come into prominence due to lack of above problems¹⁹.

In view of information mentioned previously, researchers have shown an increased interest in developing novel and potent MAO inhibitors. Structural similarity with the current drugs is one of the most used strategy for new MAO inhibitor development. The presence of arylalkylamine or benzylamine functions commonly in many MAO inhibitors may be suggested as a structural requirement for the development of a new class of MAO inhibitors (Figure 1). Resemblance in the chemical structures of MAO inhibitors and neurotransmitters or intracellular amines also cites to this requirement. Hence, it may conceivably be declared that arylalkylamine or benzylamine moieties contest with endogenous amines. This consideration has been supported by some

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Figure 1. Structure of various MAO inhibitors and synthesized compounds.



studies indicating that benzylamine moiety possesses MAO inhibitory activity^{20–22}. In addition to benzylamine, the compounds containing benzothiazole moiety have been reported to inhibit MAO enzymes²³. Besides, it has been declared that aromatic nitro compounds have an inhibition potency on MAO enzymes²⁴.

In the light of above information, herein, we report the synthesis and monoamine oxidase inhibitory activity of some novel 2-(5-substituted-benzothiazol-2-ylsulfanyl)-N-(3 or 4-nitrobenzyl)-N-(4-substitutedphenyl) acetamide derivatives (**4a–l**).

Materials and methods

All chemicals were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, OH) 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: IR, Shimadzu Affinity 1S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Bruker DPX 500 NMR spectrometer (Bruker Bioscience, Billerica, MA), in DMSO-*d*₆, using TMS as internal standard; M + 1 peaks were determined by Shimadzu LC/MS IT-TOF system (Shimadzu, Tokyo, Japan). Purity of synthesized compounds was checked by Shimadzu LC-20A prominence HPLC system, equipped with a Shimadzu DGU-14A degasser, LC-20 A dual piston pump, CTO-10 ASVP column oven and SPD-MI20A PDA detector, reodyne 7725i injection valve and a stainless steel GL Science Inertsil ODS-3 (4.6 × 250 mm) column. Mobile phase was used as solvent A, acetonitrile (95%); solvent B, water (5%) at a flow rate of 0.8 ml/min and a sample injection volume of 20 ml.

General procedure for synthesis of the compounds

Preparation of N-(3 or 4-nitrobenzylidene)-4-substituted anilines (**1a–d**)

Corresponding nitrobenzaldehyde derivative (30 mmol) appropriate four-substituted aniline (30 mmol) and catalytic amount of glacial acetic acid (0.5 ml) were refluxed in ethanol (100 ml) for 2 h. After completion of reaction, the mixture was cooled,

precipitated product was filtered and recrystallized from ethanol²⁵.

Preparation of N-(3 or 4-nitrobenzyl)-4-substituted anilines (**2a–d**)

The compounds **1a–d** (20 mmol) were dissolved in methanol (100 ml). Sodium borohydride (40 mmol, 0.76 g) was divided in to four portion (4 × 0.38 g) and added to the methanolic solution 15 min intervals. After addition of last portion, reaction mixture was allowed to stir for 1 h at room temperature. The excess of solvent was evaporated under reduced pressure, crude product was washed with water, dried and recrystallized from ethanol²⁵.

Preparation of 2-chloro-N-(3- or 4-nitrobenzyl)-N-(4-substitutedphenyl) acetamides (**3a–d**)

The compounds **2a–d** (10 mmol) were dissolved in tetrahydrofuran (50 ml) and triethylamine (11 mmol, 1.55 ml) was added. The mixture was cooled in an ice bath and chloroacetyl chloride (11 mmol, 0.88 ml) was added dropwise with stirring. After addition of chloroacetyl chloride, the reaction mixture was stirred for additional 1 h at room temperature. The solvent was evaporated under reduced pressure, product was washed with water, dried and recrystallized from ethanol²⁶.

Preparation of N-(3- or 4-nitrobenzyl)-N-(4-substitutedphenyl)-2-((5-substitutedbenzo[d]thiazol-2-yl)thio)acetamides (**4a–l**)

The compounds **3a–d** (2 mmol), appropriate 5-substituted benzothiazole-2-thiol (2 mmol) and potassium carbonate (2 mmol, 0.276 g) in acetone (40 ml) was refluxed for 12 h. After TLC screening, the solvent was evaporated under reduced pressure. The product was washed with water, dried and recrystallized from ethanol²⁶.

2-(Benzo[d]thiazol-2-yl-thio)-N-(4-chlorophenyl)-N-(4-nitrobenzyl)acetamide (**4a**)

Yield 65–67%, m.p. 119–121 °C. HPLC: 99.7% purity. IR ν_{\max} (cm⁻¹): 3104.92–3032.06 (aromatic C–H), 2951.49–2928.99 (aliphatic C–H), 1666.50 (C=O amide), 1605.98–1427.32 (C=N, C=C), 839.03 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.23 (2H, s, CO-CH₂), 5.04

(2H, s, N-CH₂), 7.38 (1H, t, *J* = 8.0 Hz, Ar-H), 7.47–7.54 (7H, m, Ar-H), 7.82 (1H, d, *J* = 8.0 Hz, Ar-H), 8.02 (1H, d, *J* = 8.0 Hz, Ar-H), 8.11 (2H, d, *J* = 8.2 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.08, 52.76, 121.54, 122.37, 123.95, 125.03, 126.82, 129.43, 130.30, 130.50, 133.46, 135.24, 140.66, 145.39, 147.19, 152.84, 166.23, 167.17. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₆ClN₃O₃S₂: 470.0394; found 470.0402.

