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ARTICLE

Synthesis of new benzothiazole derivatives bearing thiadiazole as monoamine oxidase inhibitors

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Abstract

Monoamine oxidases (MAO) are enzymes that catalyze the oxidative deamination of monoamines such as dopamine, noradrenaline, adrenaline, and serotonin. Recent studies have shown that numerous benzothiazole derivatives exhibit *h*MAO inhibitory activity in the micromolar concentration range. In this study, a novel series of benzothiazole-thiadiazole **(5a-5l)** was synthesized and characterized their chemical structures by ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy. These compounds were evaluated as inhibitors for types A and B MAO enzymes. Compounds **5f** and **5l** were the most active derivatives in the series with an IC_{50} values of 0.107 ± 0.003 and 0.128 ± 0.004, respectively. Furthermore, cytotoxicity of compounds **5f** and **5l** were investigated and found as non-cytotoxic.

1 | INTRODUCTION

Monoamine oxidase (MAO) is the FAD-dependent enzyme and is found in the outer mitochondrial membrane of the glial, neuronal other mammalian cell.[1] MAO present in the liver and intestinal epithelium and are abundant in noradrenergic nerve terminals. MAOs catalyze the oxidative deamination of exogenous and endogenous monoamines to the conforming hydrogen peroxide, aldehyde, and ammonia or substituted amine.[2] This enzymes is crucial for a regular function of synaptic neurotransmission such as serotonin (5-HT), norepinephrine (NE), and dopamine (DA).[3]

MAO consist of two isoforms namely, MAO-A and MAO-B. These isoforms have a sequence similarity of 73% but differ essentially in their inhibitor selectivity, substrate specificity, and tissue delivery.[4] The MAO-B isoform has substrate specificity for small amines like β-phenylethylamine, benzylamine while MAO-A has substrate specificity for bulkier endogenous amines, for example, serotonin, epinephrine, and NE.[5] Some substrates such as DA and tyramine are available for both enzyme isoforms.

MAOs, which play an important role in the regulation of central nervous system activity, are involved in the pathogenesis of many neurodegenerative and depressive disorders. Thus, the development of MAO inhibitors (MAOIs) characterizes a very important and beneficial approach for the treatment of various neurological and neuropsychiatric disorders.[2]

MAOIs, the first antidepressant agents to be discovered, had a number of side effects, including irreversible binding to the enzyme, and lethal food–drug interactions. New generation MAOIs, which are less toxic, selective inhibitors, reversible, have been found to be effective against a variety of neurological diseases. The MAO

enzyme is now considered to be an important drug target, and MAO-A selective inhibitors have been developed as drug candidates for the management of depression and anxiety disorders. On the other hand, MAO-B selective inhibitors have been developed as drug candidates for treatment of Alzheimer's disease and Parkinson's disease with a better safety profile than nonselective MAOIs.

The neuroprotective potential of MAOIs has been the focus of interest among researchers and neuroscientists. On the other hand, the mechanisms of action of MAOIs are not yet fully clear. Attention is now shifting towards the development of a single drug as a highly targeted agent, and this is due to the complexity of various neurogenic disorders. The expectation of these multi-targeted drugs is that they bind to different targets at the same time and therefore help in the treatment of complex neurological diseases. Computational and synthetic chemistry are tools to design ligands with properties for simultaneous inhibition of MAO enzyme and other receptors/enzymes such as cholinesterase.[6]

Heterocyclic compounds have become progressively attractive because of their potential applications in many fields. Benzothiazole belonging to an enormously important family of synthetic compounds of heterocyclic systems is a advantaged bicyclic ring system with multiple applications. Moreover, 1,3,4-thiadiazole moiety is connected to a broad spectrum of biological activities possibly due to the presence of pharmacophoric (N—C═S) moiety. 1,3,4-thiadiazole is the most extensively investigated as the sulfur atom in its ring produces an inductive effect, resulting in weak basicity and relatively high aromaticity.[7–10]

The prior studies have demonstrated that benzothiazoles have potent inhibitory activity for MAO enzymes.[11–14] In this study, a similar strategy was used for the design of target compounds. The synthesized compounds are designed to have three basic parts: (a) a 2-amino-or mercapto-substituted benzothiazole core; (b) a heteroaromatic or aromatic ring system; and (iii) a linker, including H-donor and/or H-acceptor nitrogen, between the (hetero)aromatic ring system and benzothiazole core (Figure 1).

