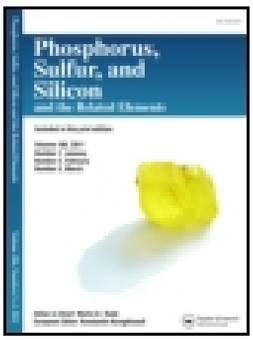

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Synthesis and monoamine oxidase A/B inhibitory evaluation of new benzothiazole-thiazolylhydrazine derivatives

Gülhan Turan[a], Derya Osmaniye[a,b], Begüm Nurpelin Sağlik[a,b], Ulviye Acar Çevik[a,b], Serkan Levent[a,b], Betül Kaya Çavuşoğlu[a,b], Ümide Demir Özkay[c], Yusuf Özkay[a,b], and Zafer Asım Kaplancikli[a]

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ABSTRACT

In this study, a novel series of benzothiazole-thiazolylhydrazine **(3a–3i)** was synthesized and their structures were characterized by ^{1}H-NMR, ^{13}C-NMR spectrometry, and mass spectroscopy. These compounds were evaluated as inhibitors of type A and type B monoamine oxidase (MAO) enzymes. The most active compound **3b** (*2-((2-(2-(4-(4-Nitrophenyl)thiazol-2-yl)hydrazineylidene)-2-phenylethyl)thio)-benzothiazole*) showed strong inhibitory activity at *h*MAO-A (IC_{50} of 0.095 ± 0.004 μM). Furthermore, compound **3i** (*2-((2-(2-(4-(2,4-dichlorophenyl)thiazol-2-yl)hydrazineylidene)-2-phenylethyl)thio)benzo-thiazole*) showed significant inhibition profile on *h*MAO-A with the IC_{50} values 0.141 ± 0.006 μM.

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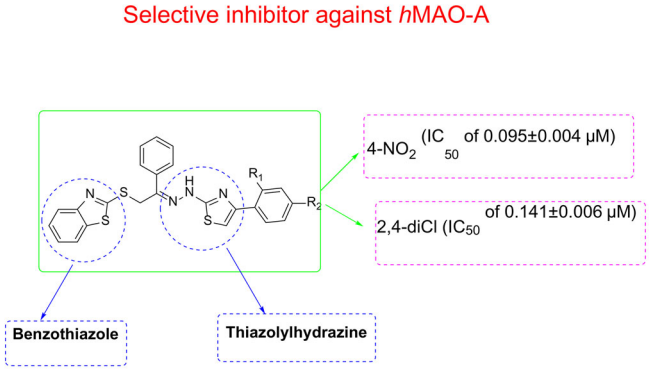
Benzothiazole; thiazolylhydrazine; monoamine oxidase

GRAPHICAL ABSTRACT



Introduction

Monoamine oxidase (MAO; EC 1.4.3.4) is a flavoprotein found in the outer membrane of mitochondria in neuronal, glial, and other cells. MAO is copiously located not only in noradrenergic nerve terminals, but also in various other tissues such as liver, intestinal mucosa, fibroblasts, and platelets [1, 2]. The function of monoamine oxidases (MAOs) in brain neurochemistry is mainly related to the oxidative deamination of biogenic amines (BA). Amines such as adrenaline, noradrenaline, melatonin, and serotonin are principally metabolized by MAO-A, whereas benzylamine and phenylethylamine are controlled by MAO-B. Both types of MAO isoforms have common substrates such as dopamine and tyramine [3–5]. The MAO-A and MAO-B are products of different genes. The MAOs are almost 70% the same at the amino acid sequence level and possess highly similar active site structures. Among 16 active site residues, only 6 differ between MAO-A and MAO-B [6, 7]. Despite the similarities in structure and function of the two isoforms, MAO-A and MAO-B have different substrate and inhibitor specificities. MAO-A inhibitors are associated with the treatment of depression and anxiety, and MAO-B inhibitors are associated with the treatment of Parkinson's and Alzheimer's disease. It is known that undesirable side effects occur when using nonselective MAO inhibitors. These side effects are listed as hepatotoxicity and hypertension, caused by the consumption of tyramine-rich food, resulting in the so-called "cheese effect." Based on these reasons, there currently exists the need for the development of compounds that are selective inhibitors of MAO [8–10]. Although some MAO-A inhibitors, including phenelzine, isocarboxazid,

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tranylcypromine, iproniazid, moclobemide, and toloxatone, have made great contributions to the depressive patients, clinic usage of MAO-A inhibitors showed some limitations due to introduction of other classes of drugs. As a result, the development of new generation of MAO-A inhibitors attracted significant attention recently, aiming at safer and better-tolerated therapeutic agents [11–13].

