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2,1-Benzothiazine – (quinolin/thiophen)yl hydrazone frameworks as new monoamine oxidase inhibitory agents; synthesis, *in vitro* and *in silico* investigation†

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Two series of new 2,1-benzothiazine derivatives have been synthesized by condensation of 4-hydrazono-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (**5**) with 2-chloroquinoline-3-carbaldehydes and acetylthiophenes to acquire new heteroaryl ethylidenes **7(a–f)** and **9(a–k)** in excellent yields. After characterization by FTIR, ^{1}H NMR, ^{13}C NMR and elemental analyses, the newly synthesized analogues were investigated against monoamine oxidase enzymes (MAO A and MAO B). The titled compounds exhibited activity in the lower micromolar range among which **9e** was the most potent compound against MAO A with IC_{50} of 1.04 ± 0.01 μM whereas **9h** proved to be the most potent derivative against MAO B with an IC_{50} value of 1.03 ± 0.17 μM. Furthermore, *in vitro* results were further endorsed by molecular docking studies to determine the interaction between the potent compounds and the enzyme active site. These newly synthesized compounds represent promising hits for the development of safer and potent lead molecules for therapeutic use against depression and other neurological diseases.

Introduction

Human monoamine oxidase (hMAO) is a flavin adenine dinucleotide (FAD) enzyme present in outer mitochondrial membrane which is responsible for the metabolism of different biogenic amines along with some neuro-transmitters.[1] To date, two types of MAOs have been identified (MAO A and MAO B) which are accountable for the metabolism of different neurotransmitters like dopamine, norepinephrine, serotonin and epinephrine found in central and peripheral tissues.[2] As a result of degradation of amines, hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) are produced which are responsible for the death of neural cells.[3] Neurological disorders (Alzheimer's and Parkinson disease) and depression originate due to unnecessary concentration of monoamine oxidase (MAO) which decreases the level of monoaminergic transmitters in the brain. The use of monoamine oxidase inhibitors (MAOI) can control the concentration of monoamine oxidase by inhibiting the excessive amount of oxidases and thus enhancing the concentration of monoaminergic transmitters.[4]

One of the primary aims of synthetic organic chemistry is the development of new, selective, and potent drug molecules. Different classes of compounds have been identified as MAOIs by various researchers among which heterocyclic organic compounds are investigated to be more effective.[5] Among different heterocyclic compounds, benzothiazine ring systems are one of the supreme targeted areas for the synthetic and clinical interest in the field of pharmacokinetics as they act as a skeleton with a rich bio-activity profile.[6–8] A number of biological potentials have marked benzothiazine based derivatives interesting as antifungal,[9,10] anti-bacterial,[10,11] anti-malarial,[12] anti-oxidant,[13] anti-hypertensive,[14] anti-neoplastic,[15] anti-viral,[16,17] and cardio-protective[18] agents. 1,2-Benzothiazines have experimentally proven to be effective as analgesic agents and different candidates have been tested to examine their respective strength as painkillers and anti-inflammatory pills.[19,20] The most overwhelming potential is exhibited by the Oxicams in this category which constitute 1,2-benzothiazine motif as the main structural feature.[20,21] 2,1-Benzothiazine also known as benzo[*c*][1,2]thiazine is considered to be the bio-isostere of 1,2-benzothiazine.[22] Different compounds bearing

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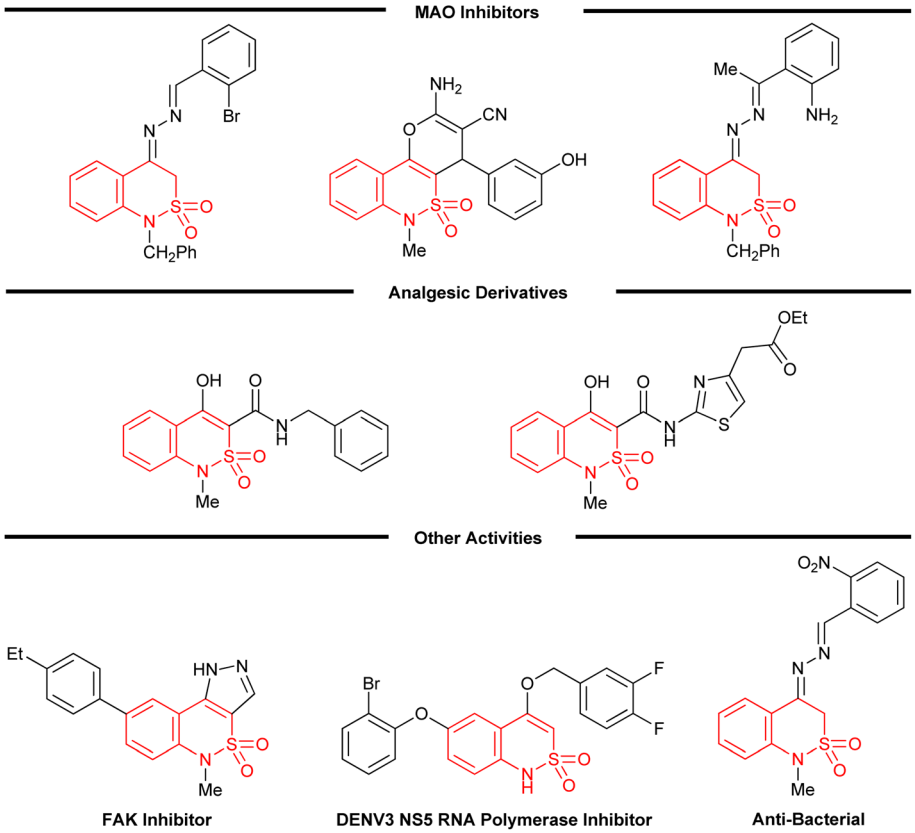


Fig. 1 Structures of some biologically effective 2,1-benzothiazine derivatives.

2,1-benzothiazine framework have been documented as antipsychotic,[22] anti-inflammatory,[23] anti-cancer,[24] and analgesic agents.[25] These have also been investigated for their lipoxygenase and RNA polymerase inhibitory potential (Fig. 1).[26,27]

In continuation of our efforts to search for new bioactive synthetic compounds,[28–30] we herein report the synthesis of new 2,1-benzothiazine 2,2-dioxide analogues and evaluation of their monoamine oxidase (MAO A and MAO B) inhibitory potential. To validate the results, *in silico* docking studies have been conducted to assess the binding interaction of the synthesized compounds inside the active site of enzyme.

Experimental

All the chemicals were purchased from Merck and Sigma Aldrich through their indigenous suppliers and were used as received. Melting points were taken by open capillary method on Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra and ^{13}C NMR spectra (300 and 75 MHz respectively) were recorded in DMSO-d_6 on Brucker Avance NMR instrument. Chemical shifts δ are reported in ppm with reference to tetramethylsilane. FTIR spectral data was obtained on an Agilent Technologies Cary 630 FTIR spectrophotometer. Elemental analyses were investigated in LECO 630-200-200 TruSpec CHNS microanalyzer and the values are established to be within ± 0.4% of the calculated results. Compounds **2–4** and **6** were synthesized by procedures reported in literature.[29,31]

Synthesis of 4-hydrazono-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (5)

A solution of 1-methyl-1*H*-benzo[*c*][1,2]thiazin-4(3*H*)-one 2,2-dioxide **4** (25 mmol; 5.275 g) and 64% hydrazine monohydrate (75 mmol; 5.86 g, 5.7 mL) in absolute ethanol (50 mL) was heated under reflux conditions for 8 hours. The progress of reaction was monitored by TLC until the reactant spot disappeared. After completion of the reaction (TLC monitoring with eluting solvent *n*-hexane – ethyl acetate (7 : 3)), excess solvent was removed on rotavapor and the concentrated solution was allowed to stand overnight after neutralizing it using 5N HCl solution. The product was obtained as yellow crystals which were filtered, washed with cold ethanol, and dried.