2-((Benzo[d]thiazol-2-yl-thio)-N-(4-fluorophenyl)-N-(4-nitrobenzyl)acetamide (**4b**)

Yield 64–65%, m.p. 149–151 °C. HPLC: 98.8% purity. IR ν_{max} (cm⁻¹): 3106.14–3056.02 (aromatic C–H), 2998.51–2939.04 (aliphatic C–H), 1662.64 (C=O amide), 1616.05–1427.98 (C=N, C=C), 856.75 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.20 (2H, s, CO-CH₂), 5.03 (2H, s, N-CH₂), 7.32 (2H, m, Ar-H), 7.38 (1H, t, *J* = 8.0 Hz, Ar-H), 7.47–7.54 (5H, m, Ar-H), 7.82 (1H, d, *J* = 8.0 Hz, Ar-H), 8.02 (2H, d, *J* = 8.0 Hz, Ar-H), 8.11 (2H, d, *J* = 8.4 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.13, 52.88, 117.14 (d, *J* = 22.6 Hz), 121.52, 122.37, 123.93, 125.02, 126.81, 129.47, 130.88 (d, *J* = 8.9 Hz), 135.23, 138.07, 145.45, 147.19, 152.86, 161.93 (d, *J* = 244.4 Hz), 166.28, 167.27. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₆FN₃O₃S₂: 454.0690; found 454.0693.

2-((Benzo[d]thiazol-2-yl-thio)-N-(4-chlorophenyl)-N-(3-nitrobenzyl)acetamide (**4c**)

Yield 66–68%, m.p. 116–118 °C. HPLC: 99.8% purity. IR ν_{max} (cm⁻¹): 3116.14–3008.52 (aromatic C–H), 2989.05–2902.29 (aliphatic C–H), 1664.57 (C=O amide), 1620.48–1439.21 (C=N, C=C), 840.96 (1,4-disubstitutedbenzene), 783.10 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.21 (2H, s, CO-CH₂), 5.06 (2H, s, N-CH₂), 7.37 (1H, t, *J* = 8.0 Hz, Ar-H), 7.42–7.47 (3H, m, Ar-H), 7.53–7.57 (3H, m, Ar-H), 7.70 (1H, d, *J* = 7.0 Hz, Ar-H), 7.77 (1H, d, *J* = 8.0 Hz, Ar-H), 8.00 (1H, d, *J* = 8.0 Hz, Ar-H), 8.10–8.12 (2H, m, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.12, 52.43, 121.48, 122.32, 122.81, 123.20, 124.97, 126.80, 130.29, 130.37, 130.60, 133.45, 135.08, 135.20, 139.79, 140.53, 148.31, 152.86, 166.21, 167.21. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₆ClN₃O₃S₂: 470.0394; found 470.0402.

2-((Benzo[d]thiazol-2-yl-thio)-N-(4-fluorophenyl)-N-(3-nitrobenzyl)acetamide (**4d**)

Yield 68–70%, m.p. 105–106 °C. HPLC: 96.5% purity. IR ν_{max} (cm⁻¹): 3112.49–3028.13 (aromatic C–H), 2997.16–2914.06 (aliphatic C–H), 1660.71 (C=O amide), 1636.62–1429.25 (C=N, C=C), 840.96 (1,4-disubstitutedbenzene), 783.10 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.19 (2H, s, CO-CH₂), 5.04 (2H, s, N-CH₂), 7.30–7.38 (3H, m, Ar-H), 7.44–7.48 (3H, m, Ar-H), 7.56 (1H, t, *J* = 7.9 Hz, Ar-H), 7.69 (1H, d, *J* = 8.0 Hz, Ar-H), 7.76 (1H, d, *J* = 8.0 Hz, Ar-H), 8.00 (1H, d, *J* = 8.0 Hz, Ar-H), 8.09–8.12 (2H, m, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.18, 52.56, 117.12 (d, *J* = 22.6 Hz), 121.46, 122.30, 122.78, 123.25, 124.95, 126.78, 130.34, 130.98 (d, *J* = 8.8 Hz), 135.14, 135.19, 137.92, 139.82, 148.30, 152.88, 161.93 (d, *J* = 244.3 Hz), 166.25, 167.29. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₆FN₃O₃S₂: 454.0690; found 454.0692.

2-((5-Chlorobenzo[d]thiazol-2-yl)thio)-N-(4-chlorophenyl)-N-(4-nitrobenzyl)acetamide (**4e**)

Yield 63–65%, m.p. 175–177 °C. HPLC: 98.0% purity. IR ν_{max} (cm⁻¹): 3101.59–3009.65 (aromatic C–H), 2994.10–2895.60 (aliphatic C–H), 1664.57 (C=O amide), 1619.91–1425.40

(C=N, C=C), 840.96 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.22 (2H, s, CO-CH₂), 5.04 (2H, s, N-CH₂), 7.43–7.55 (7H, m, Ar-H), 7.87 (1H, s, Ar-H), 8.05 (1H, d, *J* = 8.7 Hz, Ar-H), 8.13 (2H, d, *J* = 8.2, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.18, 52.72, 121.00, 123.79, 123.95, 125.00, 129.54, 130.35, 130.57, 131.71, 133.49, 134.04, 140.61, 145.37, 147.21, 153.70, 166.97, 169.13. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₅Cl₂N₃O₃S₂: 504.0005; found 504.0003.