Hydrazine–hydrazone moiety (–NH–N[dbond]C–) and their derivatives are a versatile class of compounds with interesting biological properties. The most wanted characteristics of hydrazones include simplicity of preparation, increased hydrolytic stability relative to imines, and affinity to crystallinity. These structural fragments are mainly responsible for the physical and chemical properties of the hydrazones [10, 14, 15]. The well-known biological property of hydrazine moiety is MAO inhibitor activity. The classical period of the MAO inhibitors started with hydrazine derivatives. Developed as tuberculostatic drug, iproniazid is the first modern antidepressant. Numerous compounds among the great variety of substituted hydrazines behave as MAO inhibitors. A common structural feature of substrates and inhibitors is an amino or imino group that is assumed to play an essential role in orientation and complex formation at the active site of the enzyme [16–18]. Literature studies show that arylalkylhydrazines inhibit both isoenzymes. Only the mechanism of inhibition differs. For example, the inhibition between arylalkylhydrazines and MAO-B results in irreversible alkylation at the N5 position of the flavin ring. Because of the serious side effects of iproniazid with irreversible and nonselective properties, a large number of substituted hydrazines have been studied as *h*MAO inhibitors to discover reversible and safer agents [19, 20].

Many thiazolylhydrazines show hMAO inhibitory activity in the range of micromolar concentration. 3-D-QSAR studies helped to explain experimental results and to provide robust structure–activity relationships: (i) the aryl, especially a phenyl moiety at C4 of the thiazole is responsible for the correct orientation of the inhibitor inside the active site of enzyme close to the catalytic flavin adenine dinucleotide (FAD) (aromatic cage); (ii) the aryl moiety has to be ortho and/or para substituted with an electron-withdrawing group for selective MAO-B inhibition and electron donating group for selective MAO-A inhibition; (iii) 5- or 6-membered cycloaliphatic and aromatic rings are preferred on the N1 of the hydrazine to display selective MAO-B and MAO-A inhibition, respectively [21, 22]. The different aliphatic, cycloaliphatic, heterocyclic, and bicyclic moieties on the N1 of the hydrazine allowed a better comprehension of the physicochemical effect on the enzyme–inhibitor interaction. The above studies clarified some factors responsible for selectivity against the A and B isoforms, such as the lipophilicity of the inhibitor that is important for achieving effective binding to MAO-B and the presence of electron-rich aromatic moieties, typical of selective MAO-A inhibitors [20, 23–27].

In accordance with the above information previously, we designed and synthesized novel thiazolylhydrazone derivatives with selective MAO-A inhibitory potency for further structural tuning [28]. Encouraged by these results, in this study, we designed a series of thiazolylhydrazone derivatives, in which the phenylethyl-mercaptobenzothiazole moiety on the hydrazonic N1 was retained for all homologs to evaluate their MAO inhibitor activities.

Results and discussion

Chemistry

The synthetic route to the target compounds **3a–3i** are presented in Scheme 1. 2-(Benzothiazol-2-ylthio)-1-phenylethan-1-one (**1**) was obtained with reaction between 2-mercaptobenzothiazole and 2-bromo-1-phenylethan-1-one. Compound **1** was converted to its corresponding thiosemicarbazone by reacting **1** with thiosemicarbazide. Finally, target compounds **(3a–3i)** were prepared via ring closure reaction using compound **2** and an appropriately substituted 2-bromoacetophenones.