In addition, the thiosemicarbazides (TSCs) which are open-chain counterparts of the thiadiazoles also possess MAO inhibitor activity.[11,12] TSCs, which are small molecules that can cross the blood brain barrier, can be used as potential drugs for the treatment of neurodegenerative diseases due to these properties. Moreover, it has been reported that TSCs have some common features as H-bond donors, H-bond acceptors, positive ionizable atoms, hydrophobic substructures, and aromatic rings to interact with MAO enzymes.[15,16]

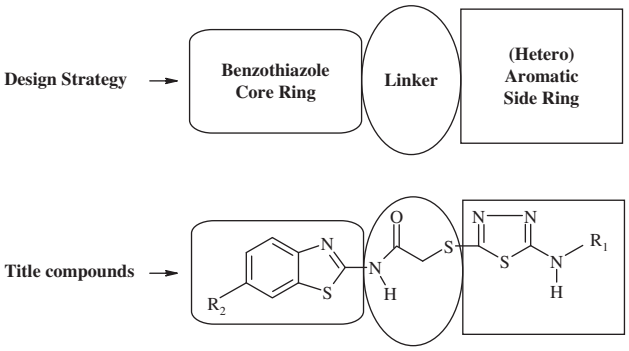


FIGURE 1 Design of the target compounds

Based on this data and our previous studies,[13,14,17] new benzothiazole derivatives as MAOIs were designed, synthesized, and biological activities were evaluated in this study.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

In this study, we have synthesized new compounds, which possessed benzothiazole-thidiazole moiety. The synthesis procedure was carried out via five steps. And synthetic pathway was outlined in Figure 2. First, suitable isothiocyanate and excess of hydrazine hydrate were reacted in order to obtained compounds **1a-1c**. Then, compounds **1a-1j** and carbondisulfide were reacted to gain thiadiazoles (**2a-2c**). Then, 5-substituebenzothiazol-2-amine (**3a-3d**) was obtained via ring closure reaction. The obtained benzothiazole-2-amine derivatives (**3a-3d**) were acetylated with chloroacetyl chloride. Finally, target compounds (**5a-5l**) were obtained with the reaction between compounds **2a-2c** and compounds **4a-4d**. The final compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, and LCMSMS) (see Data S1). The ^{1}H-NMR spectra of compounds showed signals at 4.03 to 4.20 ppm for methylene proton. Benzothiazole N—H had peaks between 12.62 ppm and 13.12 ppm. Thiadiazole NH gave peaks between 7.81 ppm and 7.93 ppm. In the ^{13}C NMR spectrum, aliphatic peaks belonging to substituents were observed between 11.79 ppm and 70.37 ppm. Aromatic carbons were gained between 119.61 ppm and 170.45 ppm. All masses were accorded with the estimated M + H values.

2.2 | Biological activity

The synthesized compounds were evaluated for their *h*MAO-A and *h*MAO-B inhibitory activities. Selegiline