Yellow crystalline solid; mp 139–140 °C (Lit mp 139–141 °C);[31] yield: 90%; IR ($\bar{\nu}$ cm^{-1}; neat): 3388, 3310 (N–H), 3070, 2954 (C–H), 1640 (C=N), 1320 & 1118 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.23 (s, 3H; N–CH_3), 4.48 (s, 2H; –CH_2–), 7.07 (s, 2H; –NH_2), 7.14 (td, 1H; J = 7.5 Hz, 1.2 Hz; Ar–H), 7.20 (dd, 1H, J = 8.1 Hz, 0.9 Hz; Ar–H), 7.32 (td, 1H; J = 8.7 Hz, 1.8 Hz; Ar–H), 7.95 (dd, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 34.7 (N–CH_3), 47.8 (–CH_2–), 120.9, 124.2, 124.7, 125.7, 129.2, 130.5, 139.9 ppm. Anal. Calcd. for $C_9H_{11}N_3O_2S$: C, 47.99;

H, 4.92; N, 18.65; S, 14.23%. Found: C, 48.13; H, 4.98; N, 18.82; S, 14.39%.

General procedure for the synthesis of 1-methyl-4-((1-(heteroaryl)ethylidene)hydrazono)-3,4-dihydro-1*H*-2,1-benzothiazine 2,2-dioxides (7,9)

To a solution of 4-hydrazono-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide **5** (1 mmol; 0.225 g) in distilled methanol (15 mL) was added 2-chloroquinoline-3-carbaldehyde (**6a**) (1 mmol; 0.192 g) and *o*-phosphoric acid (2–3 drops). The resulting mixture was refluxed until the formation of precipitates in the flask which were filtered, dried and recrystallized from absolute ethanol to obtain pure product (**7a**).

The same procedure was adopted for the condensation of **5** with substituted 2-chloroquinoline-3-carbaldehydes **6(b–f)** and substituted acetylthiophenes **8(a–k)** to achieve the derivatives **7(b–f)** and **9(a–k)**.

4-(((2-Chloroquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7a). Greenish yellow solid; mp 258–260 °C; yield: 80%; IR ($\bar{\nu}$ cm^{-1}; neat): 3106, 2980 (C–H), 1586 (C=N), 1329 & 1143 (S=O), 732 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.34 (s, 3H; N–CH_3), 5.25 (s, 2H; –SO_2CH_2–), 6.77 (s, 1H; –SO_2CH=), 7.20–7.40 (m, 2H; Ar–H), 7.52 (d, 1H; J = 8.1 Hz; Ar–H), 7.60–7.66 (m, 2H, Ar–H), 7.83–7.87 (m, 1H; Ar–H), 7.95 (d, 1H; J = 7.8 Hz; Ar–H), 8.37 (d, 1H; J = 8.1 Hz; Ar–H), 8.61, 8.69 (2s, 1H; Ar–H), 8.80, 8.95 (2s, 1H; N=CH), 10.94 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 29.6 (N–CH_3, enamine), 33.0 (N–CH_3, imine), 50.1 (SO_2CH_2), 95.1 (SO_2CH), 115.6, 116.5, 117.5, 119.4, 119.8, 122.6, 122.8, 124.1, 125.8, 129.3, 131.4, 132.3, 135.0, 139.3, 140.7, 143.3, 145.2, 155.9, 161.5 ppm. Anal. Calcd. for $C_{19}H_{15}ClN_4O_2S$: C, 57.21; H, 3.79; N, 14.05; S, 8.04%. Found: C, 57.37; H, 3.81; N, 14.20; S, 8.11%.

4-(((2-Chloro-6-methylquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7b). Light yellow solid; mp 226–228 °C; yield: 84%; IR ($\bar{\nu}$ cm^{-1}; neat): 3059, 2982 (C–H), 1584 (C=N), 1328 & 1154 (S=O), 748 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.50 (s, 3H; Ar–CH_3), 3.35 (s, 3H; N–CH_3), 5.29 (s, 2H; –SO_2CH_2–), 6.89 (s, 1H; –SO_2CH=), 7.28–7.41 (m, 2H; Ar–H), 7.64–7.74 (m, 2H, Ar–H), 7.82–7.93 (m, 2H; Ar–H), 8.39 (d, 1H; J = 7.2 Hz; Ar–H), 8.72, 8.92 (2s, 1H; Ar–H), 9.08, 9.27 (2s, 1H; N=CH), 11.13 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 21.6 (Ar-CH_3), 29.6 (N–CH_3, enamine), 33.0 (N–CH_3, imine), 50.3 (SO_2CH_2), 95.8 (SO_2CH), 116.4, 117.5, 119.7, 122.6, 125.1, 126.5, 127.6, 127.9, 132.4, 134.1, 135.4, 137.8, 139.7, 140.6, 143.4, 145.1, 146.0, 147.8, 156.8. Anal. Calcd. For $C_{20}H_{17}ClN_4O_2S$: C, 58.18; H, 4.15; N, 13.57; S, 7.77%. Found: C, 58.06; H, 4.03; N, 13.43; S, 7.63%.

4-(((2-Chloro-6-methoxyquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7c). Brown solid; mp 280–282 °C; yield: 77%; IR ($\bar{\nu}$ cm^{-1}; neat): 3106, 2950 (C–H), 1585 (C=N), 1327 & 1147 (S=O), 732 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ:3.34 (s, 3H; N–CH_3), 3.92 (s, 3H; –OCH_3), 5.29 (s, 2H; –SO_2CH_2–), 6.86 (s, 1H; –SO_2CH=), 7.34–7.41 (m, 1H; Ar–H), 7.45 (dd, 1H; J = 9.0 Hz, 2.7 Hz; Ar–H), 7.58–7.68 (m, 2H, Ar–H), 7.85 (d, 1H; J = 9.3 Hz, Ar–H), 7.95 (dd, 1H; J = 8.1 Hz, 0.9 Hz; Ar–H), 8.40 (dd, 1H; J = 7.8 Hz, 1.5 Hz; Ar–H), 8.73, 8.93 (2s, 1H; Ar–H), 9.08, 9.27 (2s, 1H; N=CH), 11.15 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 29.6 (N–CH_3, enamine), 32.9 (N–CH_3, imine), 50.2 (SO_2CH_2), 56.1 (-OCH_3), 95.7 (SO_2CH), 106.8, 116.3, 117.5, 119.7, 122.6, 124.5, 125.1, 126.7, 128.9, 129.5, 132.4, 134.7, 139.7, 140.6, 143.5, 145.1, 146.1, 158.4 ppm. Anal. Calcd. for $C_{20}H_{17}ClN_4O_3S$: C, 56.01; H, 4.00; N, 13.06; S, 7.48%. Found: C, 56.20; H, 4.10; N, 13.10; S, 7.52%.

