DESIGN, SYNTHESIS AND NEUROPHARMACOLOGICAL EVALUATION OF THIOPHENE INCORPORATED ISOXAZOLE DERIVATIVES AS ANTIDEPRESSANT AND ANTIANXIETY AGENTS

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ABSTRACT

*A series of 5-substituted phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1, 2-oxazoles **(2a-l)** was synthesized by reacting appropriate chalcones of 2-acetyl thiophene with hydroxylamine hydrochloride in the presence of dry pyridine. The newly synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral data. All the compounds were evaluated for their antidepressant and antianxiety activities in mice by forced swimming test and elevated plus maze method respectively. Test compounds and imipramine were administered intraperitoneally in antidepressant study at dose of 10 mg/kg. Similarly to study antianxiety activity, test compounds at the dose of 10 mg/kg and diazepam at the dose of 2 mg/kg were administered intraperitoneally. However, preliminary antidepressant screening of compounds (2a-l) revealed that none of the compounds showed antidepressant activity except for compound 2k which moderately ($P<0.05$) reduced the duration of immobility time. This compound was also tested in-vitro for its MAO inhibitory effect. Compound **2f** showed highest antianxiety activity compared to diazepam and did not show neurotoxicity in rotarod test. The compounds were also studied for pharmacokinetic parameters and was observed that compound **2f** displayed good ADME properties.*

Keywords: *Thiophene, isoxazole, antianxiety and antidepressant.*

INTRODUCTION

Depression is a life threatening illness that affects the major part of population across the globe [1]. It is associated with pathological change in mood state. Antidepressants can elevate mood in depressive state. Practically, all antidepressants affect monoaminergic transmission in the brain. Antidepressants affect the reuptake/ metabolism of biogenic amines. Antidepressants are generally classified as tricyclic antidepressants (including NA, 5HT reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NRIs) and MAO inhibitors [2]. Earlier MAO inhibitors were abandoned due to their side effects such as hepatotoxicity, orthostatic hypotension and the 'cheese effect' characterized by hypertensive crisis. This was due to non-selective and irreversible MAO inhibition. But identification of two isoforms i.e. MAO A and MAO B has renewed the interest in biological potential of these compounds. MAO A and MAO B are Flavin adenine dinucleotide (FAD) containing enzymes. MAO A preferably metabolizes serotonin (5-HT), adrenaline and noradrenaline whereas MAO B metabolizes β-phenyl ethylamine and benzylamine. MAO A inhibitors are useful in the treatment of depression and anxiety while MAO B could be used to treat Parkinson's disease and Alzheimer's disesase. Efforts have been oriented towards discovery of reversible and selective inhibitors of MAO A/ MAO B [3]. Similarly, anxiety is an emotional state associated with uneasiness, discomfort and fear about some future threat. Most of the anti-anxiety drugs act primarily by facilitating inhibitory GABAergic transmission [4]. The worldwide experience among clinicians and researchers is that anxiety and depression commonly co-exist in clinical samples and in general population [5]. During literature survey it was found that isoxazole nucleus is one of the important and widely exploited heterocyclic ring for the development of bioactive molecules. Increasing evidence suggests that isoxazole derivatives possess a broad spectrum of biological activities such as antianxiety, [6-7] antidepressant [8, 9] anti-stress [10], MAO inhibitory [11] and anticonvulsant [12]. Isocarboxazide is an isoxazole derivative having antidepressant action available in the market which is a non-selective MAO inhibitor. Recently antidepressant activity of isoxazole ether scaffold is also reported [13]. Many isoxazole nucleus containing compounds have also been patented for their antidepressant and antianxiety activities [14-18]. Similarly thiophene containing compounds have been reported to exhibit antidepressant [19-20] and antianxiety activities [21]. Duloxetine, Etizolam, Teniloxazine, Tigabine etc. are the thiophene nucleus containing antidepressant and antianxiety drugs being marketed. Thiophene analogues also have been well reported as MAO inhibitors [22].

However, during literature survey it was found that thiophene ring has not been linked with isoxazoles so far. This motivated us to synthesize hybrid compounds that comprise both the isoxazole and the thiophene ring systems with the hope of developing potent compounds with antianxiety and

antidepressant effects (**Figure 1**). We report herein the synthesis of a series of 5-substituted phenyl-3-(thiophen-2-yl)-4,5-dihydro-l, 2-oxazole derivatives (**2a-l).** A computational study for prediction of ADME properties of titled compounds **(2a-1)** was performed for the prediction of ADME properties. This study includes lipophilicity data also which is an important requirement for neuropharmacological activity. ADME properties of newly synthesized isoxazole derivatives were calculated using online molinspiration software. These compounds were tested for their *in-vivo* antidepressant activity by forced swimming test (FST). Compound 2k showing moderate antidepressant activity was screened for MAO inhibitory activity. All the twelve compounds were evaluated for their antianxiety activity by elevated plus maze method. Neurotoxicity was determined by rotarod toxicity test. Few of the final compounds have been reported by Ingle et al. [23] but have not been tested for their pharmacological activities.