2-((5-Chlorobenzo[d]thiazol-2-yl)thio)-N-(4-fluorophenyl)-N-(4-nitrobenzyl)acetamide (**4f**)

Yield 66–68%, m.p. 192–194 °C. HPLC: 98.2% purity. IR ν_{max} (cm⁻¹): 3171.89–3046.19 (aromatic C–H), 2982.18–2893.38 (aliphatic C–H), 1664.57 (C=O amide), 1617.98–1427.32 (C=N, C=C), 842.82 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.19 (2H, s, CO-CH₂), 5.03 (2H, s, N-CH₂), 7.32 (2H, t, *J* = 8.6 Hz, Ar-H), 7.42 (1H, dd, *J* = 8.5 Hz–1.4 Hz, Ar-H), 7.50–7.54 (4H, m, Ar-H), 7.87 (1H, d, *J* = 1.4 Hz Ar-H), 8.05 (1H, d, *J* = 8.5 Hz, Ar-H), 8.13 (2H, d, *J* = 8.5 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.27, 52.86, 117.18 (d, *J* = 22.6 Hz), 120.98, 123.79, 123.93, 124.99, 129.58, 130.91 (d, *J* = 8.8 Hz), 131.70, 134.03, 138.03 (d, *J* = 2.9 Hz) 145.44, 147.20, 153.73, 162.01 (d, *J* = 244.6 Hz), 167.06, 169.21. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₅ClFN₃O₃S₂: 488.0300; found 488.0306.

2-((5-Chlorobenzo[d]thiazol-2-yl)thio)-N-(4-chlorophenyl)-N-(3-nitrobenzyl)acetamide (**4g**)

Yield 67–68%, m.p. 164–166 °C. HPLC: 97.1% purity. IR ν_{max} (cm⁻¹): 3104.51–3005.39 (aromatic C–H), 2993.03–2921.66 (aliphatic C–H), 1672.28 (C=O amide), 1629.55–1427.32 (C=N, C=C), 839.03 (1,4-disubstitutedbenzene), 785.03 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.20 (2H, s, CO-CH₂), 5.05 (2H, s, N-CH₂), 7.40–7.47 (3H, m, Ar-H), 7.53–7.57 (3H, m, Ar-H), 7.69 (1H, d, *J* = 7.4 Hz, Ar-H), 7.78 (1H, s, Ar-H), 7.53 (1H, d, *J* = 8.6 Hz, Ar-H), 8.09–8.13 (2H, m, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.21, 52.44, 120.94, 122.32, 122.81, 123.27, 124.95, 130.33, 130.63, 131.67, 133.50, 134.06, 135.18, 139.77, 140.49, 148.30, 153.71, 167.05, 169.08. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₅Cl₂N₃O₃S₂: 504.0005; found 504.0008.

2-((5-Chlorobenzo[d]thiazol-2-yl)thio)-N-(4-fluorophenyl)-N-(3-nitrobenzyl)acetamide (**4h**)

Yield 65–67%, m.p. 134–136 °C. HPLC: 99.0% purity. IR ν_{max} (cm⁻¹): 3112.77–3010.59 (aromatic C–H), 2956.40–2891.09 (aliphatic C–H), 1660.71 (C=O amide), 1604.02–1427.32 (C=N, C=C), 842.89 (1,4-disubstitutedbenzene), 785.03 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 4.18 (2H, s, CO-CH₂), 5.04 (2H, s, N-CH₂), 7.32 (2H, t, *J* = 8.6 Hz, Ar-H), 7.41–7.48 (3H, m, Ar-H), 7.58 (1H, t, *J* = 7.9 Hz, Ar-H), 7.69 (1H, d, *J* = 7.5 Hz, Ar-H), 7.79 (1H, s, Ar-H), 8.04 (1H, d, *J* = 8.6 Hz, Ar-H), 8.09 (1H, s, Ar-H), 8.13 (1H, d, *J* = 9.1 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.31, 52.56, 117.16 (d, *J* = 22.6 Hz), 120.92, 122.36, 122.80, 123.33, 124.95, 130.33, 130.98 (d, *J* = 8.8 Hz), 131.66, 133.97, 135.25, 137.89 (d, *J* = 2.3 Hz), 139.82, 148.30, 153.74, 161.92 (d, *J* = 244.2 Hz), 167.12, 169.17. HRMS (*m/z*): [M + H]⁺ calcd for C₂₂H₁₅ClFN₃O₃S₂: 488.0300; found 488.0313.

2-((5-Methoxybenzo[d]thiazol-2-yl)thio)-N-(4-chlorophenyl)-N-(4-nitrobenzyl)acetamide (**4i**)

Yield 66–68%, m.p. 163–165 °C. HPLC: >99.9% purity. IR ν_{max} (cm⁻¹): 3097.89–3004.19 (aromatic C–H), 2982.00–2818.65

(aliphatic C–H), 1660.71 (C=O amide), 1633.49–1427.32 (C=N, C=C), 840.96 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 3.85 (3H, *s*, OCH₃), 4.22 (2H, *s*, CO-CH₂), 5.05 (2H, *s*, N-CH₂), 7.02 (1H, *dd*, *J* = 8.8 Hz–2.0 Hz, Ar-H), 7.33 (1H, *d*, *J* = 2.0 Hz, Ar-H), 7.48–7.54 (6H, *m*, Ar-H) 7.87 (1H, *d*, *J* = 8.8 Hz, Ar-H), 8.13 (2H, *d*, *J* = 8.3 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.16, 52.74, 56.00, 105.04, 114.18, 122.62, 123.96, 126.77, 129.48, 130.33, 130.51, 133.47, 140.63, 145.38, 147.19, 154.19, 159.19, 167.14, 167.20. HRMS (*m/z*): [M + H]⁺ calcd for C₂₃H₁₈ClN₃O₄S₂: 500.0500; found 500.0503.