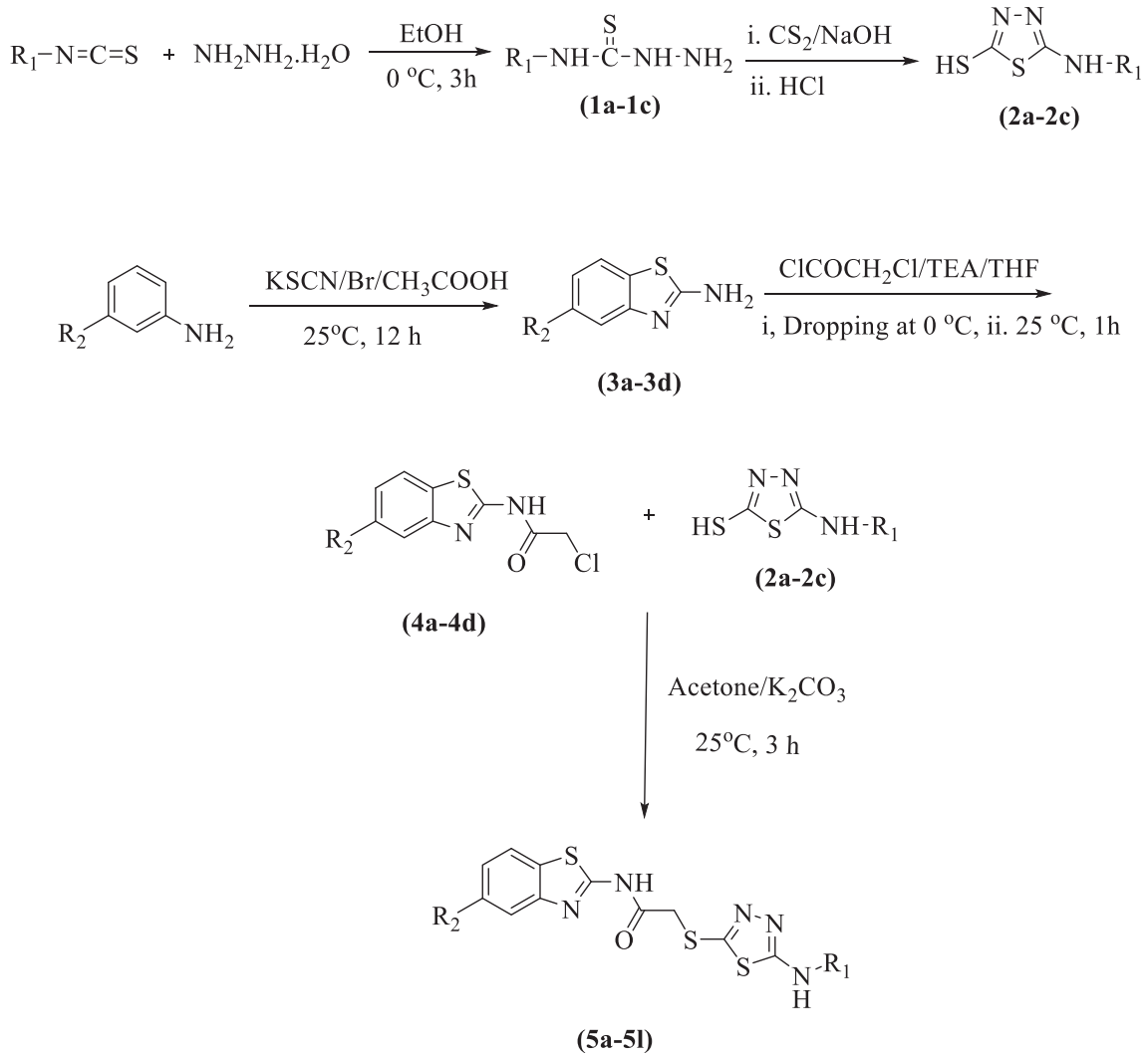


FIGURE 2 The general synthetic pathway of the target compounds (**5a-5l**)

and moclobemide were evaluated as reference compounds. The results, expressed as % inhibition and IC_{50} values are summarized in Tables 1 and 2. In the first step, the synthesized compounds were prepared at concentrations of 10^{-3} ve 10^{-4} M and inhibition values of MAO-A and MAO-B were calculated. The compounds showing >50% inhibition were selected for the second stage according to the first step results. In this second step, inhibition values and IC_{50} values of the selected compounds at concentrations of 10^{-5} to 10^{-9} M were calculated. Only compounds **5f** and **5l** passed the second step *h*MAO-A enzyme inhibition assay.

Generally, the compounds showed higher activity against *h*MAO-A than *h*MAO-B. In particular, compounds **5f** and **5l** have a significant activity value against *h*MAO-A. Compounds **5f** and **5l** showed efficient inhibition against *h*MAO-A with IC_{50} values of 0.128 ± 0.004 μM, 0.107 ± 0.003 μM, respectively. Both of them displayed more potent activity compared to reference drug. When the active compounds are examined, it is seen that both of them carry nitro groups on the benzothiazole ring. This finding proves the reported MAO inhibitory effect of the nitro group. However, compounds **5c** and **5i** did not show high activity even though they contained nitro group. The effect of this may be related to the substituent on the thiadiazole ring. In view of the active compounds, their structures include methoxyethyl and butyl groups, respectively. So, a 4-membered chain is attached to the amine of the thiadiazole ring. In this case, the 4-membered thiadiazole amine-bound group has a positive effect on activity.

2.3 | Cytotoxicity test

It is also important that a compound has minimal side effects and cytotoxicity. For this purpose, the cytotoxic effects of the most active compounds (**5f** and **5l**) on the

Comp.	MAO-A Inhibition (%)		MAO-B Inhibition (%)	
	10^{-3} M	10^{-4} M	10^{-3} M	10^{-4} M
5a	28.29 ± 0.95	21.53 ± 0.84	25.12 ± 0.62	18.11 ± 0.54
5b	41.44 ± 0.71	32.58 ± 0.55	36.65 ± 0.89	28.51 ± 0.60
5c	39.55 ± 0.67	28.61 ± 0.46	48.29 ± 0.97	26.11 ± 0.84
5d	41.58 ± 0.80	30.29 ± 0.47	24.50 ± 0.62	17.55 ± 0.48
5e	35.20 ± 0.56	30.29 ± 0.49	31.28 ± 0.49	20.23 ± 0.44
5f	**88.03 ± 1.39**	**83.30 ± 1.40**	39.55 ± 0.77	24.66 ± 0.63
5g	45.39 ± 0.99	40.21 ± 0.81	38.20 ± 0.57	19.67 ± 0.44
5h	49.33 ± 0.79	39.10 ± 0.88	36.79 ± 0.63	31.77 ± 0.59
5i	46.98 ± 0.92	42.75 ± 0.81	43.29 ± 0.85	25.18 ± 0.41
5j	23.02 ± 0.58	15.95 ± 0.55	44.07 ± 0.75	36.99 ± 0.88
5k	41.83 ± 0.89	36.74 ± 0.71	26.33 ± 0.65	20.44 ± 0.53
5l	**81.20 ± 1.05**	**74.25 ± 1.10**	29.82 ± 0.63	23.67 ± 0.51
Moclobemide	94.12 ± 2.76	82.14 ± 2.69	-	-
Selegiline	-	-	98.91 ± 1.28	96.88 ± 1.31