EXPERIMENTAL

Chemistry:

All the chemicals used were of laboratory grade and procured from E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by the open tube capillary method and are uncorrected. The Thin layer chromatography (TLC) plates (silica gel G) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reactions as well. Two different solvent systems: toluene: ethyl acetate: formic acid (5:4:1) and benzene: acetone (9:1), were used to run the TLC and spots were located under iodine vapors/UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6/$CDCl_3$ on a Bruker 300 MHz and 75 MHz spectrometer, respectively, using tetramethylsilane (TMS) as the internal reference (chemical shift was measured in δ ppm). Mass spectra (ESI-Q-TOF) were measured on a Waters mass spectrometer with an ESI (Electron spray ionization) source.

General procedure for the preparation of 3-(substituted phenyl)-1-(thiophen-2-yl)prop-2-en-1-ones (chalcones) (1a-l) A mixture of 2-acetyl thiophene (1) (0.01 mol) and substituted aromatic aldehyde (0.01 mol) in absolute ethanol (30 mL) was stirred at room temperature in the presence of base (alc. solution of KOH 40%, 15 mL) for 3 hr at room temperature. The reaction mixture was kept overnight at room temperature and then transferred to crushed ice followed by neutralization with HCl. The solid separated was filtered, dried and recrystallized with ethanol. The purity of the chalcone was confirmed using TLC.

General procedure for the preparation of 5-substituted phenyl - 3 - (thiophen – 2 - yl) - 4, 5-dihydro-1, 2-oxazoles (2a-l). To the solution of appropriate chalcone (0.01 mol) in absolute ethanol (50 ml) was added dry pyridine (1 ml) and hydroxylamine hydrochloride (0.01 mol). The contents were refluxed for 8-10 h and left overnight. The solvent was evaporated off and the residue was poured into ice cold water. The solid mass which was separated out was filtered, washed with water, dried and crystallized from methanol.

5-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2a).

FTIR [KBr, cm^{-1}]: 676 (C-S), 1356 (C-O-N stretch), 1659 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.52 (1H, *dd*, *J* = 9.3, 6.8 Hz, CH), 3.74 (1H, dd, *J* = 11.3, 6.8 Hz, CH), 7.12-7.65 (8H, m, ArH), 5.92 (1H, dd, *J* = 9.3, 11.3 Hz, CH), 7.31 and δ 7.73 (dd, 1H, (thiophene C-CH), 7.61 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 δ ppm, MHz, δ ppm, DMSO-d_6]: 44.13, 82.09, 125.67, d126.91, 127.41, d127.57, 130.31, 130.38, 138.17, 140.03, 147.82, MS: m/z 229 (M^+); Anal. Calcd for $C_{13}H_{11}NOS$: C, 68.09, H, 4.84, N, 6.11, Found: C, 68.18, H, 4.90, N, 6.16%.

5-(2-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2b).

FTIR [KBr, cm^{-1}]: 676 (C-S), 1357 (C-O-N stretch), 1662 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: δ 3.58 (1H, dd, *J* = 9.4, 7.8 Hz, CH), 3.73 (1H, dd, *J* = 11.5, 7.8 Hz, CH), 6.00 (1H, dd, *J* = 9.4,11.5 Hz, CH), 7.15-7.65 (7H, m, ArH), 7.32 and δ 7.68 (dd, 1H, (thiophene C-CH), 7.62(t, 1H, (thiophene CH-S); ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: δ 44.43, 78.63, 126.65, 127.41, 129.21, 129.36, 129.58, 130.35, 130.40, 134.32, 135.36, 138.20, 147.82, MS: m/z 263.74 (M^+), 265.74 (M+2); Anal. Calcd for $C_{13}H_{10}ClNOS$: C, 69.20, H, 3.82, N, 5.31, Found: C, 69.33, H, 3.96, N, 5.34%.

5-(3-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2c).

FTIR [KBr, cm^{-1}]: 1652 (C=N stretch), 1363 (C-O-N stretch), 669 (C-S). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.57 (1H, dd, *J* = 9.1, 7.8 Hz, CH), 3.74 (1H, dd, *J* = 11.1, 7.8 Hz, CH), 5.99 (1H, dd, *J* = 9.1, 11.1 Hz, CH), δ 6.84-7.64 (7H, m, ArH), 7.35 and δ 7.71 (dd, 1H, (thiophene C-CH), 7.67(t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.14, 82.07, 147.80, 126.49, 127.29, 127.39, 128.43, 130.31, 130.37, 130.54, 135.04, 138.16, 139.41, MS: m/z 263.74 (M^+), 265.74 (M+2); Analysis: Calcd. for $C_{13}H_{10}ClNOS$: C, 69.20, H, 3.82, N, 5.31, Found: C, 69.36; H, 3.90, N, 5.36%.

5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2d).

FTIR [KBr, cm^{-1}]: 672 (C-S), 1359 (C-O-N stretch), 1648 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.60 (1H, dd, *J* = 9.2, 7.7 Hz, CH), 3.76 (1H, dd, *J* = 11.3, 7.7 Hz, CH), 6.05 (1H, dd, *J* = 9.2, 11.3 Hz, CH), 7.29-7.62 (7H, m, ArH), 7.30 and δ 7.68 (dd, 1H, (thiophene C-CH), 7.69 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.10, 82.09, 124.36, 127.36, d129.12, d129.65, 130.28, 130.34, 134.61, 138.19, 147.85, MS: m/z 263.74 (M^+), 265.74(M+2); Anal. Calcd. for $C_{13}H_{10}ClNOS$: C, 69.20, H, 3.82, N, 5.31, Found: C, 69.32, H, 3.93, N, 5.46%.