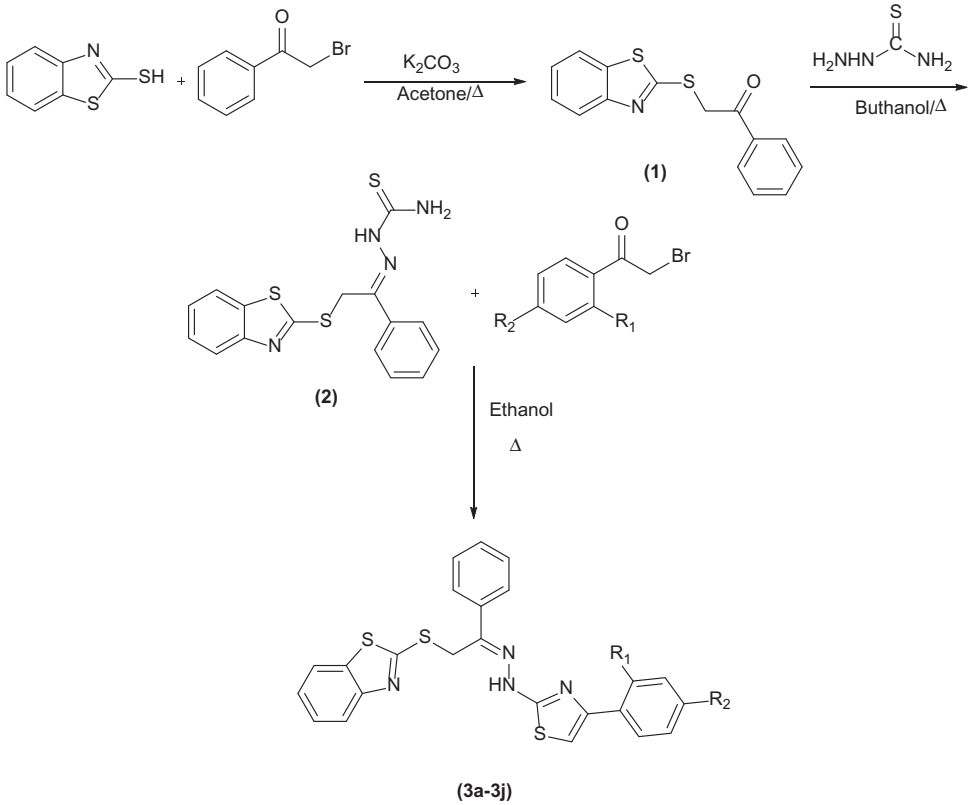
The final compounds were purified, and their structures were characterized by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, and LCMSMS). In the ^{1}H NMR spectra, the methylene protons were recorded between 4.91 and 4.93 ppm. The protons on the aromatic rings were recorded between 7.28 and 8.34 ppm. The –NH protons of thiazoylhydrazine assigned as a singlet at 12.30–12.37 ppm.

In the ^{13}C-NMR spectra, the signals for aromatic and aliphatic regions were seen at estimated areas. The aliphatic carbons were recorded between 27.74 and 27.84 ppm. The aromatic carbons were recorded between 105.21 and 172.45 ppm. The second position of the thiazole ring with the highest ppm gave a peak between 169.01 and 174.67 ppm. The carbon at the second position of the benzothiazole ring were recorded between 160.89 and 165.18 ppm. The mass spectra (ESI-MS) of the compounds [M + 1] peaks were in an agreement with their calculated molecular formula.

MAO-A and MAO-B inhibition assay

The synthesized compounds were evaluated for their *h*MAO-A and *h*MAO-B inhibitory activities. In addition, selegiline and moclobemide were evaluated as reference compounds. The results, expressed as % inhibition and IC_{50} values are summarized in supporting information Tables S1 and S2. In the first step, the synthesized compounds were prepared at concentrations of 10^{-3} to 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. Considering supporting information Table S2, only compounds **3b** and **3i** 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 **3b** and **3i** have a significant activity value against *h*MAO-A.



Compounds	R_1	R_2
3a	-H	-H
3b	-H	$-NO_2$
3c	-H	-CN
3d	-H	-Cl
3e	-H	-Br
3f	-H	$-CF_3$
3g	-H	-Phenyl
3h	-F	-F
3i	-Cl	-Cl

Scheme 1. Pathway for the synthesis of target compounds (**3a–3i**).

Compounds **3b** and **3i** showed efficient inhibition against *h*MAO-A with IC_{50} values of 0.095 ± 0.004 and $0.141 \pm 0.006\ \mu M$, respectively. Compared to the reference drug moclobemide, it is clear that they show significant activity. These findings revealed that compounds **3b** and **3i** have significant potency to inhibit *h*MAO-A compared to reference agent moclobemide. On the other hand, none of the compounds could show the same potency against *h*MAO-B compared to reference agent selegeline. It was determined that the synthesized compounds have selective inhibition potency against *h*MAO-A.