2-((5-Methoxybenzo[d]thiazol-2-yl)thio)-N-(4-fluorophenyl)-N-(4-nitrobenzyl)acetamide (4j)

Yield 66–68%, m.p. 175–177 °C. HPLC: >99.9% purity. IR ν_{max} (cm^{−1}): 3071.46–3029.03 (aromatic C–H), 2919.18–2895.38 (aliphatic C–H), 1662.64 (C=O amide), 1617.98–1419.61 (C=N, C=C), 846.75 (1,4-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 3.85 (3H, *s*, OCH₃), 4.19 (2H, *s*, CO-CH₂), 5.03 (2H, *s*, N-CH₂), 7.02 (1H, *dd*, *J* = 8.7 Hz–1.7 Hz, Ar-H), 7.30–7.33 (3H, *m*, Ar-H), 7.49–7.55 (4H, *m*, Ar-H), 7.87 (1H, *d*, *J* = 8.7 Hz, Ar-H), 8.13 (2H, *d*, *J* = 8.3 Hz, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.24, 52.86, 55.99, 105.03, 114.15, 117.16 (*d*, *J* = 22.6 Hz), 122.67, 123.94, 126.75, 129.52, 130.90 (*d*, *J* = 8.9 Hz), 138.04 (*d*, *J* = 2.6 Hz), 145.45, 147.18, 154.21, 159.18, 161.93 (*d*, *J* = 244.5 Hz), 167.16, 167.22. HRMS (*m/z*): [M + H]⁺ calcd for C₂₃H₁₈FN₃O₄S₂: 484.0796; found 484.0790.

2-((5-Methoxybenzo[d]thiazol-2-yl)thio)-N-(4-chlorophenyl)-N-(3-nitrobenzyl)acetamide (4k)

Yield 62–65%, m.p. 140–143 °C. HPLC: >99.9% purity. IR ν_{max} (cm^{−1}): 3093.67–3031.09 (aromatic C–H), 2941.18–2891.01 (aliphatic C–H), 1662.64 (C=O amide), 1625.69–1429.25 (C=N, C=C), 840.96 (1,4-disubstitutedbenzene), 783.10 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 3.84 (3H, *s*, OCH₃), 4.20 (2H, *s*, CO-CH₂), 5.05 (2H, *s*, N-CH₂), 7.00 (1H, *dd*, *J* = 8.8 Hz–1.8 Hz, Ar-H), 7.28 (1H, *d*, *J* = 1.8 Hz, Ar-H), 7.45 (2H, *d*, *J* = 8.2 Hz, Ar-H), 7.53–7.58 (3H, *m*, Ar-H) 7.69 (1H, *d*, *J* = 8.8 Hz, Ar-H), 7.85 (1H, *d*, *J* = 8.8 Hz, Ar-H), 8.10–8.13 (2H, *m*, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.22, 52.43, 56.00, 104.97, 114.17, 122.56, 122.82, 123.22, 126.71, 130.31, 130.38, 130.61, 133.47, 135.13, 139.77, 140.48, 148.31, 154.21, 159.15, 167.12, 167.20. HRMS (*m/z*): [M + H]⁺ calcd for C₂₃H₁₈ClN₃O₄S₂: 500.0500; found 500.0497.

2-((5-Methoxybenzo[d]thiazol-2-yl)thio)-N-(4-fluorophenyl)-N-(3-nitrobenzyl)acetamide (4l)

Yield 68–70%, m.p. 140–143 °C. HPLC: >99.9% purity. IR ν_{max} (cm^{−1}): 3108.25–3003.99 (aromatic C–H), 2992.71–2831.04 (aliphatic C–H), 1670.35 (C=O amide), 1619.91–1419.61 (C=N, C=C), 848.68 (1,4-disubstitutedbenzene), 783.10 (1,3-disubstitutedbenzene). ¹H-NMR (500 Mhz, DMSO-*d*₆, ppm) δ 3.83 (3H, *s*, OCH₃), 4.18 (2H, *s*, CO-CH₂), 5.04 (2H, *s*, N-CH₂), 7.00 (1H, *dd*, *J* = 8.8 Hz–2.0 Hz, Ar-H), 7.28–7.33 (3H, *m*, Ar-H), 7.46–7.47 (2H, *m*, Ar-H), 7.58 (1H, *t*, *J* = 7.9 Hz, Ar-H), 8.86 (1H, *d*, *J* = 8.8 Hz, Ar-H), 8.10–8.13 (2H, *m*, Ar-H). ¹³C-NMR (125 Mhz, DMSO-*d*₆, ppm) δ 37.31, 52.55, 55.98, 104.97, 114.13, 117.14 (*d*, *J* = 22.5 Hz), 122.54, 122.80, 123.26, 126.70, 130.35, 130.99 (*d*, *J* = 8.7 Hz), 135.20, 137.87, 139.80, 148.30, 154.24, 159.15, 161.93 (*d*, *J* = 244.5 Hz), 167.18, 167.27. HRMS (*m/z*): [M + H]⁺ calcd for C₂₃H₁₈FN₃O₄S₂: 484.0796; found 484.0786.