5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2e).

FTIR [KBr, cm^{-1}]: 672 (C-S), 1361 (C-O-N stretch), 1648 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: δ 3.64 (1H, dd, *J* = 9.3, 7.6 Hz, CH), 3.73 (1H, dd, *J* = 11.2, 7.6 Hz, CH), 6.00 (1H, dd, *J* = 9.3, 11.2 Hz, CH), 7.16-7.65 (7H, m, ArH), 7.31 and δ 7.69 (dd, 1H, (thiophene C-CH), 7.65 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.15, 82.11, 125.06, 127.37, d128.50, 130.31, 130.35, d132.43, 138.13, 147.75, MS: m/z 308.19 (M^+), 310.19 (M+2); Anal. Calcd. for $C_{13}H_{10}BrNOS$: C, 50.66, H, 3.27, N, 4.54, Found: C, 50.75, H, 3.39, N, 4.63%.

5-(4-fluorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2f).

FTIR [KBr, cm^{-1}]: 668 (C-S), 1353 (C-O-N stretch), 1654 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.66 (1H, dd, *J* = 9.6, 7.9 Hz, CH), 3.75 (1H, dd, *J* = 11.4, 7.9 Hz, CH), 6.04 (1H, dd, *J* = 9.6, 11.4 Hz, CH), 6.69-7.60 (7H, m, ArH), 7.29 and δ 7.71 (dd, 1H, (thiophene C-CH) 7.69 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.17, 82.05, d115.65, 122.57, 127.40, d127.51, 130.32, 130.38, 138.15, 147.82, 165.93, MS: m/z 247.29 (M^+), 249.29 (M+2); Anal. Calcd for $C_{13}H_{10}FNOS$: C, 63.14, H, 4.08, N, 5.66, Found: C, 63.25, H, 4.19, N, 5.73%.

5-(4-hydroxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2g).

FTIR [KBr, cm^{-1}]: 668 (C-S), 1355 (C-O-N stretch), 1652 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 2.32 (s, 3H, Ar-CH_3), 3.56 (1H, dd, *J* = 9.4, 7.9 Hz, CH), 3.69 (1H, dd, *J* = 11.1, 7.9 Hz, CH), 5.97 (1H, dd, *J* = 9.4, 11.1 Hz, CH), 6.15 (s, 1H, Ar-OH), 6.75-7.67 (7H, m, ArH), 7.33 and δ 7.67 (dd, 1H, (thiophene C-CH), 7.63 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.11, 82.00, d118.26, 119.32, 127.39, d129.64, 130.32, 130.36, 138.15, 147.83, 157.83, MS: m/z 245.30 (M^+); Anal. Calcd for $C_{13}H_{11}NO_2S$: C, 63.65, H, 4.52, N, 5.71, Found: C, 63.73, H, 4.65, N, 5.86%.

5-(4-methylphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2h).

FTIR [KBr, cm^{-1}]: 672 (C-S), 1350 (C-O-N), 1659 (C=N). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 2.32 (s, 3H, Ar-CH_3), 3.55 (1H, dd, *J* = 9.2, 5.7 Hz, CH), 3.71 (1H, dd, *J* = 11.3, 5.7 Hz, CH), 5.98 (1H, dd, *J* = 9.2, 11.3 Hz, CH), 7.18-7.63 (7H, m, ArH), 7.37 and δ 7.70 (dd, 1H, (thiophene C-CH), 7.64(t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 20.30, 44.16, 82.03, 124.51, 127.36, d129.48, 130.25, 130.40, d130.88, 138.26, 138.74, 147.78, MS: *m/z* 243.32 (M^+); Anal. Calcd for $C_{14}H_{13}NOS$: C, 67.11, H, 5.39, N, 5.76, Found: C, 67.23, H, 5.46, N, 5.84%.

5-(4-methoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2i).

FTIR [KBr, cm^{-1}]: 672 (C-S), 1352 (C-O-N stretch), 1656 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.42 (3H, s, OCH_3), 3.56 (1H, dd, *J* = 9.2, 7.6 Hz, CH), 3.71 (1H, dd, *J* = 11.3, 7.6 Hz, CH), 5.98 (1H, dd, *J* = 9.2, 11.3 Hz, CH), 6.85-7.63 (7H, m, ArH), 7.29 and δ 7.57 (dd, 1H, (thiophene C-CH), 7.64 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.16, 55.26, 82.06, d114.89, 119.68, 127.40, d130.10, 130.28, 130.32, 138.17, 147.83, 158.78, MS: m/z 259.32 (M^+); Anal. Calcd for $C_{14}H_{13}NO_2S$: C, 64.84, H, 5.50, N, 5.40, Found: C, 64.93, H, 5.56, N, 5.54%.

4-[3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazol-5-yl]aniline (2j).