In terms of SARs, considering the structural properties of the phenyl group of thiazole in C4; the 4-nitro and 2,4-dichloro substituents of the phenyl group appear to be most active derivatives in the series. The absence of activity in the compound **3d** bearing the 4-chlorine substituent suggests that the chlorine substituent placed in position 2 increases activity.

The N1 of hydrazine moiety is another significant section of the compounds for an intrinsic enzyme inhibition. According to literature, N1 of the hydrazine group has steric and electronic influences on enzyme–inhibitor interaction [20, 21]. And as we mentioned in our previous study [28], the presence of an aromatic structure in this region increases the selectivity on MAO-A enzyme. From this knowledge in this study, we incorporated 4-(3- or 4-methyl-piperidin-1-yl)benzylidene fragment to N1 of hydrazine. We added a benzothiazole ring to this region, which we have previously seen as a MAO enzyme inhibitor [29]. Our aim was to increase the activity with a benzothiazole ring on the thiazolylhydrazine structure. However, according to the activity results, we obtained less active derivatives than our

previous studies. This suggests that the steric hindrance from the aromatic ring reduces the activity value.

Enzyme kinetic studies

The mechanism of hMAO-A inhibition was examined by enzyme kinetic studies. Lineweaver–Burk graphs were used to assessment the type of inhibition. Data were analyzed by recording substrate–velocity curves in the various concentrations ($IC_{50}/2$, IC_{50} and $2 \times IC_{50}$) of the most active compound, **3b**. The velocity measurements were performed by using different substrate concentrations (20–0.625 µM). The Ki (intercept on the *x*-axis) value of compound **3b** was determined from the secondary plot of the $1/V$ versus varying concentrations. The graphical analysis of inhibition type for compound **3b** is displayed in supporting information Figure S39. The Lineweaver–Burk graphics present the inhibition type as a mixed-type, competitive, uncompetitive, or noncompetitive. In the mix-typed inhibition, the control and inhibitor lines cross neither *x*- nor *y*-axis at the same point. Competitive inhibitors possess the same intercept on *y*-axis, but there are diverse slopes and intercepts on *x*-axis between the two data sets, as seen in supporting information Figure S37. There are no crosses between the lines in an uncompetitive-type inhibition. Noncompetitive inhibition has plots with the same intercept on the *x*-axis, but there are different slopes and intercepts on the *y*-axis. Therefore, this pattern indicates that the compound **3b** is a competitive inhibitor, namely, have similar inhibition features with the substrate. The Ki value for compound **3b** was calculated as 0.019 µM, for the inhibition of *h*MAO.

Conclusion

To develop biologically active derivatives, the presence of a pharmacophore group in the compounds whose synthesis is planned is a basic approach in pharmaceutical chemistry. Based on this approach, benzothiazole and thiazolylhydrazine rings in which we reported MAO inhibitor activities in previous studies were combined at the same molecule. MAO inhibitor activities and kinetic studies of the obtained compounds were performed. According to the results of the *in vitro* MAO enzyme inhibition assay, it was found that the compounds **3b** and **3i** were not effective against MAO-B enzyme while they were found to be the most active derivatives in the series against the MAO-A enzyme. Compound **3i** carries a 2,4-dichlorobenzene ring. This information may suggest that the chlorine substituent contributes to the activity through its halogen bond. However, compound **3d** with a chlorine substituent at the 4-position does not show the same level of activity. Therefore, it can be considered that a chlorine substituent at the 2-position significantly contributes to activity. The other active derivative (**3b**) contains a nitro substituent. The nitro group has been shown to increase activity on MAO enzyme in our previous studies. With the results of the study, compounds derived from **3b** and **3i** can be found to be effective drug candidates for depression and other neurological diseases.