4-(((2-Chloro-7-methylquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7d). Greenish yellow solid; mp 198–200 °C; yield: 78%; IR ($\bar{\nu}$ cm^{-1}; neat): 3106, 2927 (C–H), 1662 (C=N), 1330 & 1149 (S=O), 750 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.52 (s, 3H; Ar–CH_3), 3.34 (s, 3H; N–CH_3), 5.29 (s, 2H; –SO_2CH_2–), 6.88 (s, 1H; –SO_2CH=), 7.04–7.14 (m, 1H; Ar–H), 7.20–7.41 (m, 1H; Ar–H), 7.51–7.73 (m, 2H, Ar–H), 7.95 (d, 1H; J = 7.2 Hz; Ar–H), 8.08 (d, 1H; J = 8.4 Hz, 2.7 Hz; Ar–H), 8.36 (d, 1H; J = 8.4 Hz; Ar–H), 8.74, 8.93 (2s, 1H; Ar–H), 9.15, 9.28 (2s, 1H; N=CH), 11.12 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 22.1 (Ar-CH_3), 29.6 (N–CH_3, enamine), 32.9 (N–CH_3, imine), 50.1 (SO_2CH_2), 95.7 (SO_2CH), 116.4, 117.7, 119.8, 122.6, 124.1, 125.7, 130.4, 130.7, 133.5, 135.8, 138.8, 139.9, 140.7, 142.5, 143.4, 145.1, 148.6, 155.7, 156.9, 161.2 ppm. Anal. Calcd. for $C_{20}H_{17}ClN_4O_2S$: C, 58.18; H, 4.15; N, 13.57; S, 7.77%. Found: C, 58.24; H, 4.21; N, 13.63; S, 7.89%.

4-(((2-Chloro-7-methoxyquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7e). Greenish yellow solid; mp 251–253 °C; yield: 80%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2920 (C–H), 1653 (C=N), 1338 & 1148 (S=O), 1025 (C–O–C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.33 (s, 3H; N–CH_3), 3.93 (s, 3H; –OCH_3), 5.23 (s, 2H; –SO_2CH_2–), 6.71 (s, 1H; –SO_2CH=), 6.84–6.91 (m, 1H; Ar–H),7.31–7.39 (m, 1H; Ar–H), 7.56–7.65 (m, 1H; Ar–H), 7.76 (dd, 1H; J = 8.7 Hz, 3.0 Hz; Ar–H), 7.94 (d, 1H; J = 8.1 Hz; Ar–H), 8.09 (d, 1H; J = 8.7 Hz; Ar–H), 8.35 (d, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H), 8.60, 8.77 (2s, 1H; Ar–H), 9.11, 9.27 (s, 1H; N=CH), 11.86 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 29.5 (N–CH_3, enamine), 33.0 (N–CH_3, imine), 50.0 (SO_2CH_2), 56.0 (–OCH_3), 98.3 (SO_2CH), 112.5, 113.8, 116.5, 117.4, 119.7, 121.5, 122.3, 124.1, 130.9, 131.6, 133.4, 140.6, 141.3, 142.6, 143.2, 145.3, 155.4, 157.0, 161.8 ppm. Anal. Calcd. for $C_{20}H_{17}ClN_4O_3S$: C, 56.01; H, 4.00; N, 13.06; S, 7.48%. Found: C, 56.13; H, 4.16; N, 13.12; S, 7.60%.

4-(((2,7-Dichloroquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (7f). Yellow solid; mp 223–225 °C; yield: 89%; IR ($\bar{\nu}$ cm^{-1}; neat): 3106, 2980 (C–H), 1659 (C=N), 1322 & 1150 (S=O), 766 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ:3.35 (s, 3H; N–CH_3), 5.27 (s, 2H; –SO_2CH_2-), 6.88 (s, 1H; –SO_2CH=), 7.21–7.37 (m, 2H; Ar–H), 7.60–7.71 (m, 1H, Ar–H), 7.92 (d, 1H; J = 7.8 Hz; Ar–H), 7.99 (s, 1H; Ar–H), 8.19 (d, 1H; J = 9.0 Hz; Ar–H), 8.35 (d, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H), 8.70, 8.89 (2s, 1H; Ar–H), 9.19, 9.33 (s, 1H; N=CH), 11.16 (s, 1H; NH) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 29.6 (N–CH_3, enamine), 32.8 (N–CH_3, imine), 50.3 (SO_2CH_2), 96.0 (SO_2CH), 116.3, 117.5, 119.7, 122.6, 125.0, 126.1, 126.2, 127.1, 128.9, 131.1, 135.9, 136.5, 140.6, 145.0, 147.5, 149.9, 157.2, 161.3 ppm. Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O_2S$: C, 52.67; H,

3.26; N, 12.93; S, 7.40%. Found: C, 52.73; H, 3.40; N, 13.09; S, 7.58%.

1-Methyl-4-((1-(thiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9a). Yellow crystalline solid; mp 152–154 °C; yield: 81%; IR ($\bar{\nu}$ cm^{-1}; neat): 3090, 2999 (C–H), 1584 (C=N), 1331 & 1150 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ:2.52 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.95 (s, 2H; –CH_2-), 7.19 (t, 1H; J = 4.5 Hz; Ar–H), 7.28 (t, 1H; J = 7.5 Hz; Ar–H), 7.35 (d, 1H; J = 8.4 Hz; Ar–H), 7.60 (t, 1H; J = 8.1 Hz; Ar–H), 7.74 (d, 1H; J = 3.9 Hz; Ar–H), 7.77 (d, 1H; J = 5.1 Hz; Ar–H), 8.36 (d, 1H; J = 8.1 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.9 (-CH_3), 33.3 (N–CH_3), 49.8 (–CH_2–), 120.1, 122.7, 124.3, 126.4, 128.6, 130.8, 131.4, 133.2, 142.9, 143.3, 151.9, 160.7 ppm. Anal. Calcd. for $C_{15}H_{15}N_3O_2S_2$: C, 54.03; H, 4.53; N, 12.60; S, 19.23%. Found: C, 54.19; H, 4.71; N, 12.72; S, 19.41%.

1-Methyl-4-((1-(3-methylthiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9b). Greenish yellow solid; mp 154–156 °C; yield: 70%; IR ($\bar{\nu}$ cm^{-1}; neat): 3094, 2970 (C–H), 1584 (C=N), 1329 & 1144 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ:2.53 (s, 3H; –CH_3), 2.54 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.94 (s, 2H; –CH_2–), 7.06 (d, 1H; J = 5.1 Hz; Ar–H), 7.28 (td, 1H; J = 7.5 Hz, 1.2 Hz; Ar–H), 7.35 (d, 1H; J = 7.5 Hz; Ar–H), 7.59 (td, 1H; J = 7.8 Hz, 1.8 Hz; Ar–H), 7.65 (d, 1H; J = 5.1 Hz; Ar–H), 8.36 (dd, 1H; J = 7.8 Hz, 1.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 17.9 (–CH_3), 18.2 (–CH_3), 33.4 (N–CH_3), 50.2 (–CH_2–), 120.1, 123.0, 124.4, 126.4, 128.6, 133.0, 133.5, 136.4, 140.3, 142.8, 151.5, 161.7 ppm. Anal. Calcd. for $C_{16}H_{17}N_3O_2S_2$: C, 55.31; H, 4.93; N, 12.09; S, 18.46%. Found: C, 55.29; H, 4.87; N, 12.01; S, 18.38%.