Note: The most effective derivatives are shown in bold.

TABLE 1 % Inhibition of compounds **5a-5l**, moclobemide and selegiline against MAO-A and MAO-B

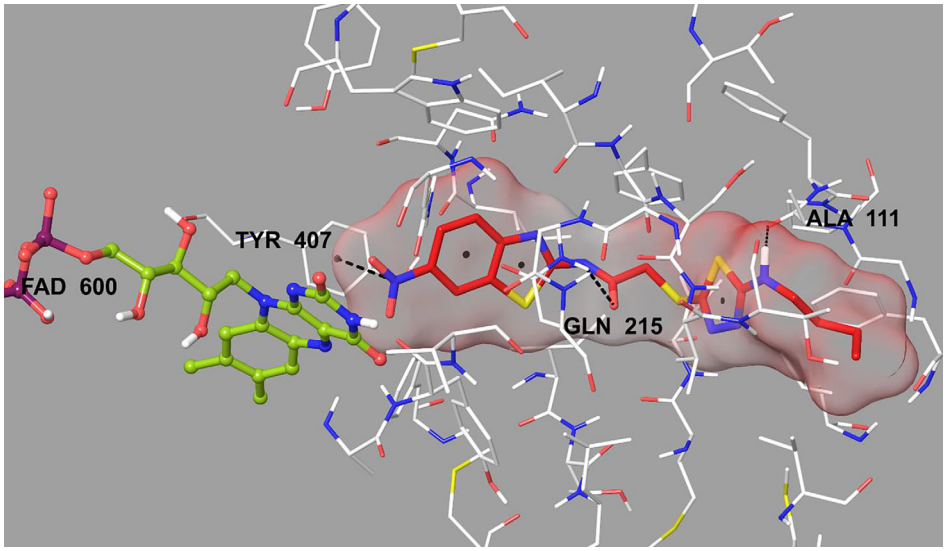


FIGURE 3 The interacting mode of compound **5l** in the active region of *h*MAO-A. The inhibitor, colored with red, and the important residues in the active site of the enzyme are presented by tube model. The FAD molecule is colored green with ball and stick model

NIH3T3 cell line were investigated. Compounds **5f** and **5l** displayed non-cytotoxicity. The IC_{50} value of the **5f** was calculated as 0.316 ± 0.013 μM. And the IC_{50} of compound **5I** was found to be greater than one.

2.3.1 | Molecular docking studies

The compound **5l** was found to be the most active and selective *h*MAO-A inhibitor as mentioned in MAO inhibition assay. Docking studies were conducted to learn more about the binding modes of compound **5l** and to evaluate the effects of structural modifications on the inhibitory activity against *h*MAO-A. X-ray crystal structure of *h*MAO-A (PDB ID: 2Z5X) [18] was obtained from Protein Data Bank server (www.pdb.org). The docking pose of compound **5l** on *h*MAO-A is presented in Figure 3.

The compounds **5l** adequately binds to amino acid residues lining the cavity and are located very near the FAD cofactor. When analyzed docking pose of this compound, it is clearly seen that nitrogen atom of nitro moiety at sixth position of benzothiazole ring is in interaction with phenyl of Tyr407 by doing cation–π interaction. A hydrogen bond formation is seen between carbonyl of amide group near to benzothiazole ring and amino of Gln215. Also, another hydrogen bond observed is belonging to amino group between thiadiazole ring