Experimental

Chemistry

All chemicals used in the syntheses were purchased either from Merck Chemicals (Merck KGaA, Darmstadt, Germany) or Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA). The IR spectra of the compounds were recorded using an IRAffinity-1S Fourier transform IR (FTIR) spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA) in DMSO-d_6, respectively. In the NMR spectra splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Coupling constants (J) were reported as Hertz. Melting points of the obtained compounds were measured by MP90 digital melting point apparatus (Mettler Toledo, OH) and were presented as uncorrected. LC-MS-MS studies were performed on a Schimadzu, 8040 LCMSMS spectrophotometer (Shimadzu, Tokyo, Japan). The purities of compounds were checked by TLC on silica gel 60 F254 (Merck KGaA, Darmstadt, Germany). Elemental analyses were also performed on a Leco TruSpec Micro CHN/CHNS elemental analyzer (Leco, St. Joseph, MI). The Supplemental Materials contains sample ^{1}H and ^{13}C NMR and IR spectra for products 3 (supporting information Figures S1–S36).

Materials and methods

Synthesis of 2-(benzothiazol-2-ylthio)-1-phenylethan-1-one (1)

To a stirred solution of 2-mercaptobenzothiazole (1.66 g, 0.01 mol) in acetone (20 mL) was added 2-bromo-1-phenylethan-1-one (1.97 g, 0.01 mol), followed by potassium carbonate. The resulting mixture was heated to reflux at 70 °C. After completion of reaction, acetone was evaporated by means of reduced pressure. The precipitated product was washed with water, dried, and recrystallized from ethanol.

Synthesis of 2-(2-(benzothiazol-2-ylthio)-1-phenylethylidene)hydrazine-1-carbothioamide (2)

A mixture of 2-(benzothiazol-2-ylthio)-1-phenylethan-1-one **(2)** (2.76 g, 0.0097 mol), thiosemicarbazide (0.88 g, 0.0097 mol), and catalytic quantity of acetic acid (CH_3COOH) was refluxed in butanol (50 mL) for 24 h. After completion of the reaction, the mixture was cooled, precipitated product was filtered, washed with deionized water, dried, and recrystallized from ethanol.

General procedure for the synthesis of target compounds (3a-3j)

To a stirred solution of 2-(2-(benzothiazol-2-ylthio)-1-phenylethylidene)hydrazine-1-carbothioamide **(2)** (0.297 g, 0.00083 mol) in EtOH (20 mL) was added appropriate 2-bromo-1-(2,4-substitutedphenyl)ethan-1-one derivatives

(0.00083 mol), refluxed for 10 h. The precipitated product was filtered, washed with cold-ethanol, dried, and recrystallized from ethanol.

2-((2-Phenyl-2-(2-(4-phenylthiazol-2-yl)hydrazineylidene)ethyl)thio)benzothiazole (3a)

Powder, pale yellow. Yield: 83%, M.P.=174.2–175.5 °C, FTIR (ATR, cm^{-1}): 2916 (C–H), 1585 (C[dbond]N), 1132 (C–N), 746, 707. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) : 4.93 (2 H, s, –CH_2–), 7.32-7.51 (10 H, m, Ar–H), 7.86 (2 H, d, J = 6.8 Hz, Ar–H), 7.94 (1 H, d, J = 7.1 Hz, Ar–H), 8.04 (1 H, d, J = 7.4 Hz, Ar–H), 8.18 (1 H, d, J = 7.4 Hz, Ar–H), 12.31 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.79 (–CH_2–), 105.28 (Ar–C), 112.89 (Ar–C), 121.70 (Ar–C), 122.53 (Ar–C), 125.31 (Ar–C), 125.95 (Ar–C), 126.49 (Ar–C), 126.85 (Ar–C), 127.64 (Ar–C), 128.18 (Ar–C), 129.05 (Ar–C), 129.20 (Ar–C), 129.54 (Ar–C), 135.38 (Ar–C), 136.10 (Ar–C), 152.29 (Ar–C), 153.55 (Ar–C), 160.89 (Ar–C), 170.90 (Ar–C). ESI-MS $[M+H]^+$: 459. HRMS (m/z): $[M+Na]^+$ calcd for $C_{24}H_{18}N_4S_3$: 481.0586; found: 481.0504.