1-Methyl-4-((1-(4-methylthiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9c). Greenish yellow solid; mp 149–151 °C; yield: 78%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2950 (C–H), 1590 (C=N), 1324 & 1143 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.49 (s, 3H; –CH_3), 2.57 (s, 3H; –CH_3), 3.32 (s, 3H; N–CH_3), 4.98 (s, 2H; –CH_2–), 7.28 (td, 1H; J = 7.5 Hz, 0.9 Hz; Ar–H), 7.33–7.38 (m, 2H; Ar–H), 7.58–7.65 (m, 2H; Ar–H), 8.36 (dd, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.9 (–CH_3), 23.4 (–CH_3), 33.3 (N–CH_3), 49.8 (–CH_2–), 120.1, 122.6, 124.4, 126.6, 127.4, 132.3, 133.1, 137.0, 142.8, 143.1, 151.8, 160.7 ppm. Anal. Calcd. for $C_{16}H_{17}N_3O_2S_2$: C, 55.31; H, 4.93; N, 12.09; S, 18.46%. Found: C, 55.37; H, 4.99; N, 12.11; S, 18.50%.

1-Methyl-4-((1-(5-methylthiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9d). Bright yellow solid; mp 173–175 °C; yield: 71%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2978 (C–H), 1585 (C=N), 1325 & 1150 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.47 (s, 3H; –CH_3), 2.49 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.94 (s, 2H; –CH_2–), 6.89 (dd, 1H; J = 3.6 Hz, 1.2 Hz; Ar–H), 7.27 (td, 1H; J = 7.5 Hz, 0.9 Hz; Ar–H), 7.34 (d, 1H; J = 8.4 Hz; Ar–H), 7.55 (d, 1H; J = 3.9 Hz; Ar–H), 7.59 (td, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H), 8.35 (dd, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.4 (–CH_3), 15.9 (–CH_3), 33.3 (N–CH_3), 49.7 (–CH_2–), 120.0, 122.8, 124.3, 126.4, 127.1, 131.2, 133.1, 141.0, 142.8, 145.6, 151.8, 160.9 ppm. Anal. Calcd. for $C_{16}H_{17}N_3O_2S_2$: C, 55.31; H, 4.93; N, 12.09; S, 18.46%. Found: C, 55.43; H, 5.05; N, 12.21; S, 18.60%.

4-((1-(3-Chlorothiophen-2-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9e). Greenish yellow solid; mp 174–176 °C; yield: 86%; IR ($\bar{\nu}$ cm^{-1}; neat): 3105, 2975 (C–H), 1586 (C=N), 1325 & 1144 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.60 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.92 (s, 2H; –CH_2–), 7.19 (d, 1H; J = 5.4 Hz; Ar–H), 7.29 (t, 1H; J = 7.8 Hz; Ar–H), 7.36 (d, 1H; J = 8.4 Hz; Ar–H), 7.61 (td, 1H; J = 8.1 Hz, 1.2 Hz; Ar–H), 7.85 (d, 1H; J = 5.1 Hz; Ar–H), 8.36 (dd, 1H; J = 8.1 Hz, 1.2 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 17.8 (–CH_3), 33.4 (N–CH_3), 50.1 (–CH_2–), 120.1, 122.6, 124.4, 125.5, 126.5, 130.4, 130.8, 133.4, 135.5, 143.0, 152.4, 159.4 ppm. Anal. Calcd. for $C_{15}H_{14}ClN_3O_2S_2$: C, 48.97; H, 3.84; N, 11.42; S, 17.43%. Found: C, 49.11; H, 4.00; N, 11.66; S, 17.59%.

4-((1-(3-Bromothiophen-2-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9f). Greenish yellow solid; mp 164–166 °C; yield: 74%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2978 (C–H), 1585 (C=N), 1332 & 1150 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.62 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.94 (s, 2H; –CH_2–), 7.25 (d, 1H; J = 5.4 Hz; Ar–H), 7.28 (t, 1H; J = 7.5 Hz; Ar–H), 7.36 (d, 1H; J = 8.4 Hz; Ar–H), 7.61 (td, 1H; J = 8.1 Hz, 1.2 Hz; Ar–H), 7.83 (d, 1H; J = 5.4 Hz; Ar–H), 8.36 (dd, 1H; J = 8.1 Hz, 1.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 17.9 (–CH_3), 33.4 (N–CH_3), 50.2 (–CH_2–), 111.3, 120.1, 122.6, 124.4, 126.6, 130.9, 133.4, 133.6, 137.1, 143.0, 152.3, 159.5 ppm. Anal. Calcd. for $C_{15}H_{14}BrN_3O_2S_2$: C, 43.69; H, 3.42; N, 10.19; S, 15.55%. Found: C, 43.71; H, 3.50; N, 10.33; S, 15.73%.

4-((1-(5-Chlorothiophen-2-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9g). Greenish yellow solid; mp 171–173 °C; yield: 80%; IR ($\bar{\nu}$ cm^{-1}; neat): 3100, 2923 (C–H), 1585 (C=N), 1328 & 1148 (S=O); 761 (C–Cl). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.46 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.93 (s, 2H; –CH_2-), 7.25–7.30 (m, 2H; Ar–H), 7.35 (d, 1H; J = 8.4 Hz; Ar–H), 7.58–7.63 (m, 2H; Ar–H), 8.35 (d, 1H; J = 7.5 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.0 (–CH_3), 33.3 (N–CH_3), 49.9 (–CH_2–), 120.1, 122.6, 124.5, 126.5, 128.5, 130.6, 131.6, 132.9, 138.9, 142.3, 152.5, 160.3 ppm. Anal. Calcd. for $C_{15}H_{14}ClN_3O_2S_2$: C, 48.97; H, 3.84; N, 11.42; S, 17.43%. Found: C, 48.89; H, 3.76; N, 11.38; S, 17.31%.

4-((1-(5-Bromothiophen-2-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9h). Light green solid; mp 203-205 °C; yield: 84%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2978 (C–H), 1587 (C=N), 1325 & 1148 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.47 (s, 3H; –CH_3), 3.33 (s, 3H; N–CH_3), 4.93 (s, 2H; –CH_2–), 7.27 (t, 1H; J = 7.5 Hz; Ar–H), 7.36–7.40 (m, 2H; Ar–H), 7.61–7.68 (m, 2H; Ar–H), 8.35 (d, 1H; J = 7.8 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.2 (–CH_3), 33.3 (N–CH_3), 49.9 (–CH_2–), 117.7, 120.0, 122.7, 124.3, 126.5, 131.4, 131.9, 133.3, 134.4, 143.0, 152.5, 160.2 ppm. Anal. Calcd. for $C_{15}H_{14}BrN_3O_2S_2$: C, 43.69; H, 3.42; N, 10.19; S, 15.55%. Found: C, 43.77; H, 3.54; N, 10.27; S, 15.69%.