FTIR [KBr, cm^{-1}]: 674 (C-S), 1357 (C-O-N stretch), 1653 (C=N stretch), ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.32 (s, 2H, Ar-NH_2), 3.59 (1H, dd, *J* = 9.5, 7.7 Hz, CH), 3.74 (1H, dd, *J* = 11.2, 7.7 Hz, CH), 5.97 (1H, dd, *J* = 9.5, 11.2 Hz, CH), 6.50-7.80 (7H, m, ArH), 7.30 and δ 7.73 (dd, 1H, (thiophen C-CH), 7.68 (t, 1H, (thiophen CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: 44.18, 82.09, 116.43, 120.46, d127.03, 127.35, 130.30, d130.38, 138.17, 145.34, 147.72, MS: m/z 244.31 (M^+); Anal. Calcd for $C_{13}H_{12}N_2OS$: C, 63.91, H, 4.95, N, 11.47, Found: C, 63.99, H, 4.98, N, 11.54%.

N,N-dimethyl-4-[3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazol-5-yl]aniline (2k).

FTIR [KBr, cm^{-1}]: 677 (C-S), 1358 (C-O-N stretch), 1659 (C=N stretch), ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 2.86 (s, 6H, Ar-N(CH_3)$_2$), 3.57 (1H, dd, *J* = 9.5, 7.9 Hz, CH), 3.76 (1H, dd, *J* = 11.4, 7.9 Hz, CH), 6.03 (1H, dd, *J* = 9.5, 11.4 Hz, CH), 6.44-7.69 (7H, m, ArH), 7.31 and δ 7.68 (dd, 1H, (thiophene C-CH), 7.69 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-d_6]: d40.33, 44.14, 82.09, d113.48, 119.01, 127.41, d128.61, 130.33, 130.39, 138.18, 147.83, 148.58, MS: m/z 272.37 (M^+); Anal.

Calcd for $C_{15}H_{16}N_2OS$: C, 66.15, H, 5.92, N, 10.29, Found: C, 66.21, H, 5.98, N, 10.35%.

5-(3,4-dimethoxyphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole (2l).

FTIR [KBr, cm^{-1}]: 669 (C-S), 1348 (C-O-N stretch), 1650 (C=N stretch). ^{1}H NMR [300 MHz, δ ppm, DMSO-d_6]: 3.38 (s, 6H, Ar-$(OCH_3)_2$), 3.57 (1H, dd, *J* = 9.4, 7.6 Hz, CH), 3.76 (1H, dd, *J* = 11.5, 7.6 Hz, CH), 6.00 (1H, dd, *J* = 9.4, 11.5 Hz, CH), 6.70-7.66 (6H, m, ArH), 7.31 and δ 7.62 (dd, 1H, (thiophene C-CH), 7.65 (t, 1H, (thiophene CH-S). ^{13}CNMR [75 MHz, δ ppm, DMSO-*d*6]: 44.15, d56.03, 83.16, 110.25, 113.32, 121.17, 127.40, 130.32, 130.38, 130.87, 138.17, 147.81, 148.26, 149.84, MS: m/z 289.35 (M^+); Anal. Calcd for $C_{15}H_{15}NO_3S$: C, 62.26, H, 5.23, N, 4.84, Found: C, 62.36, H, 5.32, N, 4.96%.

Pharmacology:

Prediction of Pharmacokinetic (ADME) Parameters

A computational study for the prediction of ADME properties of the compounds **(2a-l)** was performed. The percentage of absorption (%ABS) was calculated using topological polar surface area (TPSA)[24]. Absorption (%ABS) was calculated by: % ABS = 109-(0.345xTPSA) [25]. The excellent pharmacokinetic parameters of these compounds make them potentially promising agents for neuropharmacological activity. The pharmacokinetic parameters of the titled compounds **(2a-l)** are presented in **Table 1**.

Antidepressant activity (Forced swim test in mice)

Behavioral despair or forced swim test (FST) was suggested that mice or rats when forced to swim in restricted space from where they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. This behavioral despair test was employed to assess the antidepressant activity of newly synthesized derivatives. Albino mice of 20-25 g in a group of six each were used and on the first day of the experiment (pretest session), mice were individually placed in a cylindrical recipient (Plexiglass cylinder) of dimensions (diameter 10 cm, height 25 cm) containing 10 cm of water 25 °C. The animals were left to swim for 6 min before being removed, dried and returned to their cages. The procedure was repeated 24 h later, in 5 min swim session (test session). The synthesized compounds (10 mg kg^{-1}), and imipramine, as a reference antidepressant drug (10 mg kg^{-1}) were suspended in a 1% aqueous solution of Tween 80. The drugs were injected intraperitoneally (*ip*) in a standard volume of 0.5 ml/20 g body weight, 1 h prior to the test. Control animals received 1% aqueous solution of Tween 80. Then, the mice were dropped individually into the Plexiglass cylinder and left in the water for 6 min. During 6 min test session, the duration of immobility was recorded. Immobility time is the time spent by mice floating in water without struggling, making only those moment necessary to keep the head above the water. The total duration of immobility was recorded during the last 4 min of the 6 min test session [26]. The results of FST have been summarized in **Table 2.**