TABLE 2 IC_{50} values of **5f**, **5l** and moclobemide against MAO-A

Comp.	MAO-A Inhibition (%)							MAO-A IC_{50} (μM)
	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	
5f	81.20 ± 1.05	74.25 ± 1.10	66.35 ± 0.97	57.17 ± 0.86	48.95 ± 0.82	41.28 ± 0.49	34.85 ± 0.57	0.128 ± 0.004
5l	88.03 ± 1.39	83.30 ± 1.40	75.59 ± 1.06	66.38 ± 1.00	42.77 ± 0.87	38.63 ± 0.75	31.52 ± 0.68	0.107 ± 0.003
Moclobemide	94.121 ± 2.760	82.143 ± 2.691	60.458 ± 2.559	36.151 ± 1.984	22.135 ± 1.337	18.166 ± 0.812	14.128 ± 0.725	6.061 ± 0.262

and butyl alkyl chain. This amino group forms a hydrogen bond with carbonyl of Ala111. Besides, it is thought that the presence of extended alkyl chain such as butyl substituents of this amino group contribute to bind to active site positively via van der Waals interactions. All these findings could help to explain the potent in vitro inhibition profile of compound **5l**.

3 | EXPERIMENTAL

3.1 | Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. Spectroscopic data were recorded with the following instruments: ^{1}H-NMR (nuclear magnetic resonance) Bruker DPX 300 FT-NMR spectrometer; ^{13}C-NMR, Bruker DPX 75 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) and M + 1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan). Elemental analyses were performed on a Leco 932 CHNS analyzer (Leco, Michigan, USA).

3.1.1 | Preparation of *N*-substitutedhydrazinecarbothioamides (1a-1c)

A mixture of suitable isothiocyanate derivatives (0.02 mol) and hydrazine hydrate (0.04 mol) were stirred in ethanol (30 mL) for 4 h. After completion of the reaction, the precipitated product was filtered and washed with cold-ethanol.

3.1.2 | Preparation of 5-(substituteamino)-1,3,4-thiadiazole-2-thiols (2a-2c)

Carbon disulfide (0.019 mol) was added in a solution of compounds **1a**-**1c** (0.018 mol) in EtOH in presence of sodium hydroxide (0.019 mol) and then the mixture was refluxed for 8 h.

After this period, the solution was cooled and acidified to pH 4 to 5 with hydrochloric acid solution and crystallized from ethanol.

3.1.3 | Preparation of 5-substituebenzothiazol-2-amine (3a-3d)

3-substitueanilines (0.01 mol) and potassium thiocyanate (0.01 mol) were dissolved in acetic acid. This mixture was cooled in an ice bath and brome solution (0.012 mol) in acetic acid was added dropwise with stirring. After completion of the reaction, the precipitated product was filtered and dissolved with water at 100°C. After cooling, the heated mixture was neutralized with ammonia. The precipitated product was filtered and crystallization with ethanol.

3.1.4 | Preparation of 2-chloro-*N*-(5-substituebenzothiazol-2-yl)acetamide (4a-4d)

5-Substituebenzothiazol-2-amine (0.01 mol) and triethyl amine (0.011 mol) were dissolved in tetrahydrofuran. This mixture was cooled in an ice bath and chloroacetyl chloride (0.012 mol) in tetrahydrofuran was added dropwise with stirring. After completion of reaction, tetrahydrofuran was removed under reduced pressure, precipitated product was washed with water in order to removed obtained salts.

3.1.5 | Preparation of target compounds (5a-5l)

2-chloro-*N*-(5-substituebenzothiazol-2-yl)acetamide (**4a-4d**) (0.0013 mol), 5-(substituteamino)-1,3,4-thiadiazole-2-thiols **(2a-2c)** (0.0013 mol) and potassium carbonate (0.0013 mol) as catalyst were stirred for 4 h in acetone. After completion of the reaction, acetone was evaporated under reduced pressure. The precipitated product was washed with water in order to removed potassium carbonate, dried and recrystallized from EtOH. The synthesized derivatives are presented in Table 3.

3.1.6 | *N*-(benzothiazol-2-yl)-2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl) thio)acetamide (5a)

Yield: 81%, M.P. = 242.6 to 244.9°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 1.13 (t, 3H, J = 7.2 Hz, —CH_3), 3.25 (q, 2H, J = 5.4 Hz, —$\underline{CH_2}CH_3$—), 4.16 (s, 2H, —CH_2—), 7.32 (td, 1H, J_1 = 0.9 Hz, J_2 = 7.0 Hz, H-benzothiazole), 7.45 (td, 1H, J_1 = 1.0 Hz, J_2 = 7.1 Hz, H-benzothiazole), 7.77 (d, 1H, J = 7.9 Hz, H-benzothiazole), 7.83 (t, 1H, J = 5.3 Hz, —NH), 7.99 (d, 1H, J = 7.8 Hz, H-benzothiazole), 12.64 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 14.64, 38.00, 39.83, 121.11, 122.24, 124.16, 126.67, 131.91, 148.61, 148.95, 158.21, 167.78, 170.22. ESI-MS $[M + H]^+$: 352.