MAO activity assay

Enzyme activity assay was performed according to the procedure reported by Matsumoto et al.,²⁷ with slight modifications. The entire materials used in enzymatic assay were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO). All of the pipettings in the assay were performed by Biotek Precision robotic system (BioTek Instruments, Inc., Winooski, VT). In order to prevent light effect on MAO enzymes the assay was performed in 96-well black plates. Phosphate buffer (0.1 M, pH = 7.4) was used for the preparation of stock solutions (5 mg/ml) of both human recombinant MAO-A and MAO-B enzymes. Enzyme stock solutions was further diluted with assay buffer to get a final concentration 0.006 mg/ml for MAO-A and 0.015 mg/ml for MAO-B. Kynuramine was dissolved in sterilized distilled water to get stock solution (25 mM) and then diluted with assay buffer to get a final concentration of 40 μM for MAO-A enzyme and 20 μM for MAO-B enzyme. Synthesized compounds and reference drugs (moclobemide for MAO-A enzyme and selegiline for MAO-B enzyme) were diluted to 10^{−3} M and 10^{−4} M concentrations (100 μl/well) using 2% DMSO. The addition of the compounds dilutes was followed by the addition of either MAO-A or MAO-B enzyme (50 μl/well). After 10-min incubation at 37 °C, kynuramine (50 μl/well) was added and enzyme–substrate reaction was initiated. The plate was incubated for 20 min at 37 °C, and then, reaction was terminated by using of 2 N NaOH (75 μl/well). The fluorimetric read from top was performed by BioTek-Synergy H1 multimode microplate reader (BioTek Instruments, Inc., Winooski, VT) at 310/380 nm excitation/emission wavelength pair. The same procedure was followed for further concentrations (10^{−5}–10^{−9} M, 100 μl/well) of the inhibitors and selected compounds indicating ≥50% inhibition at initial concentrations (10^{−3} and 10^{−4} M, 100 μl/well). The IC₅₀ value was calculated from the plots of enzyme activity against concentrations by applying regression analyses on GraphPad Prism Version 5.

Enzyme kinetic studies

The same materials were used in MAO-inhibition assay. The compound **4h** was prepared at IC₅₀ concentration that calculated in enzyme assay and then added to the wells (100 μl/well). Stock solution (25 mM) of kynuramine 0.1 M phosphate buffer was diluted to final concentrations of 40, 20, 10, 5, 2.5 and 1.25 μM and then added to the wells (50 μl/well) that contain the test compound. The monoamine oxidase-B enzyme was added to the plate (50 μl/well) and this step was followed by a 20-min incubation period at 37 °C. The plate was read at 310/340 nm excitation/emission wavelength pair²⁸. Control measurement without inhibitor was also determined simultaneously. The results were analysed as Lineweaver–Burk plots using Microsoft Office Excel 2013.

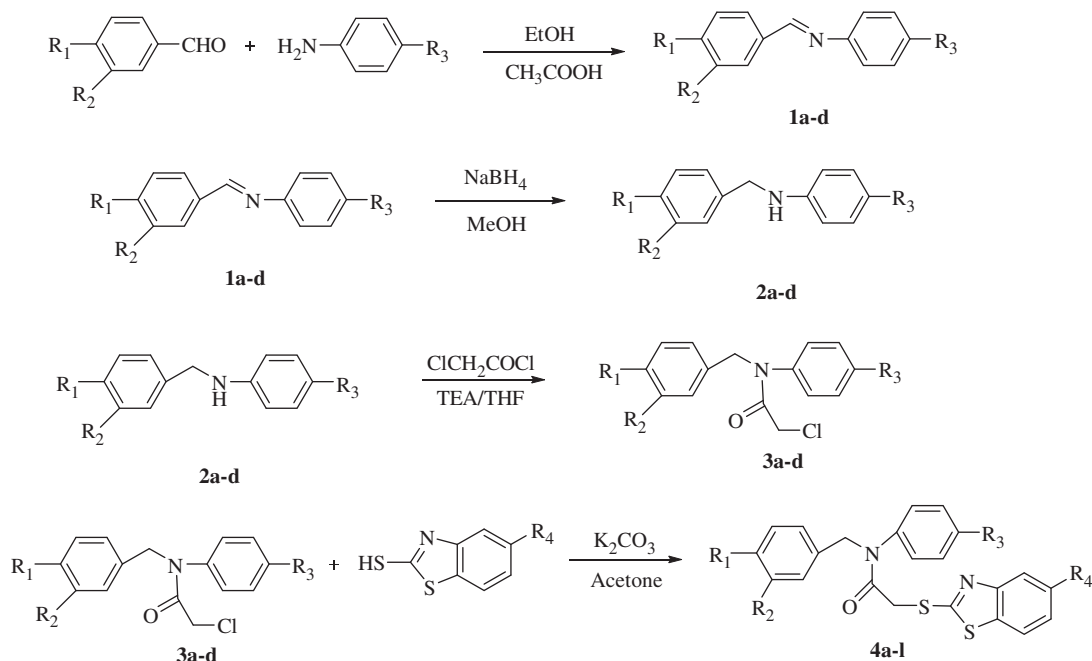
Theoretical calculation of ADME parameters

In order to evaluate pharmacokinetic profiles of the synthesized compounds, some physicochemical parameters that are used for determination of ADME properties were calculated using the Molinspiration property calculation program²⁹.