MAO inhibitory activity

MAO activity was determined by Spectrophotofluorometric method. Kynuramine was used as substrate to evaluate MAO activity of rat brain homogenate [27]. Rats were sacrificed by cervical dislocation. Brains from freshly killed rats were plunged into phosphate buffer. The obtained brain tissue was homogenized in phosphate buffer pH 7.4 (2 g/10.00 mL). The homogenate were centrifuged at 4 °C for 15 min. at 500-3000 rpm. The precipitate was rejected (unbroken cells, cell debris nuclei etc.) and the supernatant was collected. The incubation was carried out as follows. To 1.0 mL supernatant were added kynuramine (0.5 mL, 0.1 mM), Phosphate buffer (0.5 mL, pH 7.4) and test/ standard (tranylcypromine) (1.0 mL, $5x\ 10^{-4}$ M) and the volume was made up to 3.0 mL using distilled water. The above mixture was incubated at 37 °C for 30 min. After this the enzyme reaction was stopped by adding perchloric acid solution (1.0 ml of 100 g/L). The mixture was centrifuged at 1000 rpm for 5 min.and NaOH solution (2 mL, 1 N) was added to the 1.0 mL of the supernatant obtained above, in a quartz cuvette. This solution was activated at 315 nM and the fluorescence was measured at 389 nM with a spectrofluorimeter (RF-5301PC Shimadzu). A blank experiment was also performed where the standard/test sample was replaced with distilled water. MAO results were expressed as % inhibition (**TABLE 3**). %inhibition was calculated using the following formula:

%inhibition= (control fluorescence – test fluorescence or standard fluorescence/ control fluorescence) × 100. (The 4-hydroxy quinolone formed during oxidative deamination of kynuramine was measured fluorometrically. MAO is responsible for the oxidative deamination of a given amine to form corresponding aldehyde).

Antianxiety activity (Elevated plus maze test in mice)

Swiss albino mice, weighing 20–24 g each, were selected from the stock colony maintained in our animal facility with free access to food and water. Animals were maintained in an air-conditioned room. The room was maintained at 25 ± 2 °C with natural day time. Concentration of each compound (10 mg/kg) was used in the form of freshly prepared suspensions in 1% tween 80. All solutions were prepared freshly

on test days and given intraperitoneally (*ip*) in a volume of 2 ml/kg body weight of mice. The experimental animals were treated with Diazepam (2 mg/ kg, n = 6), or the test compounds (10 mg/kg) 60 min before evaluation in the maze. The control group was given saline with 1% tween 80. Plus maze for mice [28] consisted of two open (16 x 5 cm^2) and two closed arms (16 x 5 x 12 cm^3) facing each other with an open roof. The entire maze is elevated a height of 25 cm. In the test, mice were individually examined in 5-min sessions in this apparatus. Each mouse was placed in the central platform facing one open arm. The numbers of entries into open and closed arms and the time spent in the respective arms were recorded during a 5-min period. The percentage of time spent in the open arms [(open/open + closed) x 100] was calculated for each mice[29]. The results of EPM have been summarized in **Table 4.**

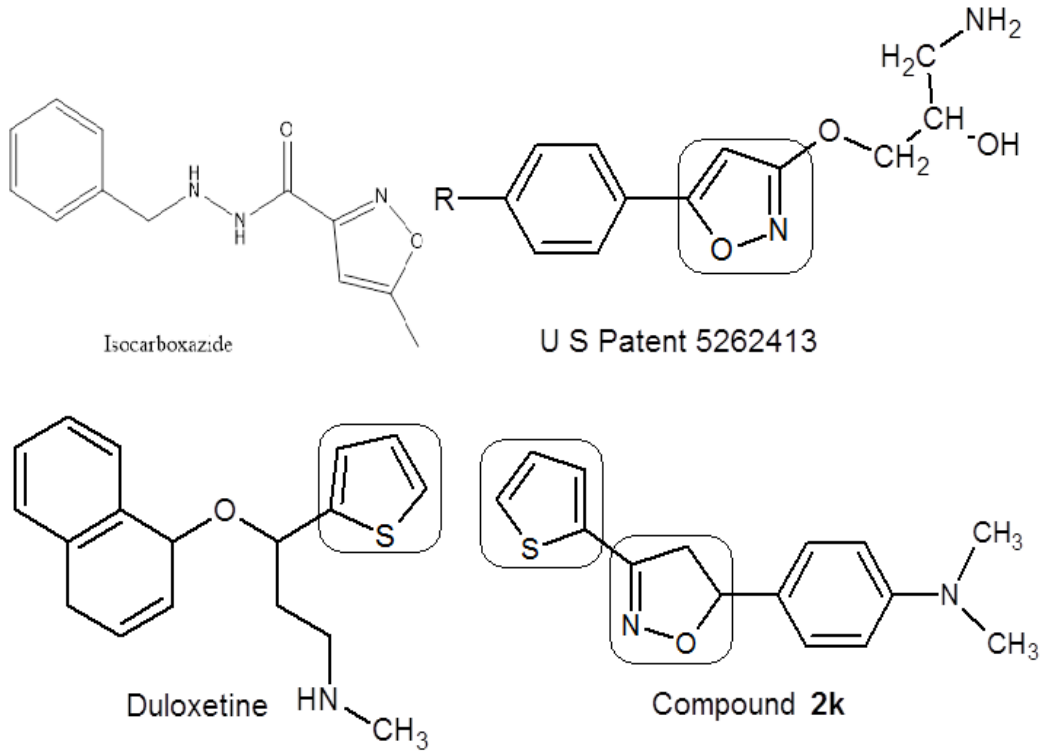


Figure 1: Structures of isoxazole and thiophene containing drugs (isocarboxazide, duloxetine), antidepressant patent and newly synthesized compound 2k.