3.1.7 | *N*-(6-chlorobenzothiazol-2-yl)-2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl) thio)acetamide (5b)

Yield: 84%, M.P. = 281.1 to 282.8°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 1.13 (t, 3H, J = 7.1 Hz, —CH_3), 3.19 to 3.28 (m, 2H, —$\underline{CH_2}CH_3$—), 4.15 (s, 2H, —CH_2—), 7.46 (d, 1H, J = 8.6 Hz, H-benzothiazole), 7.75 (d, 1H, J = 8.6 Hz, H-benzothiazole), 7.81 (br.s., 1H, —NH), 8.13 (br.s., 1H, H-benzothiazole), 12.71 (1H, s, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 14.63, 37.92, 121.97, 122.35, 127.03, 128.02, 133.61, 147.85, 148.56, 159.01, 167.98, 170.22. ESI-MS $[M + H]^+$: 385.

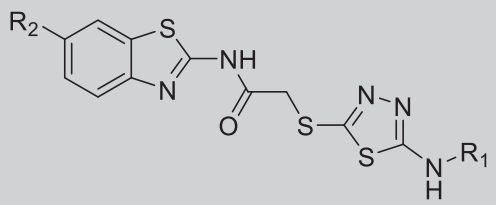
3.1.8 | 2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(6-nitrobenzothiazol-2-yl)acetamide (5c)

Yield: 79%, M.P. = 282.9 to 283.9°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 1.13 (t, 3H, J = 7.1 Hz, —CH_3), 3.22 to 3.26 (m, 2H, —$\underline{CH_2}CH_3$—), 4.20 (s, 2H, —CH_2—), 7.85 (br. s., 1H, —NH), 7.91 (d, 1H, J = 8.9 Hz, H-benzothiazole), 8.28 (d, 1H, J = 8.8 Hz, H-benzothiazole), 9.06 (br.s., 1H, H-benzothiazole), 13.04 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 14.62, 37.93, 119.61, 121.23, 122.30, 132.66, 143.55, 148.57, 153.84, 163.71, 168.55, 170.21. ESI-MS $[M + H]^+$: 397.

3.1.9 | *N*-(benzothiazol-2-yl)-2-((5-((2-methoxyethyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (5d)

Yield: 78%, M.P. = 241.2 to 243.0°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 3.24 (s, 3H, —OCH_3), 3.39 to 3.47 (m, 4H, —CH_2CH_2—), 4.16 (s, 2H, —CH_2—), 7.31 (td, 1H, J_1 = 1.1 Hz, J_2 = 6.9 Hz, H-benzothiazole), 7.44 (td, 1H, J_1 = 1.2 Hz, J_2 = 7.3 Hz, H-benzothiazole), 7.76 (d, 1H, J = 7.9 Hz, H-benzothiazole), 7.93 (t, 1H, J = 5.3 Hz, —NH), 7.99 (d, 1H, J = 7.4 Hz, H-benzothiazole), 12.64 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 37.89, 44.40, 58.40, 70.37, 121.14, 122.25, 124.18, 126.68, 131.91, 148.96, 149.03, 158.14, 167.73, 170.16. ESI-MS $[M + H]^+$: 382.

TABLE 3 Synthesized compounds **(5a-5l)**



Compounds	R_1	R_2
5a	-Ethyl	—H
5b	-Ethyl	—Cl
5c	-Ethyl	—NO_2
5d	-Methoxyethyl	—H
5e	-Methoxyethyl	—Cl
5f	-Methoxyethyl	—NO_2
5g	-Propyl	—H
5h	-Propyl	—Cl
5i	-Propyl	—NO_2
5j	-Butyl	—H
5k	-Butyl	—Cl
5l	-Butyl	—NO_2

3.1.10 | *N*-(6-chlorobenzothiazol-2-yl)-2-((5-((2-methoxyethyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (5e)

Yield: 80%, M.P. = 258.9 to 261.5°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 3.24 (s, 3H, -OCH_3), 3.39 to 3.45 (m, 4H, -CH_2CH_2-), 4.16 (s, 2H, —CH_2—), 7.47 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.6 Hz, H-benzothiazole), 7.76 (d, 1H, J = 8.6 Hz, H-benzothiazole), 7.93 (t, 1H, J = 5.3 Hz, —NH), 8.14 (d, 1H, J = 2.0 Hz, H-benzothiazole), 12.74 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 37.83, 44.39, 58.40, 70.36, 121.99, 122.36, 127.04, 128.21, 133.61, 147.86, 148.96, 159.02, 167.97, 170.16. ESI-MS $[M + H]^+$: 416.