Results and discussion

Chemistry

Target molecules (**4a–g**) were synthesized in four steps as shown in Figure 2. Initially, *N*-(3 or 4-nitrobenzylidene)-4-substituted anilines (**1a–d**) were synthesized via condensation of appropriate benzaldehyde and aniline in ethanol with catalytic amount of

Figure 2. The synthetic pathway of the compounds (**4a-l**).

glacial acetic acid. Secondly, compounds **1a-d** in methanol was reduced to *N*-(3 or 4-nitrobenzyl)-4-substituted anilines (**2a-d**) by using NaBH_4 . In the third step, 2-chloro-*N*-(3- or 4-nitrobenzyl)-*N*-(4 substitutedphenyl)acetamides (**3a-d**) were synthesized by acetylation of the compounds **2a-d** in tetrahydrofuran (THF) with chloroacetyl chloride. Finally, bimolecular nucleophilic substitution ($\text{S}_{\text{N}}2$) reaction between appropriate 5-substituted benzothiazole-2-thiol derivatives and the compounds **3a-d** give the 12 new *N*-(3- or 4-nitrobenzyl)-*N*-(4-substitutedphenyl)-2-((5-substituted-benzothiazol-2-yl)thio)acetamides (**4a-l**).

Structures of the obtained compounds were elucidated by spectral data. In the IR spectra, significant stretching bands belonging to $\text{C}=\text{O}$ were observed between $1670\text{--}1660\text{ cm}^{-1}$. The stretching bands for $\text{C}=\text{N}$ and $\text{C}=\text{C}$ were recorded in the region of $1593\text{--}1419\text{ cm}^{-1}$. Stretching bands at $783\text{--}786\text{ cm}^{-1}$ and $839\text{--}857\text{ cm}^{-1}$ were observed for 1,3-disubstituted and 1,4-disubstituted benzenes, respectively. In the $^1\text{H-NMR}$ spectra, a singlet peak between $4.18\text{--}4.23\text{ ppm}$ was assigned for the protons of acetamide ($-\text{NHCOCH}_2$) moiety. A singlet peak at $5.03\text{--}5.06\text{ ppm}$ was recorded for methylene ($-\text{CH}_2$) protons of benzylamine ($-\text{NCH}_2\text{Ph}$). The methoxy ($-\text{OCH}_3$) protons in compounds **4g-l** were assigned as a singlet at about 3.84 ppm . The protons belonging to nonsubstituted benzothiazole were observed at $7.38\text{--}8.05\text{ ppm}$ as two doublets and one triplet. Substituted benzothiazole peaks were determined as two doublet and one doublet of doublet at $7.01\text{--}8.05\text{ ppm}$. Nitrobenzene protons near to nitro group observed at $8.11\text{--}8.13\text{ ppm}$ as a doublet or multiplet depending on 1,4 or 1,3-disubstituted nitrobenzene, respectively. Other aromatic protons recorded as multiplet around 7.50 ppm . In the $^{13}\text{C-NMR}$ spectra, peaks between $37.08\text{--}37.31\text{ ppm}$ and $52.43\text{--}52.88\text{ ppm}$ was observed for the methylene ($-\text{CH}_2$) carbons of acetamide ($-\text{NHCOCH}_2$) and benzylamine ($-\text{NCH}_2\text{Ph}$) moieties. The peaks belonging to methoxy ($-\text{OCH}_3$) carbons in compounds **4g-l** were observed at about 56.00 ppm . Aromatic carbons were generally observed at $104.97\text{--}162.02\text{ ppm}$. The carbonyl of acetamide and second carbon of benzothiazole were observed between $166.21\text{--}167.18\text{ ppm}$ and $169.21\text{--}167.17\text{ ppm}$, respectively. According to HPLC analysis, purity ratio was found between $96.5\text{--}99.9\%$. Peak purity index of all compounds were also checked and no impurity was determined in observed peaks.

Monoamine oxidase inhibitory activity

The synthesized 2-(5-substituted-benzothiazol-2-ylsulfanyl)-*N*-(substitutedbenzyl)-*N*-(4-substitutedphenyl)acetamide derivatives (**4a-l**) were investigated for their MAO-A and MAO-B inhibitory activity by an *in vitro* fluometric method. The fundamental of the activity determination is based on the ability of both MAO-A and MAO-B enzymes to metabolize non-fluorescent kynuramine, which is a suitable substrate for both isozymes, to the fluorescent product 4-hydroxyquinoline. Both enzymes were pre-incubated with test compounds and the MAO-inhibitory effect of the test compounds was correlated with the amount of 4-hydroxyquinoline formed. The read of the activity was performed by using proper excitation/emission wavelength pair²⁷. DMSO was considered as control (100% activity). Moclobemide and selegiline were used as reference drugs. The inhibitory activity results are listed in Tables 1 and 2.

In general, it was observed that the synthesized compounds are more potent against MAO-B enzyme when compared with MAO-A enzyme. The compounds **4h**, **4k** and **4l** displayed considerable inhibitory activity against MAO-A enzyme, whereas compounds **4c**, **4d** and **4h** indicated remarkable inhibition profile against MAO-B enzyme. Due to their inhibition potency ($>50\%$) at 10^{-3} and 10^{-4} M , related compounds were tested at further concentrations ($10^{-5}\text{--}10^{-9}\text{ M}$) and their IC_{50} values were calculated (Tables 1 and 2). Compound **4h** was the most active derivative with a $17.00\text{ }\mu\text{M}$ and $2.95\text{ }\mu\text{M}$ IC_{50} values against MAO-A and MAO-B enzymes, respectively.