Table 1: **Pharmacokinetic parameters important for good oral bioavailability of compounds**

Compound	MW	MV	n-atoms	% ABS	n-ROTB	TPSA (A^2)	nOHNH donors	milogP	n-ON acceptors	Lipinski's violations
Lipinski Rule	**<500**	-	-	-	-	-	**<5**	**<5**	**<10**	**≤1**
2a	243.331	217.758	17.0	101.54	2	21.598	0	3.864	2	0
2b	263.749	214.732	17.0	101.54	2	21.598	0	4.045	2	0
2c	263.749	214.732	17.0	101.54	2	21.598	0	4.069	2	0
2d	263.749	214.732	17.0	101.54	2	21.598	0	4.093	2	0
2e	308.2	219.082	17.0	101.54	2	21.598	0	4.225	2	0
2f	247.294	206.128	17.0	101.54	2	21.598	0	3.579	2	0
2g	245.303	209.214	17.0	94.57	2	41.826	1	2.936	3	0
2h	259.33	226.742	18.0	98.36	3	30.832	0	3.472	3	0
2i	289.356	252.288	20.0	95.18	4	40.066	0	3.062	4	0
2j	244.319	212.485	17.0	92.58	2	47.621	2	2.491	3	0
2k	272.373	247.103	19.0	100.43	3	24.836	0	3.518	3	0
2l	274.301	224.531	19.0	85.94	3	67.422	0	3.374	5	0

MW, molecular weight; MV, molecular volume; n-atoms, number of atoms; %ABS, percentage of absorption; n-ROTB, number of rotatable bonds; TPSA, topological polar surface area; n-OHNH, number of hydrogen bond donors; milogP, logarithm of compound partition coefficient between octanol and water calculated as per molinspiration online property toolkit; n-ON, number of hydrogen bond acceptors; %ABS = 109 – (0.345 x TPSA)

Table 2: Results of antidepressant activity of compounds (2a-l) by Forced swim test in mice

Compounds No	Antidepressant activity	
	Immobility time (s) (mean ± SEM)	Change from control (%)
2a	187.66±6.78	-5.14
2b	188.66±6.36	-4.63
2c	186.83±5.70	-5.56
2d	180.00±4.69	-9.01
2e	190.50±4.84	-3.70
2f	177.66±4.67	-10.19
2g	184.16±4.43	-6.90
2h	191.83±4.11	-3.03
2i	186.00±3.58	-5.97
2j	189.66±6.28	-4.12
2k	173.00±5.00*	-12.55
2l	187.16±6.08	-5.39
Imipramine (10 mg/kg, ip)	152.00±4.20**	-23.16
Control	197.83±5.91	-

Values represent the mean ±SEM (n=6), *Significantly compared to control (Dunnet's test; *p<0.05, **p<0.01)

Table 3: Results of in vitro inhibition activity and neurotoxicity study of selected isoxazoline derivatives.

S. No.	Compound No.	Neurotoxicity Coordination time in sec.[c] (mean ± SEM)	MAO Inhibition Monoamine oxidase inhibition[a] (%)
1	2f	56.00 ± 1.06	Nt
2	2k	Nt	27.05
3	Tranylcypromine[b]	Nt	84.50
4	Imipramine	Nt	Nt
5	Control	58.33 ± 1.70	Nt

[a] Each value is the mean from three separate experiments with SE of mean. Compound 2k was used at a final concentration of $5x10^{-4}$ M. [b] Concentration of tranylcypromine used 5.0 $x10^{-4}$ M.
Nt = denotes not tested, [c] Values represent the mean ±SEM (n = 6)

Table 4: Results of anti-anxiety activity of compounds (2a-l) by Elevated plus maze test in mice

Compounds	% preference to open arm	Open arm	
		No. of entries (mean ± SEM)	Average time spent (mean ± SEM)
2a	10.28	5.83±0.16	26.50±2.21**
2b	7.61	4.83±0.30	19.16±1.16
2c	11.97	6.33±0.33	29.16±1.40**
2d	19.61	7.33±0.49*	47.66±2.21**
2e	7.14	4.33±0.21	18.00±1.15
2f	20.44	7.50±0.22**	48.83±1.99**
2g	16.08	6.50±0.42*	39.83±1.47**
2h	6.84	4.83±0.30	18.33±1.16
2i	17.81	6.33±0.33	42.50±2.01**
2j	10.03	5.33±0.21	25.16±1.74**
2k	16.53	6.83±0.30**	39.83±1.24**
2l	16.87	6.50±0.34*	40.33±2.76**
Control	6.09	5.16±0.16	16.16±1.42
Diazepam ($2 mg kg^{-1}$, ip)	21.34	8.50±0.34**	54.33±2.60**