1-Methyl-4-((1-(5-nitrothiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9i). Orange yellow solid; mp 210–212 °C; yield: 94%; IR ($\bar{\nu}$ cm^{-1}; neat): 3089, 2934 (C–H), 1585 (C=N), 1325 & 1150 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.64 (s, 3H; –CH_3), 3.34 (s, 3H; N–CH_3), 4.97 (s, 2H; –CH_2–), 7.29 (t, 1H; J = 7.5 Hz; Ar–H), 7.36 (d, 1H; J = 7.5 Hz;

Ar–H), 7.63 (t, 1H; $J = 8.1$ Hz; Ar–H), 7.80 (d, 1H; $J = 4.5$ Hz; Ar–H), 8.19 (d, 1H; $J = 4.5$ Hz; Ar–H), 8.36 (d, 1H; $J = 7.8$ Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.3 (–CH_3), 33.2 (N–CH_3), 50.2 (–CH_2–), 120.2, 121.9, 124.4, 127.6, 129.0, 131.9, 134.1, 137.1, 143.7, 149.8, 152.8, 159.6 ppm. Anal. Calcd. for $C_{15}H_{14}N_4O_4S_2$: C, 47.61; H, 3.73; N, 14.81; S, 16.95%. Found: C, 47.69; H, 3.81; N, 14.85; S, 17.10%.

4-((1-(2,5-Dimethylthiophen-3-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide(9j). Light yellow solid; mp 150–152 °C; yield: 82%; IR ($\bar{\nu}$ cm^{-1}; neat): 3095, 2919 (C–H), 1594 (C=N), 1327 & 1146 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.40–2.42 (2s, 6H; –CH_3), 2.61 (s, 3H; –CH_3), 3.32 (s, 3H; N–CH_3), 4.90 (s, 2H; –CH_2–), 7.12 (s, 1H; Ar–H), 7.28 (t, 1H; $J = 7.5$ Hz; Ar–H), 7.34 (d, 1H; $J = 8.4$ Hz; Ar–H), 7.58 (td, 1H; $J = 8.1$ Hz, 1.2 Hz; Ar–H), 8.35 (dd, 1H; $J = 8.1$ Hz, 1.2 Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 15.1 (–CH_3), 16.8 (–CH_3), 18.2 (–CH_3), 33.4 (N–CH_3), 50.3 (–CH_2–), 120.1, 123.1, 124.4, 126.4, 127.3, 132.9, 135.0, 135.3, 139.3, 142.8, 150.8, 162.5 ppm. Anal. Calcd. for $C_{17}H_{19}N_3O_2S_2$: C, 56.48; H, 5.30; N, 11.62; S, 17.74%. Found: C, 56.56; H, 5.45; N, 11.70; S, 17.86%.

4-((1-(2,5-Dichlorothiophen-3-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (9k). Light green solid; mp 149–151 °C; Yield: 76%; IR ($\bar{\nu}$ cm^{-1}; neat): 3080, 2978 (C–H), 1585 (C=N), 1325 & 1150 (S=O). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 2.43 (s, 3H; –CH_3), 3.32 (s, 3H; N–CH_3), 4.94 (s, 2H; –CH_2–), 7.28 (t, 1H; $J = 7.5$ Hz; Ar–H), 7.35 (d, 1H; $J = 8.4$ Hz; Ar–H), 7.60 (t, 1H; $J = 7.8$ Hz; Ar–H), 7.69 (s, 1H; Ar–H), 8.34 (d, 1H; $J = 8.1$ Hz; Ar–H) ppm. ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 17.8 (–CH_3), 33.3 (N–CH_3), 50.2 (–CH_2–), 120.1, 122.6, 124.3, 125.6, 126.6, 128.6, 133.3, 136.5, 142.9, 143.0, 151.6, 158.6 ppm. Anal. Calcd. for $C_{15}H_{13}Cl_2N_3O_2S_2$: C, 44.78; H, 3.26; N, 10.44; S, 15.94%. Found: C, 44.90; H, 3.42; N, 10.60; S, 16.08%.

Biological activity

Monoamine oxidase (MAO A and MAO B) inhibition assay. For the newly synthesized compounds, MAO A and MAO B inhibitory activity was measured as per previously reported protocol.[32] Fresh enzyme was prepared 15–20 min before and cooled to room temperature. Clorgyline (60 nM) or deprenyl (300 nM) were used accordingly to block of MAO A and MAO B activity irreversibly. For performing assay white 96 well plates were used. The assay volume was 100 μL having 60 μL buffer (Na_2HPO_4, pH 7.4), 10 μL test compound (0.1 mM, 10% DMSO) followed by adding enzyme 10 μL (26 μg of protein for MAO A and 5.0 μg for MAO B). The mixture was incubated for 15 and 20 min for MAO B and MAO A respectively, after incubation 10 μL of substrate (0.3 mM) and 10 μL of freshly prepared Amplex red was added in the mixture and reading was noted (pre read). The final concentration of clorgyline and deprenyl was 0.1 mM used to determine non-MAO A and MAO B activity accordingly. After 20–25 min of incubation, the reading was noted again and the change in the fluorescence was determined using fluorescence plate reader (BMG Labtech GmbH, orten berg Germany). The compounds which exhibited over 50% inhibition of either the MAO A or MAO B activity were further evaluated for determination of IC_{50} values. All experiments were repeated twice in triplicate. IC_{50} values were calculated by non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

Docking studies

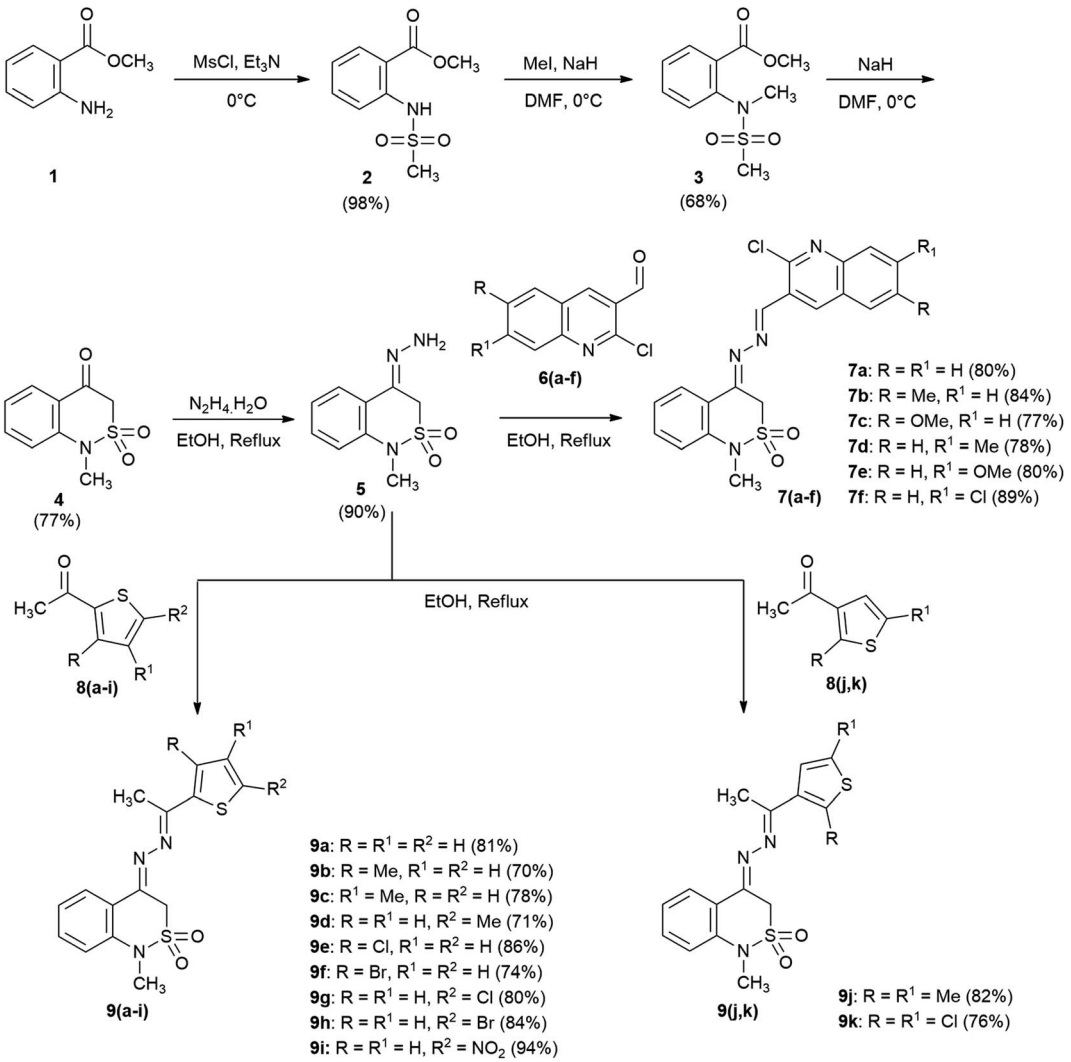
In order to look into the protein–ligand interactions of the newly synthesized analogues, docking studies were carried out against human monoamine oxidase A and B with PDB-ID 2Z5Y and 2V5Z, respectively, using Molecular Operating Environment (MOE 2014) software package. MAOs showed three functional areas in the active site, *i.e.*, the aromatic-cage (formed from Tyr435, Tyr398, and FAD), the substrate-cavity, and the entrance-cavity. Once the protein was prepared, the cognate ligand was a docked to validate the docking protocol by reproducing the experimentally determined orientation within an RMSD value of 1.5 Å.