3.1.11 | 2-((5-((2-methoxyethyl)amino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(6-nitrobenzothiazol-2-yl)acetamide (5f)

Yield: 77%, M.P. = 263.0 to 264.7°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 3.24 (s, 3H, —OCH_3), 3.38 to 3.45 (m, 4H, —CH_2CH_2—), 4.20 (s, 2H, —CH_2—), 7.89 to 7.92 (m, 2H, H-benzothiazole —NH), 8.28 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.9 Hz, H-benzothiazole), 9.05 (d, 1H, J = 2.3 Hz, H-benzothiazole), 13.04 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 37.99, 44.42, 58.40, 70.38, 119.55, 121.16, 122.27, 132.69, 143.48, 148.91, 153.92, 163.92, 168.68, 170.19. ESI-MS $[M + H]^+$: 427.

3.1.12 | *N*-(benzothiazol-2-yl)-2-((5-(propylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (5g)

Yield: 82%, M.P. = 232.6 to 234.7°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.87 (t, 3H, J = 7.3 Hz —CH_3), 1.53 (q, 2H, J = 7.1 Hz, —CH_2—), 3.18 (q, 2H, J = 6.9 Hz, —CH_2—), 4.15 (s, 2H, —CH_2—), 7.32 (td, 1H, J_1 = 1.1 Hz, J_2 = 6.9 Hz, H-benzothiazole), 7.45 (td, 1H, J_1 = 1.2 Hz, J_2 = 7.3 Hz, H-benzothiazole), 7.76 (d, 1H, J = 7.9 Hz, H-benzothiazole), 7.84 (t, 1H, J = 5.4 Hz, —NH), 7.99 (d, 1H, J = 7.4 Hz, H-benzothiazole), 12.62 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 11.79, 22.19, 37.97, 46.81, 121.14, 122.25, 124.18, 126.68, 143.29, 148.48, 149.05, 158.13, 167.75, 170.45. ESI-MS $[M + H]^+$: 366.

3.1.13 | *N*-(6-chlorobenzothiazol-2-yl)-2-((5-(propylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (5h)

Yield: 78%, M.P. = 265.5 to 267.0°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.87 (t, 3H, J = 7.4 Hz —CH_3), 1.53 (q, 2H, J = 7.2 Hz, —CH_2—), 3.17 (q, 2H, J = 6.9 Hz, —CH_2—), 4.15 (s, 2H, —CH_2—), 7.46 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.6 Hz, H-benzothiazole), 7.75 (d, 1H, J = 8.6 Hz, H-benzothiazole), 7.84 (t, 1H, J = 5.3 Hz, —NH), 8.13 (d, 1H, J = 2.1 Hz, H-benzothiazole), 12.72 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 11.79, 22.18, 37.94, 46.81, 121.97, 122.34, 127.02, 128.21, 133.62, 147.85, 148.41, 159.04, 168.01, 170.45. ESI-MS $[M + H]^+$: 400.

3.1.14 | *N*-(6-nitrobenzothiazol-2-yl)-2-((5-(propylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (5i)

Yield: 75%, M.P. = 265.5 to 267.6°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.86 (t, 3H, J = 7.3 Hz —CH_3), 1.52 (q, 2H, J = 7.2 Hz, —CH_2—), 3.17 (q, 2H, J = 6.8 Hz, —CH_2—), 4.19 (s, 2H, —CH_2—), 7.84 (t, 1H, J = 5.3 Hz, —NH), 7.91 (d, 1H, J = 8.9 Hz, H-benzothiazole), 8.28 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.9 Hz, H-benzothiazole), 9.06 (d, 1H, J = 2.3 Hz, H-benzothiazole), 13.04 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 11.79, 22.18,

38.03, 46.81, 119.57, 121.17, 122.28, 132.68, 143.50, 148.34, 153.90, 163.88, 168.66, 170.45. ESI-MS [M + H]$^+$: 411.

3.1.15 | *N*-(benzothiazol-2-yl)-2-((5-(butylamino)-1,3,4-thiadiazol-2-yl) thio)acetamide (5j)

Yield: 81%, M.P. = 229.6 to 231.4°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.86 (t, 3H, J = 7.3 Hz —CH_3), 1.30 (q, 2H, J = 7.6 Hz, —CH_2—), 1.44 to 1.54 (m, 2H, —CH_2—), 3.20 (q, 2H, J = 6.9 Hz, —CH_2—), 4.15 (s, 2H, —CH_2—), 7.31 (td, 1H, J_1 = 1.1 Hz, J_2 = 8.1 Hz, H-benzothiazole), 7.44 (td, 1H, J_1 = 1.3 Hz, J_2 = 7.3 Hz, H-benzothiazole), 7.76 (d, 1H, J = 7.9 Hz, H-benzothiazole), 7.83 (t, 1H, J = 5.4 Hz, —NH), 7.99 (d, 1H, J = 7.3 Hz, H-benzothiazole), 12.62 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 14.07, 19.97, 30.96, 37.99, 44.68, 121.11, 122.25, 124.15, 126.66, 131.91, 148.48, 148.95, 158.23, 167.81, 170.39. ESI-MS [M + H]$^+$: 380.