2-((2-(2-(4-(4-Nitrophenyl)thiazol-2-yl)hydrazineylidene)-2-phenylethyl)thio)benzothiazole (3b)

Powder, yellow. Yield: 85%, M.P.=216.2–218.6 °C, FTIR (ATR, cm^{-1}): 2953 (C–H), 1577 (C[dbond]N), 1141 (C–N), 852. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.92 (2 H, s, –CH_2–), 7.38-7.52 (6 H, m, Ar–H), 7.82-7.84 (2 H, m, Ar–H), 8.03 (1 H, d, J = 7.7 Hz, Ar–H), 8.13 (1 H, d, J = 8.2 Hz, Ar–H), 8.19 (2 H, d, J = 8.7 Hz, Ar–H), 8.34 (2 H, d, J = 8.9 Hz, Ar–H), 12.37 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.83 (–CH_2–), 104.67 (Ar–C), 110.23 (Ar–C), 121.65 (Ar–C), 122.26 (Ar–C), 122.53 (Ar–C), 124.72 (Ar–C), 125.35 (Ar–C), 126.58 (Ar–C), 126.78 (Ar–C), 126.99 (Ar–C), 127.62 (Ar–C), 129.06 (Ar–C), 129.66 (Ar–C), 136.04 (Ar–C), 144.29 (Ar–C), 149.24 (Ar–C), 152.44 (Ar–C), 162.40 (Ar–C), 174.67 (Ar–C). ESI-MS $[M+H]^+$: 504. HRMS (m/z): $[M+Na]^+$ calcd for $C_{24}H_{17}N_5O_2S_3$: 526.0437; found: 526.0345.

4-(2-(2-(2-(Benzothiazol-2-ylthio)-1-phenylethylidene) hydrazineyl)thiazol-4-yl)benzonitrile (3c)

Powder, yellowish cream. Yield: 81%, M.P.=205.1–207.1 °C, FTIR (ATR, cm^{-1}): 2929 (C–H), 2218 (C[tbond]N), 1575 (C[dbond]N), 1163 (C–N), 835. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.92 (2 H, s, –CH_2–), 7.35–7.53 (6 H, m, Ar–H), 7.75 (1 H, s, Ar–H), 7.85 (2 H, d, J = 6.5 Hz, Ar–H), 7.93 (2 H, d, J = 8.5 Hz, Ar–H), 8.03 (1 H, d, J = 7.3 Hz, Ar–H), 8.12 (2 H, d, J = 8.6 Hz, Ar–H), 12.37 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.76 (–CH_2–), 109.16 (Ar–C), 110.17 (Ar–C), 119.47 (Ar–C), 121.64 (Ar–C), 122.56 (Ar–C), 125.35 (Ar–C), 126.55 (Ar–C), 126.57 (Ar–C), 126.94 (Ar–C), 129.06 (Ar–C), 129.66 (Ar–C), 133.31 (Ar–C), 135.37 (Ar–C), 135.96 (Ar–C), 139.21 (Ar–C), 144.24 (Ar–C), 149.52 (Ar–C), 152.19 (Ar–C), 166.76 (Ar–C), 170.01 (Ar–C). ESI-MS $[M+H]^+$: 484.

2-((2-(2-(4-(4-chlorophenyl)thiazol-2-yl) hydrazineylidene)-2-phenylethyl)thio)benzothiazole (3d)

Powder, green. Yield: 78%, M.P.=180.1–182.9 °C, FTIR (ATR, cm^{-1}): 2953 (C–H), 1577 (C[dbond]N), 1141 (C–N), 835. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.91 (2 H, s, –CH_2–), 7.35–7.54 (8 H, m, Ar–H), 7.85 (2 H, d, J = 6.5 Hz, Ar–H), 7.95 (2 H, d, J = 8.3 Hz, Ar–H), 8.02 (1 H, d, J = 7.5 Hz, Ar–H), 8.16 (1 H, d, J = 7.9 Hz, Ar–H), 12.32 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.76 (–CH_2–), 106.02 (Ar–C), 112.89 (Ar–C), 121.68 (Ar–C), 122.53 (Ar–C), 124.69 (Ar–C), 125.33 (Ar–C), 126.51 (Ar–C), 126.93 (Ar–C), 127.65 (Ar–C), 129.05 (Ar–C), 129.23 (Ar–C), 129.59 (Ar–C), 132.56 (Ar–C), 133.98 (Ar–C), 135.36 (Ar–C), 136.03 (Ar–C), 148.18 (Ar–C), 152.19 (Ar–C), 166.86 (Ar–C), 171.98 (Ar–C). ESI-MS $[M+H]^+$: 493.