Values represent the mean ±SEM (n = 6)
p < 0.05, ** p < 0.01 (Dunnet's test compared to control)

Neurotoxicity

The rotarod test was used to evaluate neurotoxicity. The mice were trained to stay on a 1 inch diameter knurled wooden rod rotating at 6 rpm for 1 min. The animal was placed on rotating at 6 rpm. The trained animals were injected intraperitoneally with the test compounds **2f** at doses of 50 mg/kg, 30 min prior to the test session. Neurotoxicity was indicated by the inability of the animal to maintain equilibrilium on the rotating rod and results are reported as duration for which the animal is able to balance on the moving rod (i.e. till the animal falls) is noted as coordination time (mean±S.E.M) [30].

STATISTICAL ANALYSIS

The obtained experimental data were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's test and used to evaluate the results, using InStat Graph Pad (version 3.06, raph Pad Software Inc., San Diego, CA, USA). The results are expressed as mean S.E.M and n represents the number of animals. Differences between data sets were considered as significant when p value was less than 0.05.

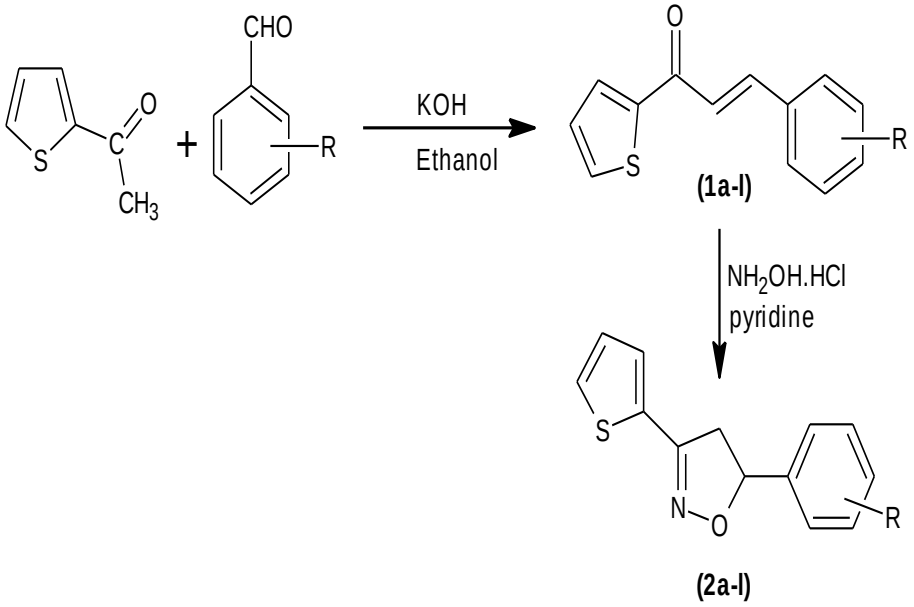
RESULTS AND DISCUSSION

Chemistry

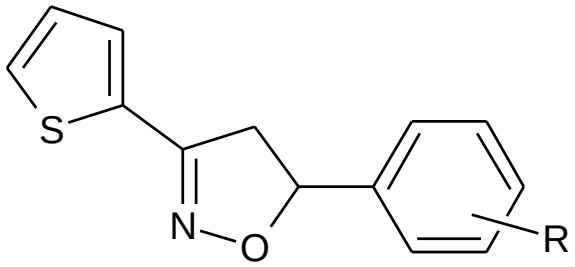
The physical constants of isoxazoline derivatives (**2a-l**) are shown in **Table 5** and a reaction sequence for the preparation is outlined in **Scheme 1**. Previously reported desired chalcones (**1a-l)** were obtained through Claisen-Schmidt condensation by reacting 2-acetyl thiophene with different substituted aldehydes in the presence of a base (KOH). Reaction between (**1a-l**) and hydroxylamine hydrochloride in the presence of dry pyridine afforded 5-substituted phenyl-3-(thiophen-2-yl)-4, 5-dihydro-1,2-oxazoles (**2a-l**) in 50-78% yield. TLC and elemental analyses were done to confirm the purity of the compounds. Spectral data [IR, ^{1}H-NMR and ^{13}CNMR] of the synthesized compounds were found in full agreement with the proposed structures, which was further confirmed by mass spectral data.

The IR spectrum of compound (**2a**) showed absorption peak at 1356 cm^{-1} due to C-O-N, 1659 cm^{-1} for C=N and 676 cm^{-1} for (C-S) stretching vibrations. The structure was further confirmed by its ^{1}H NMR spectrum, which showed two double doublets at δ 3.52 (1H, *dd*, *J* = 9.3, 6.8 Hz) and δ 3.74 (1H, dd, *J* = 11.3, 6.8 Hz) for CH_2 protons of isoxazoline ring. The CH proton at C-5 of isoxazoline was obtained as double doublet at δ 5.92 (1H, dd, *J* = 9.3, 11.3 Hz). Thus disappearance of signals of the olefinic protons of chalcone and appearance of CH_2 and CH proton signals in the spectrum confirmed the formation of isoxazoline ring. Further in ^{13}C NMR spectra the characteristic CH_2 and CH carbon of isoxazoline were observed at δ 44.13 and 82.09 ppms respectively.