Results and discussion

Chemistry

The titled 2,1-benzothiazine derivatives **7(a–f)** and **9(a–k)** were achieved by practicing the synthetic route presented in Scheme 1. The *N*-sulphonylated ester **2** obtained by solvent-free *N*-mesylation of methyl anthranilate **1** upon *N*-methylation lead to the formation of ester **3**.[29] Subsequent base catalyzed ring closure of the ester **3** resulted in the formation of ketone **4** with 2,1-benzothiazine framework which was further condensed with hydrazine monohydrate to yield hydrazone **5** in excellent yield. Finally, the acid catalyzed reaction of hydrazone **5** with (un)substituted 2-chloroquinoline carbaldehydes **6(a–f)** and methyl-thiophenyl ketones **8(a–k)** in methanol resulted in the formation of new titled compounds **7(a–f)** and **9(a–k)** correspondingly in reasonably good yields.

The necessary techniques like FTIR, ^{1}H NMR, ^{13}C NMR and elemental analyses were used to characterize the synthesized compounds. After determination of functional groups from the FTIR spectra and confirmation of composition from elemental analyses, the synthesized compounds were studied by NMR spectroscopy. The ^{1}H NMR spectra of the compounds **9(a–k)** exhibited three conspicuous singlet peaks referring to –SO_2CH_2– methylene protons (4.90 and 4.98 ppm), N–CH_3 (near 3.33 ppm) and methyl protons of ketone (near 2.50 ppm). The aromatic proton signals on the other hand exhibited chemical shifts in a range 6.89–8.36 ppm with multiplicities depending upon the environment of the corresponding hydrogen atoms. In ^{13}C NMR spectra of these compounds, N–CH_3 and –SO_2CH_2– methylene carbon showed signals around 33.3 ppm and 50.0 ppm respectively. The aromatic and imine (N=C) signals appeared in aromatic region *i.e.*, 111.3–162.5 ppm. The ^{1}H NMR spectra of compounds **7(a–f)** too were in agreement with the proposed structures showing N–CH_3 singlet near 3.33 ppm and aromatic signals between 6.84 and 8.93 ppm. However, these spectra exhibited duplicate signals for few protons including N=CH, H-4 of quinoline ring and –SO_2CH_2– protons. The presence of a deshielded singlet around 10.94–11.86 ppm



Scheme 1 Synthetic route for the titled 2,1-benzothiazine derivatives.

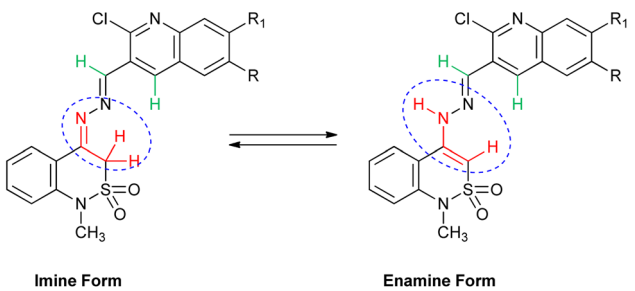


Fig. 2 Isomeric forms of compounds **7(a–f)** in solution resulting in duplicate peaks.

referring to NH proton indicated the formation of tautomers in the solution *i.e.*, imine and enamine forms (Fig. 2). The imine form showed the singlet for methylene protons (–SO_2CH_2–) around 5.25 ppm while enamine form exhibited the singlet peak for NH around 10.94–11.86 ppm and methine (SO_2CH=) singlet around 6.71–6.89 ppm. The N=CH proton due to neighbouring isomeric system, appeared as two discrete singlets for the two forms in a range 8.80–9.33 ppm. Conversely, ^{13}C NMR spectra too, showed duplicated signals for few carbon atoms. The imine form gave signal for N–CH_3 near 33.0 ppm however the enamine form exhibited this signal around 29.6 ppm. Methylene (SO_2CH_2) carbon atom showed up near 50.2 ppm for imine form while enamine form gave signal of methine (SO_2CH=) carbon atom near 95.0 ppm. The N=CH signal appeared near 150.0 ppm in aromatic region with other aromatic carbon atoms (106.8–161.8 ppm).

Monoamine oxidase activity

Monoamine oxidase inhibition studies. Newly synthesized compounds were tested against rat monoamine oxidase. For MAO A and MAO B Clorgyline and Deprenyl were used as standard inhibitors, respectively. The IC_{50} values of all compounds are summarized in Table 1. All tested compounds exhibited inhibitory activity in lower micromolar range. Against MAO A, the most potent compound **9e** was found to have an IC_{50}

Table 1 IC_{50} values of synthesized compounds against monoamine oxidase

		MAO A	MAO B
Entry	Code	IC $_{50}$ (μM) & % inhibition	
1	**7a**	38.8[a]	43.6[a]
2	**7b**	2.32 ± 0.91[b]	1.43 ± 0.89[b]
3	**7c**	2.10 ± 0.09[b]	35.75[a]
4	**7d**	1.82 ± 0.24[b]	1.05 ± 0.66[b]
5	**7e**	3.43 ± 0.52[b]	2.22 ± 0.94[a]
6	**7f**	2.03 ± 0.91[a]	1.21 ± 0.17 [b]
7	**9a**	2.09 ± 0.89[a]	1.98 ± 0.14 [b]
8	**9b**	38.5[a]	2.38 ± 0.19 [b]
9	**9c**	39.1[a]	3.83 ± 0.48 [b]
10	**9d**	1.27 ± 0.10 [b]	31.09[a]
11	**9e**	1.04 ± 0.01 [b]	41.93[a]
12	**9f**	41.6[a]	3.82 ± 0.37 [b]
13	**9g**	1.52 ± 0.56 [b]	1.25 ± 0.28 [b]
14	**9h**	28.4[a]	1.03 ± 0.17 [b]
15	**9i**	39.8[a]	1.21 ± 0.13 [b]
16	**9j**	26.3[a]	2.61 ± 0.37 [b]
17	**9k**	2.48 ± 0.70 [b]	2.58 ± 0.48 [b]
19	Clorgyline[c]	0.0045 ± 0.03	61.35 ± 1.13
20	Deprenyl[c]	67.25 ± 1.02	0.0196 ± 0.001

[a] Percentage inhibition. [b] IC_{50}. [c] Positive control.

value of 1.04 μM whereas the most potent inhibitor of MAO B was **7d** with an IC_{50} value of 1.05 μM. The compounds **9d** and **7c** exhibited significant selective inhibition toward MAO A (Fig. 3).