2-((2-(2-(4-(4-Bromophenyl)thiazol-2-yl) hydrazineylidene)-2-phenylethyl)thio) benzothiazole (3e)

Powder, green. Yield: 85%, M.P.=190.0–192.3 °C, FTIR (ATR, cm^{-1}): 2949 (C–H), 1583 (C[dbond]N), 1141 (C–N), 823. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.91 (2 H, s, –CH_2–), 7.35–7.53 (7 H, m, Ar–H), 7.65 (2 H, d, J = 8.6 Hz, Ar–H), 7.85 (2 H, d, J = 8.3 Hz, Ar–H), 7.88 (1 H, d, J = 8.4 Hz, Ar–H), 8.02 (1 H, d, J = 7.4 Hz, Ar–H), 8.16 (1 H, d, J = 7.9 Hz, Ar–H), 12.32 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.74 (–CH_2–), 106.18 (Ar–C), 121.16 (Ar–C), 121.67 (Ar–C), 122.27 (Ar–C), 122.53 (Ar–C), 125.34 (Ar–C), 126.51 (Ar–C), 126.93 (Ar–C), 127.96 (Ar–C), 129.05 (Ar–C), 129.59 (Ar–C), 132.13 (Ar–C), 135.36 (Ar–C), 136.02 (Ar–C), 143.93 (Ar–C), 150.87 (Ar–C), 152.22 (Ar–C), 166.78 (Ar–C), 169.81 (Ar–C). ESI-MS $[M+H]^+$: 536.

2-((2-Phenyl-2-(2-(4-(4-(trifluoromethyl)phenyl)thiazol-2-yl)hydrazineylidene)ethyl)thio)benzothiazole (3f)

Powder, cream. Yield: 79%, M.P.=201.2–202.9 °C, FTIR (ATR, cm^{-1}): 2943 (C–H), 1585 (C[dbond]N), 1141 (C–N), 850. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.92 (2 H, s, –CH_2–), 7.35–7.54 (6 H, m, Ar–H), 7.68 (1 H, s, Ar–H), 7.82–7.87 (4 H, m, Ar–H), 8.03 (1 H, d, J = 7.4 Hz, Ar–H), 8.15 (1 H, d, J = 8.1 Hz, Ar–H), 12.36 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.84 (–CH_2–), 108.11 (Ar–C), 121.65 (Ar–C), 122.27 (Ar–C), 122.54 (Ar–C), 125.34 (Ar–C), 126.24 (Ar–C), 126.48 (Ar–C), 126.54 (Ar–C), 126.96 (Ar–C), 129.06 (Ar–C), 129.63 (Ar–C), 135.37 (Ar–C), 136.00 (Ar–C), 138.70 (Ar–C), 144.10 (Ar–C), 149.69 (Ar–C), 152.22 (Ar–C), 166.65 (Ar–C), 169.99 (Ar–C). ESI-MS $[M+H]^+$: 527.

2-((2-(2-(4-([1,1′-biphenyl]-4-yl)thiazol-2-yl) hydrazineylidene)-2-phenylethyl)thio)benzothiazole (3g)

Powder, light brown. Yield: 80%, M.P.=172.2–174.1 °C, FTIR (ATR, cm^{-1}): 2918 (C–H), 1577 (C[dbond]N), 1136 (C–N), 844. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.93 (2 H, s, –CH_2–), 7.36–7.55 (10 H, m, Ar–H), 7.75 (2 H, d, J = 7.3 Hz, Ar–H), 7.78 (2 H, d, J = 8.5 Hz, Ar–H), 7.86 (2 H, d, J = 6.7 Hz, Ar–H), 8.03 (2 H, d, J = 8.1 Hz, Ar–H), 8.21 (1 H, d, J = 7.1 Hz, Ar–H), 12.36 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.80 (–CH_2–), 117.66 (Ar–C), 121.73 (Ar–C), 122.54 (Ar–C), 125.33 (Ar–C), 126.51 (Ar–C), 126.93 (Ar–C), 126.99 (Ar–C), 127.42 (Ar–C), 128.01 (Ar–C), 129.06 (Ar–C), 129.45 (Ar–C), 129.56 (Ar–C), 135.37 (Ar–C), 136.09 (Ar–C), 139.66 (Ar–C), 140.05 (Ar–C), 143.95 (Ar–C), 147.90 (Ar–C), 152.39 (Ar–C), 166.86 (Ar–C), 172.45 (Ar–C). ESI-MS $[M + H]^+$: 535.