The MAO-B enzyme kinetic of the most active compound **4h** was studied. The nature of MAO-B inhibition, caused by this compound, was investigated by the graphical analysis of steady-state inhibition data (Figure 3). According to reciprocal plots (Lineweaver-Burk plots) compound **4h** was found as a mixed type inhibitor, due to different intercepts on both the y- and x-axes. The values of K_{m} and V_{max} were calculated by non-linear regression according to literature²⁸ and found as 185.19 and 578.57 , respectively.

In the design of the target compounds (**4a-l**), the substitution pattern was performed on three regions to discuss substitution effect on biological activity. These were 3- or 4-nitrobenzyl,

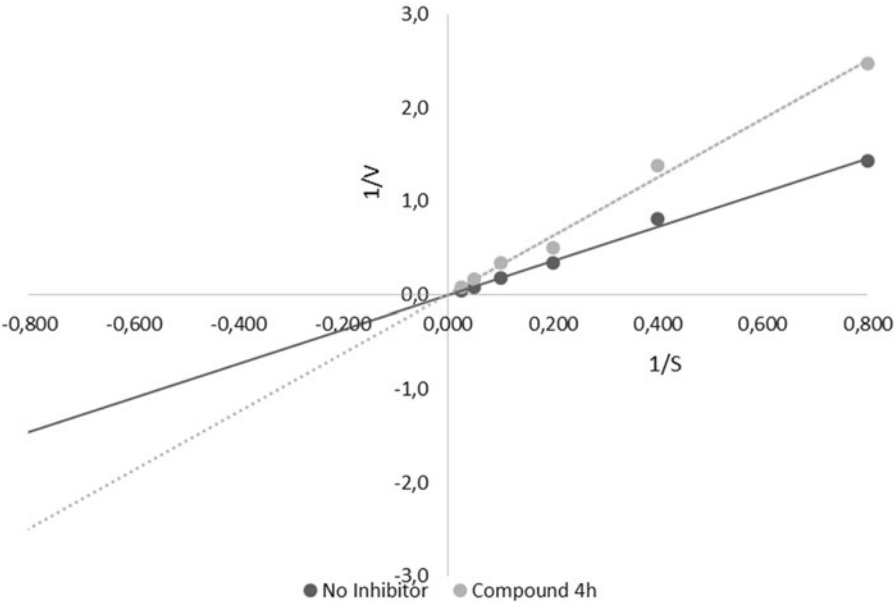
Table 1. Inhibitory activity (%) of the compounds against MAO-A enzyme.

Comp.	MAO-A Inhibition %							IC ₅₀ (μM)
	10 ^{−3} M	10 ^{−4} M	10 ^{−5} M	10 ^{−6} M	10 ^{−7} M	10 ^{−8} M	10 ^{−9} M	
4a	37.04 ± 1.95	34.09 ± 1.27	n.d	n.d	n.d	n.d	n.d	>1000
4b	49.09 ± 2.82	44.77 ± 1.78	n.d	n.d	n.d	n.d	n.d	>1000
4c	44.18 ± 1.19	39.54 ± 1.24	n.d	n.d	n.d	n.d	n.d	>1000
4d	52.23 ± 2.98	42.77 ± 2.04	n.d	n.d	n.d	n.d	n.d	>100
4e	42.32 ± 0.88	37.76 ± 1.21	n.d	n.d	n.d	n.d	n.d	>1000
4f	43.59 ± 2.52	41.57 ± 1.75	n.d	n.d	n.d	n.d	n.d	>1000
4g	64.18 ± 3.21	45.48 ± 1.53	n.d	n.d	n.d	n.d	n.d	>100
4h	71.39 ± 2.98	54.83 ± 1.96	43.15 ± 2.25	35.63 ± 1.22	28.65 ± 0.96	25.49 ± 1.57	18.77 ± 0.59	17.00 ± 0.89
4i	58.16 ± 2.45	40.69 ± 1.98	n.d	n.d	n.d	n.d	n.d	>100
4j	55.42 ± 2.13	40.96 ± 1.86	n.d	n.d	n.d	n.d	n.d	>100
4k	60.14 ± 2.66	50.82 ± 2.04	31.47 ± 1.51	27.62 ± 0.95	23.17 ± 0.86	17.48 ± 0.72	16.04 ± 0.77	26.03 ± 1.22
4l	58.37 ± 2.01	51.32 ± 1.96	36.12 ± 1.43	30.47 ± 1.21	27.67 ± 1.05	21.98 ± 0.89	20.54 ± 0.91	18.54 ± 1.04
Moclobemide	94.12 ± 2.76	82.14 ± 2.69	60.45 ± 2.55	36.15 ± 1.98	22.13 ± 1.33	18.16 ± 0.81	14.12 ± 0.72	6.06 ± 0.26

Table 2. Inhibitory activity (%) of the compounds against MAO-B enzyme.