On the other hand, several compounds showed selective inhibition toward the MAO B enzyme including **9(a–c)**, **9f**, **9h**, **9i** and **9j**. While, some of these analogues *i.e.*, **7b**, **7d**, **7e**, **7f**, **9g** and **9k** displayed dual inhibition on MAO A as well as MAO B.

Structure-activity relationship. The role of different substituted groups at the benzylidene ring and their bioactivities were studied to get insights regarding the identification of selective inhibitors of monoamine oxidases (MAOs). Among the two series **7(a–f)** and **9(a–k)**, analogues showed distinguished activities against the MAOs inhibition. While different functional groups were introduced into the basic pharmacophore 4-(((2-Chloroquinolin-3-yl)methylene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (**7a**) and 1-Methyl-4-((1-(thiophen-2-yl)ethylidene)hydrazono)-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (**9a**), the inhibition was increased toward both isoforms of MAO (Fig. 4).

The unsubstituted compound **7a** showed less activity toward both isoforms, while the introduction of the methyl group *i.e.*, compound **7b** and methoxy group *i.e.*, compound **7c** at position 6 on quinoline ring made these derivatives potent inhibitor against MAO A (with IC_{50} values 2.10 ± 0.09 and 2.32 ± 0.91 μM respectively) as well as against MAO B (IC_{50} values 1.43 ± 0.89 μM, however, less activity for methoxy substituted analogue). Furthermore, the presence of methyl *i.e.*, compound **7d**, methoxy *i.e.*, compound **7e** and chloro group *i.e.*, compound **7f** at position 7 on same ring showed activities 1.82 ± 0.24 μM, 3.43 ± 0.5 μM and 2.03 ± 0.9 μM, respectively on MAO A

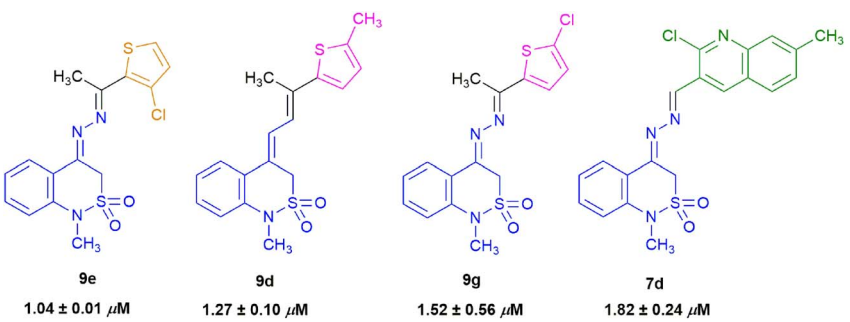


Fig. 3 Most potent MAO A inhibitory compounds.

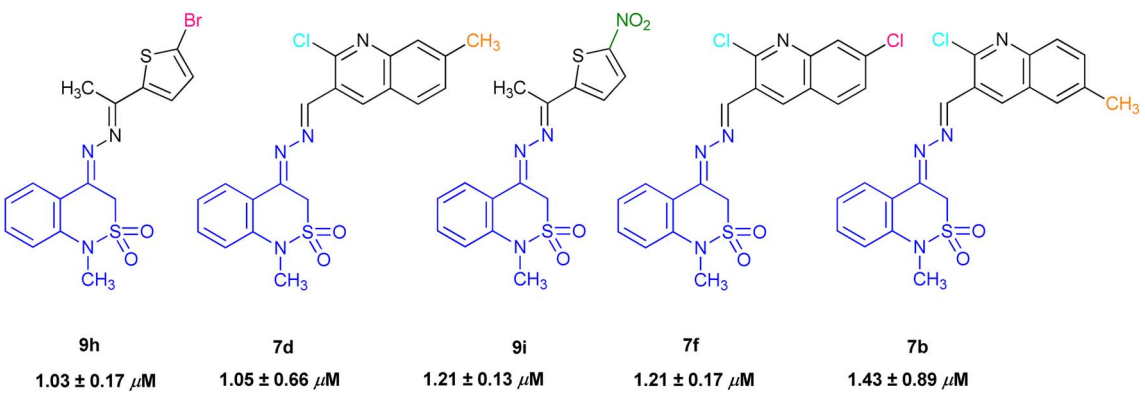


Fig. 4 Most potent inhibitors of MAO B.

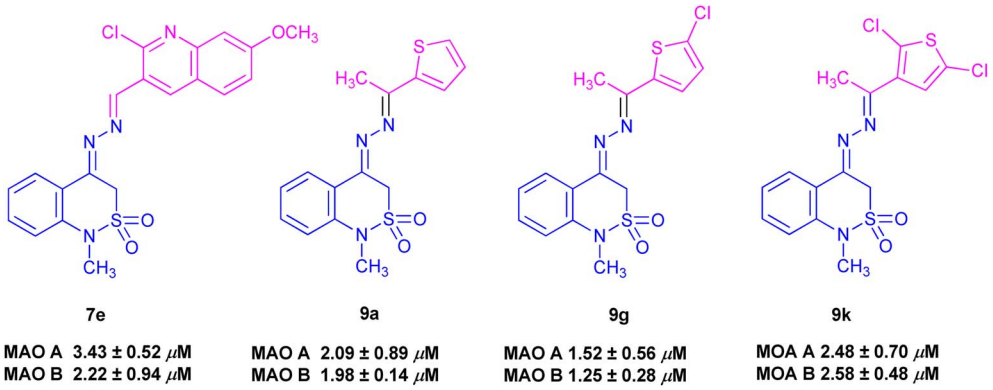


Fig. 5 Dual inhibitors of MAO A and MAO B.

whereas inhibition against MAO B was observed 1.05 ± 0.66 μM, 2.22 ± 0.94 μM and 1.21 ± 0.17 μM, respectively (Fig. 5).

In 2nd series, the introduction of chloro-substituent at thiophene ring led the compound **9e** to be the most potent derivative against MAO A having IC_{50} 1.04 ± 0.01 μM. The presence of methyl group at position 5 on same ring showed activity 1.27 ± 0.10 μM whereas the introduction of chloro group at same position showed nearly same IC_{50} value 1.52 ± 0.56 μM toward MAO A. The introduction of bromo- (**9g**), nitro- (**9h**) and chloro- (**9i**) at the same position exhibited inhibition of MAO B with IC_{50} values 1.03 ± 0.17 μM, 1.21 ± 0.13 μM and 1.25 ± 0.28 μM respectively.