2-((2-(2-(4-(2,4-difluorophenyl)thiazol-2-yl) hydrazineylidene)-2-phenylethyl)thio)benzothiazole (3h)

Powder, cream. Yield: 77%, M.P.=187.4–189.9 °C, FTIR (ATR, cm^{-1}): 2920 (C–H), 1583 (C[dbond]N), 1136 (C–N), 754, 692. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.92 (2 H, s, –CH_2–), 7.28–7.52 (9 H, m, Ar–H), 7.85 (2 H, d, J = 7.7 Hz, Ar–H), 8.03 (1 H, d, J = 8.1 Hz, Ar–H), 8.13 (1 H, d, J = 8.0 Hz, Ar–H), 12.31 (1 H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.77 (–CH_2–), 105.21 (Ar–C), 111.17 (Ar–C), 121.65 (Ar–C), 122.28 (Ar–C), 122.59 (Ar–C), 125.37 (Ar–C), 126.62 (Ar–C), 126.98 (Ar–C), 129.04 (Ar–C), 129.62 (Ar–C), 130.61 (Ar–C), 136.07 (Ar–C), 137.46 (Ar–C), 140.82 (Ar–C), 144.13 (Ar–C), 149.55 (Ar–C), 152.82 (Ar–C), 155.97 (Ar–C), 159.35 (Ar–C), 166.54 (Ar–C), 169.25 (Ar–C). ESI-MS $[M + H]^+$: 495.

2-((2-(2-(4-(2,4-dichlorophenyl)thiazol-2-yl) hydrazineylidene)-2-phenylethyl)thio)benzothiazole (3i)

Powder, cream. Yield: 83%, M.P.=191.9–194.8 °C, FTIR (ATR, cm^{-1}): 2954 (C–H), 1583 (C[dbond]N), 1138 (C–N), 765, 692. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): 4.91 (2 H, s, –CH_2–), 7.35–7.50 (8 H, m, Ar–H), 7.59 (1 H, dd, J_1=2.2 Hz, J_2 = 8.5 Hz, Ar–H), 7.74 (1 H, d, J = 2.2 Hz, Ar–H), 7.85 (2 H, d, J = 8.1 Hz, Ar–H), 8.02 (1 H, d, J = 7.2 Hz, Ar–H), 8.06 (1 H, d, J = 7.9 Hz, Ar–H), 12.30 (1 H, s, –NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ = 27.84 (–CH_2–), 110.85 (Ar–C), 112.89 (Ar–C), 121.66 (Ar–C), 122.27 (Ar–C), 122.51 (Ar–C), 124.69 (Ar–C), 125.32 (Ar–C), 126.53 (Ar–C), 126.90 (Ar–C), 127.64 (Ar–C), 128.06 (Ar–C), 129.05 (Ar–C), 129.59 (Ar–C), 130.32 (Ar–C), 132.61 (Ar–C), 135.37 (Ar–C), 136.06 (Ar–C), 143.87 (Ar–C), 152.27 (Ar–C), 165.18 (Ar–C), 169.01 (Ar–C). ESI-MS $[M + H]^+$: 526.

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 [28–31]. All pipetting procedures in the enzyme inhibition assay were carried out using a robotic system, Biotek Precision XS (Winooski, VT). Selegiline and moclobemide were used as reference drugs. The IC_{50} values were calculated from a dose–response curve gained by plotting the percentage inhibition versus the log concentration with the use of GraphPad PRISM software (version 5.0, GraphPad Software Inc., La Jolla, CA). The results are shown as the mean ± standard deviation (SD).

Kinetic studies of enzyme inhibition

In order to determine the type of inhibition on *h*MAO-A, enzyme kinetic studies were carried out for compound **3b**. The enzyme kinetic assay was performed by means of Ellman's method, in keeping with the previous studies reported by our research group [28–31].

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