Comp.	MAO-B Inhibition %							IC ₅₀ (μM)
	10 ^{−3} M	10 ^{−4} M	10 ^{−5} M	10 ^{−6} M	10 ^{−7} M	10 ^{−8} M	10 ^{−9} M	
4a	72.66 ± 2.13	41.91 ± 2.06	n.d	n.d	n.d	n.d	n.d	>100
4b	59.16 ± 2.11	48.16 ± 1.87	n.d	n.d	n.d	n.d	n.d	>100
4c	80.24 ± 2.77	57.80 ± 2.11	40.34 ± 1.99	28.66 ± 0.86	21.14 ± 0.77	18.77 ± 0.45	11.00 ± 0.42	24.11 ± 1.42
4d	77.24 ± 2.01	59.71 ± 1.88	42.42 ± 1.34	34.54 ± 1.32	23.22 ± 1.04	16.69 ± 0.60	13.08 ± 0.56	13.54 ± 0.70
4e	61.21 ± 1.78	45.92 ± 1.71	n.d	n.d	n.d	n.d	n.d	>100
4f	70.32 ± 1.56	38.38 ± 1.36	n.d	n.d	n.d	n.d	n.d	>100
4g	61.05 ± 1.59	45.07 ± 1.25	n.d	n.d	n.d	n.d	n.d	>100
4h	91.14 ± 1.92	76.27 ± 1.65	62.14 ± 1.43	46.47 ± 1.05	31.47 ± 0.98	27.27 ± 0.75	15.69 ± 0.62	2.95 ± 0.09
4i	58.30 ± 1.25	48.91 ± 1.12	n.d	n.d	n.d	n.d	n.d	>100
4j	53.80 ± 1.45	45.80 ± 1.43	n.d	n.d	n.d	n.d	n.d	>100
4k	71.41 ± 2.01	41.65 ± 1.76	n.d	n.d	n.d	n.d	n.d	>100
4l	68.15 ± 1.48	44.61 ± 1.28	n.d	n.d	n.d	n.d	n.d	>100
Selegiline	98.94 ± 2.06	94.73 ± 1.97	86.96 ± 1.82	79.22 ± 1.71	65.36 ± 1.12	43.27 ± 1.02	14.70 ± 0.25	0.038 ± 0.002

Figure 3. Lineweaver–Burk plots for compound **4h** (IC₅₀=2.95 μM). Substrate (kynuramine) concentrations used: 40, 20, 10, 5, 2.5 and 1.25 μM. 1/V: 1/velocity of reaction [1/(nmoles/min/mg protein)], 1/S: 1/ substrate concentration (1/μM).



4-chloro or 4-florophenyl and 5-methoxy or 5-chlorobenzothiazole-2-thiol substructures. It was observed that there is no significant relationship between enzyme inhibitory activity and substituent type on benzothiazole and phenyl rings. On the other hand, an important finding was observed with 3- or 4-nitrobenzyl fragment. It was determined that 3-nitro substitution at this part increases the inhibitory activity significantly against both enzyme types when compared 4-nitro substitution.

Table 3. Substituent pattern and some physicochemical parameters of the compounds **4a–l** used in prediction of ADME profiles.

Comp.	R_1	R_2	R_3	R_4	MW	$\log p$	TPSA	HBA	HBD	Vol	Vio
4a	–NO ₂	–H	–Cl	–H	469.98	6.18	79.03	6	0	377.78	1
4b	–NO ₂	–H	–F	–H	453.52	5.66	79.03	6	0	369.17	1
4c	–H	–NO ₂	–Cl	–H	469.98	6.15	79.03	6	0	377.78	1
4d	–H	–NO ₂	–F	–H	453.52	5.66	79.03	6	0	369.17	1
4e*	–NO ₂	–H	–Cl	–Cl	504.42	6.83	79.03	6	0	391.31	2
4f	–NO ₂	–H	–F	–Cl	487.96	6.32	79.03	6	0	382.78	1
4g*	–H	–NO ₂	–Cl	–Cl	504.42	6.81	79.03	6	0	391.31	2
4h	–H	–NO ₂	–F	–Cl	487.96	6.29	79.03	6	0	382.71	1
4i*	–NO ₂	–H	–Cl	–OCH ₃	500.00	6.21	88.26	7	0	403.32	2
4j	–NO ₂	–H	–F	–OCH ₃	483.55	5.69	88.26	7	0	394.72	1
4k*	–H	–NO ₂	–Cl	–OCH ₃	500.00	6.18	88.26	7	0	403.32	2
4l	–H	–NO ₂	–F	–OCH ₃	483.55	5.67	88.26	7	0	394.72	1

The data were determined with Molinspiration calculation software²⁷. $\log p$: octanol/water partition coefficient, TPSA: topological polar surface area, H-BA: number of hydrogen acceptor, H-BD: number of hydrogen donor, Vol: molecular volume, Vio: number of violations,

*Not possess a pharmacokinetic profile that sticks the Lipinski's rule.

Theoretical determination of ADME properties

The theoretical prediction of ADME properties (molecular weight, $\log p$, topological polar surface area (TPSA), number of hydrogen donors and acceptors, volume) of all target compounds (**4a–l**) was carried out and presented in Table 3 along with violations of Lipinski's rule^{30,31}. This rule suggests that, an orally active drug has no more than one violation. Thus, according to the data in Table 3, compounds **4e**, **4g**, **4i** and **4k** does not stick the Lipinski's rule due to two violations. On the other hand, all calculated physicochemical parameters for the compound **4h** are compatible with the Lipinski's rule except $\log p$ value. Although the $\log p$ (6.29) of compound **4h** exceeds Lipinski's limit it shows that the related compound has a lipophilic character which is suitable to cross central nervous system (CNS). Furthermore, TPSA, described to be a predictive indicator of membrane penetration, is positive (79.03) and as MAO inhibitors have to pass different membranes and reach the CNS, this supports the potential of compound **4h**.

Declaration of interest

The authors declare no conflicts of interest.

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