The mass spectrum of the compound **2a** showed molecular ion peak M^{+} at m/z 229 corresponding to molecular formula $C_{13}H_{11}NOS$.



Scheme 1



(2a-l)

Table 5: Physicochemical parameters of the synthesized compounds (2a-l)

Compound No.	R	Molecular Formula	Molecular Weight	Yield[a] %	Melting Point °C
2a	4-H	$C_{13}H_{11}NOS$	229.29	78	141-143
2b	2-Cl	$C_{13}H_{10}ClNOS$	263.74	58	92-94
2c	3-Cl	$C_{13}H_{10}ClNOS$	263.74	52	110-112
2d	4-Cl	$C_{13}H_{10}ClNOS$	263.74	76	260-261
2e	4-Br	$C_{13}H_{10}BrNOS$	308.19	65	233-234
2f	4-F	$C_{13}H_{10}FNOS$	247.28	68	75-77
2g	4-OH	$C_{13}H_{11}NO_2S$	245.29	50	68-70
2h	4-CH_3	$C_{14}H_{13}NOS$	243.32	62	114-116
2i	4-OCH_3	$C_{14}H_{13}NO_2S$	259.32	68	80-82
2j	4-NH_2	$C_{13}H_{12}N_2OS$	244.31	70	132-134
2k	4-$N(CH_3)_2$	$C_{15}H_{16}N_2OS$	272.36	58	135-136
2l	3,4-$(OCH_3)_2$	$C_{15}H_{15}NO_3S$	289.34	64	59-60

[a] After recrystallization from ethanol,

Prediction of ADME Properties:

A computational study for prediction of ADME properties of titled compounds **(2a-l)** was performed and it was observed that all titled compounds exhibited an excellent %ABS ranging from 85.94 to 101.54 % Table **1**. The lipophilicity data also suggested that the derivatives were fairly lipophilic and hence able to cross BBB. It was encouraging to note that among the series none of the compounds violated any Lipinski's parameter. The excellent pharmacokinetic parameters of these compounds make them potentially promising agents for neuropharmacological therapy.

Antidepressant activity:

All the newly synthesized compounds (**2a-l**) were tested for their antidepressant activity by forced swimming test (FST) in mice (Behavioral despair test). Antidepressant activity was assessed as mean immobility time in seconds and data has been presented as mean ±S.E.M in **Table 2**. It was found that only compound 2k has moderate antidepressant activity.

MAO inhibition activity:

Compound showed very weak MAO inhibition (27.05%) at a final concentration of $5x10^{-4}$ M compared to standard drug tranylcypromine (84.50%) **Table 3**.

Anti-anxiety activity:

The antianxiety activity of the synthesized compounds was evaluated by elevated plus maze test in mice The test compounds showed antianxiety activity ranging from 6.84% to 20.44**%** preference to open arm, whereas diazepam showed 21.34**%** preference to open arm (**Table 3**). Among 12 compounds tested, three compounds **2d**, **2f** and **2i** showed promising antianxiety activity (>17% preference to open arm). Compound **2f** (*p-fluoro* substituted) showed highest antianxiety activity 20.44% preference to open arm. When the fluoro group of this compound was replaced by *p-chloro*

group the obtained compound **2d** was also found to have a good antianxiety activity 19.61% preference to open arm. Replacement of these substituents with *p-methoxy* group slightly decreased the activity and obtained compound **2i** exhibited good activity (17.81%). Compounds with *p-hydroxy* (**2g**), *p-N,N-dimethyl amino* group (**2k**) and *3,4-dimethoxy* (**2l**) groups showed moderate antianxiety activity. Other compounds did not show encouraging results. It was interesting to note that compound **2f** possessing excellent antianxiety activity did not show significant antidepressant activity (**Table 4**).

CONCLUSION

A new series of 5-substituted phenyl-3-(thiophen-2-yl)-4,5-dihydro-1,2-oxazole **(2a-l)** was synthesized with the aim to develop new antidepressant and antianxiety agents having good safety margin. Out of 12 newly synthesized compounds only one compound (2k) exhibited only moderate antidepressant activity by forced swim test in mice and it showed weak MAO inhibitory activity. This finding suggested a different mechanism of action of this compound besides inhibition of MAO. Compound **2f** displayed excellent results when studied for antianxiety activity. The study is also supported by Prediction of Pharmacokinetic (ADME) Parameters and it was observed that compounds **2f** and **2k** displayed good ADME properties. Compound **2f** did not show any neurotoxicity in the rotarod test. Therefore, it can be concluded that compound **2f** would constitute a useful model for further investigations in the development of antianxiety compound.

ACKNOWLEDGMENTS

Authors are thankful to Dr. G. N. Qazi, Vice Chancellor Jamia Hamdard for providing necessary facilities and also to Neuro-behavioral Pharmacology Laboratory, Hamdard University for help in conducting pharmacological activities. One of the author (Jagdish Kumar) pay sincere thanks to University Grants Commission (UGC), New Delhi, India, for providing financial assistance.

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