3.1.16 | 2-((5-(butylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(6-chlorobenzothiazol-2-yl)acetamide (5k)

Yield: 79%, M.P. =239.0 to 240.9°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.87 (t, 3H, J = 7.3 Hz —CH_3), 1.31 (q, 2H, J = 7.8 Hz, —CH_2—), 1.46 to 1.53 (m, 2H, —CH_2—), 3.20 (q, 2H, J = 6.9 Hz, —CH_2—), 4.03 (s, 2H, —CH_2—), 7.33 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.6 Hz, H-benzothiazole), 7.57 (d, 1H, J = 8.6 Hz, H-benzothiazole), 7.82 (t, 1H, J = 5.3 Hz, —NH), 7.93 (d, 1H, J = 2.1 Hz, H-benzothiazole). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz): δ = 14.09, 19.99, 30.99, 39.38, 44.64, 121.18, 121.27, 125.95, 126.51, 134.35, 148.89, 149.59, 157.75, 163.44, 170.02. ESI-MS [M + H]$^+$: 414.

3.1.17 | 2-((5-(butylamino)-1,3,4-thiadiazol-2-yl)thio)-*N*-(6-nitrobenzothiazol-2-yl)acetamide (5l)

Yield: 82%, M.P. = 239.1 to 241.5°C, ^{1}H-NMR (DMSO-d_6, ppm, 300 MHz,): 0.86 (t, 3H, J = 7.3 Hz —CH_3), 1.30 (q, 2H, J = 7.6 Hz, —CH_2—), 1.44 to 1.52 (m, 2H, —CH_2—), 3.20 (q, 2H, J = 6.8 Hz, —CH_2—), 4.17 (s, 2H, —CH_2—), 7.84 to 7.92 (m, 3H, H-benzothiazole, —NH), 8.26 (dd, 1H, J_1 = 2.3 Hz, J_2 = 8.9 Hz, H-benzothiazole), 9.01 (d, 1H, J = 2.3 Hz, H-benzothiazole), 13.12 (s, 1H, —NH). ^{13}C-NMR (DMSO-d_6, ppm, 75 MHz,): δ = 14.07, 19.98, 30.96, 38.57, 44.64, 119.35, 120.79, 122.14, 132.79, 143.11, 148.72, 154.33, 165.12, 169.42, 170.28. ESI-MS [M + H]$^+$: 425.

3.2 | MAO-A and MAO-B inhibition assay

The inhibitory activities of the obtained compounds against MAO-A and MAO-B enzymes were evaluated in black-bottom 96-well plates by method as defined in our previous studies.[17,19,20]

3.2.1 | Cytotoxicity test

Metabolic activity of viable cells were measured by MTT assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt to formazan product, which can be quantified spectrophotometrically to determine percent of viable cells. The cytotoxicity of compounds **5a** and **5l** were screened according to the MTT assay. The MTT assay was performed as previously described.[21,22] NIH3T3 cell line was used in the MTT assay.

3.2.2 | Molecular docking studies

A structure based in silico procedure was applied to discover the binding modes of compound **5l** to *h*MAO-A enzyme active site. The crystal structures of *h*MAO-A (PDB ID: 2Z5X),[18] which was crystallized with harmine, was retrieved from the Protein Data Bank server (www.pdb.org).

The structures of ligands were built using the *Schrödinger Maestro* [23] interface and then were submitted to the *Protein Preparation Wizard* protocol of the *Schrödinger Suite 2016 Update 2*.[24] The ligands were prepared by the *LigPrep 3.8* [25] to assign the protonation states at pH 7.4 ± 1.0 and the atom types, correctly. Bond orders were assigned, and hydrogen atoms were added to the structures. The grid generation was formed using *Glide 7.1*.[26] The grid box with dimensions of 20 Å x 20 Å x 20 Å was centered in the vicinity of the flavin (FAD) N5 atom on the catalytic site of the protein to cover all binding sites and neighboring residues.[27–29] Flexible docking runs were performed with single precision docking mode (SP).

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CONFLICT OF INTEREST

Authors have no conflict of interest regarding this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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