Docking studies

The newly synthesized compound 4-((1-(3-chlorothiophen-2-yl) ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2] thiazine 2,2-dioxide (**9e**) showed activity toward MAO A in micromolar range (IC_{50} = 0.04 ± 0.01 μM). The presence of the two oxygen atoms in thiazine 2,2-dioxide were found to make hydrogen bond interactions with the amino acid Tyr69 and Ala68 same as already reported.[32–34] Furthermore, the benzene ring of the inhibitor was found to form π–π interactions with the amino acid residues Tyr444 and Tyr407 aromatic rings. Moreover, the presence of halogen *i.e.*, chloro-group at thiophene ring and the methyl group exhibited π–alkyl interactions with the amino acid residues Ile180, Leu337 and Ile335, respectively. Similarly, the presence of chlorine at position 3 of thiophene and sulfur in thiophene ring made halogens and π–sulfur interaction with the amino acid residues Phe208 and Phe352 respectively, comparable to the reference ligand interactions. Hence **9e** interacts with other residues as well as with those observed in case of reference cognate ligand. The putative binding mode of compound **9e** is shown in Fig. 6.

4-((1-(5-Bromothiophen-2-yl)ethylidene)hydrazono)-1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxide (**9h**) showed highest inhibitory activity against MAO B in micromolar range with IC_{50} value 1.03 ± 0.17 μM. The presence of the oxygen at thiazine 2,2-dioxide was found to show hydrogen bond interaction with the amino acid residue Tyr60, Ser59 and Lys296. The presence of benzene ring made π–sigma interaction with the amino acid Tyr398. The presence of bromo group at thiophene ring allowed π–alkyl interaction with the amino acid residue Ile199. 5-Bromothiophene ring made π–alkyl and π–sulfur interaction with amino acid residues Leu171 and Cys172, respectively. The presence of methyl group made π–

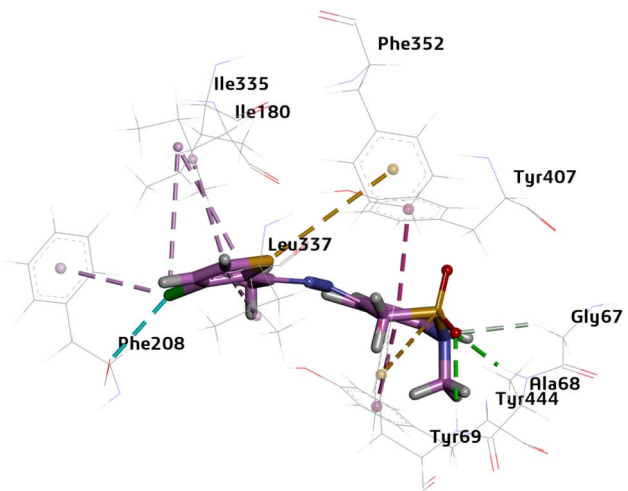


Fig. 6 Interaction of **9e** with MAO A protein.

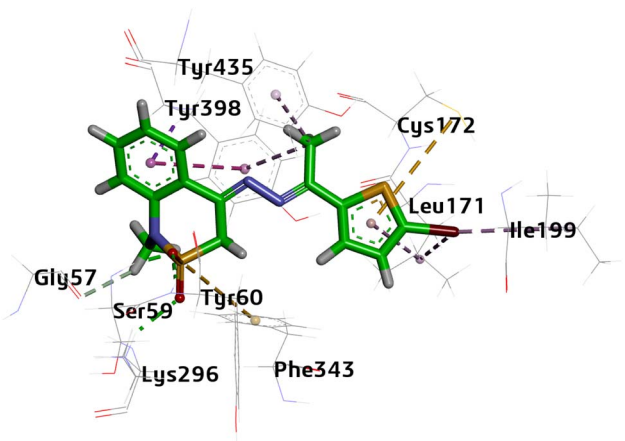


Fig. 7 Interaction of **9h** with MAO B protein.

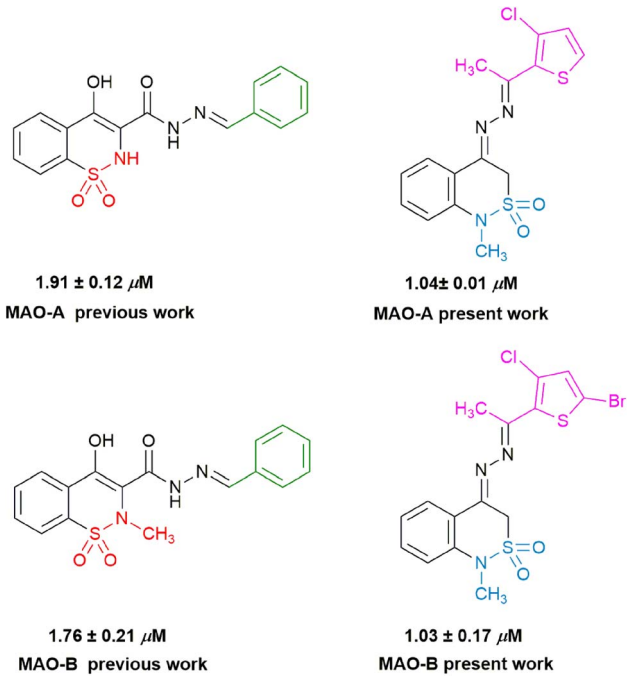


Fig. 8 Current work compared to previous work.

alkyl interaction with Tyr435 as reported previously.[32–34] While discussing the type of interaction displayed by reference ligand, the synthesized inhibitor (**9h**) has displayed same kind of interactions at the active site of the enzyme. The putative binding mode of compound **9h** is shown in Fig. 7.

Previously reported work and current study

If we compare our work with previously or already reported monoamine oxidase inhibitors, it was well known that already reported analogues of benzylidenethiazine-3-carbohydrazide 1,1-dioxide showed distinguished inhibition against monoamine oxidases. Whereas present study showed that the derivatives of benzylidenethiazine 2,2-dioxide showed better results than already reported derivatives (Fig. 8). The introduction of different groups (–F, –Cl, –Br, $–NO_2$,$–CH_3$,$–OCH_3$) in basic pharmacophore made it more potent and selective towards the targeted enzymes.

Conclusion

A library of 1-methyl-3,4-dihydro-1*H*-benzo[*c*][1,2]thiazine 2,2-dioxides with different substituent was synthesized and investigated against MAOs. All the synthesized compounds showed MAO inhibition activity in the lower micromolar range. Compound **9e**, having an IC_{50} value of 1.04 ± 0.01 μM, was the most potent MAO A inhibitor, while compound **9h**, with an IC_{50} value of 1.03 ± 0.17 μM, was the most active MAO B inhibitor. Furthermore, the docking studies further verified the binding site interactions of the inhibitors. Moreover, the pivotal role played by this target in AD and PD pathogenesis suggests that these potent compounds may act as promising new chemical entity in the design of multi-target-directed-ligands.

Author contributions

Noman Javid: conceptualization, methodology, formal analysis, validation. Saquib Jalil: investigation (bioactivity), writing – original draft preparation (bioactivity part), software. Rubina Munir: supervision, resources (synthesis), data curation, writing – original draft preparation (synthesis), project administration. Muhammad Zia-ur-Rehman: reviewing and editing, software. Amna Sahar: investigation (synthesis). Sara Arshad: investigation (synthesis). Jamshed Iqbal: supervision, resources (bioactivity and docking), funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no significant conflict of interest.

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