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Syntheses and anti-depressant activity of 5-amino-1, 3, 4-thiadiazole-2-thiol imines and thiobenzyl derivatives

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ABSTRACT

A number of new imine derivatives of 5-amino-1, 3, 4-thiadiazole-2-thiol have been synthesized, and their anti-depressant activity was tested using imipramine as reference drug. Two compounds namely 5-{[1-(4-chlorophenyl)-3-(4-methoxy-phenyl)prop-2-en-1-ylidene]-amino}- 5-benzylthio-1, 3,4 -thiadiazole **4i(b)** and 5-{[1-(4-chlorophenyl)-3-(4-dimethyl-aminophenyl)-prop-2-en-1-ylidene]amino}-5-benzylthio-1,3,4-thiadiazole **4i(c)** have shown significant anti-depressant activity, which decreased immobility time by 77.99% and 76.26% compared to the standard imipramine (82%). All the compounds in the series have passed neurotoxicity tests.

1. Introduction

During recent years intense investigations on different classes of thiadiazole compounds for CNS disorders have been reported.[1–3] Brufani et al.[1] have synthesized a series of 2-amino-5-sulfanyl-1, 3, 4-thiadiazoles as aldehyde dehydrogenase inhibitors and observed significant effects on the CNS as well as anti-depressant activity. Clerici et al.[2] reported the synthesis of 2-amino-5-sulfanyl-1, 3, 4-thiadiazoles and their anti-depressant and anxiolytic activities. Srivastava et al.[3] reported triazolo-thiadiazole and carbazolyl-thiadiazole-2-oxaazetidines as alternate compounds with moderate CNS activity. The reports on the diathiazole substitution prompted us to investigate the imine substitution in the moiety. A preliminary SAR, as discussed for the diathiazoles[1], has suggested the 2-amino substitution for better activity. Our efforts have been on the major substitution on the imine. The substitution of bulkier aliphatic group on the diathiazoles does not have much effect on the antidepressant activity. However, the benzyl substitution provided much impetus to the activity profile, and a substitution in the aromatic ring proved more beneficial to the activity enhancement. The features for the diathiazole-linked substitution for desired level of bioactivity have been retained in all the derivatives. The findings suggested designing amino substitution with bulkier aromatic groups with the electron-donating substitution for activity mapping. We have designed a series of amino-substituted compounds with electron-withdrawing and electron- donating groups on both terminals of the two aromatic substitutions. A topographical comparison also provided the chlorobenzyl difference in the series developed by us. Keeping this in view, we have synthesized some new imine derivatives of 5-amino-1, 3, 4-thiadiazole-2-thiol for their anti-depressant activity.

2. Results and discussion

The compounds were synthesized starting from chalcones and 5-amino-1, 3, 4-thiadiazole-2-thiols. The 5-amino-1, 3, 4-thiadiazole-2-thiol was synthesized from carbon disulfide addition to thiosemicarbazide under reflux. The series **2a**–**2e** were synthesized in single step by addition of different chalcones to the 2-amino-5-mercapto-1, 3, 4-thiadiazoles under reflux for 5 to 8 hrs. The compounds of **2a**–**2e** and benzyl chloride/4-chloro-benzyl chloride in ethanolic alkali were refluxed to give the desired **4i** and **4ii** series of compounds (Fig. 1). Concerning the characterization of compounds, infrared spectra were scanned in KBr on Bio-Red FTIR-spectrophotometer. Nuclear magnetic resonance spectra were scanned on Bruker model DRX-400 MHz, in $CDCl_3$ using tetramethylsilane (TMS) as the internal reference and Mass spectra was recorded on a JOEL SX 102\DA-6000 mass spectrometer using Argon\Xenon as FAB gas in matrix of *m*-nitro benzyl alcohol with an accelerating voltage of 10 kV.

The IR spectra of compounds exhibited absorption bands in the range of 3390–3318 cm^{-1} due to *trans*-ene C—H stretching bonds, 2600–2550 cm^{-1} due to SH, 1600–1550 cm^{-1} due to aromatic C—H groups, 1540–1500 cm^{-1} due to imine C=N stretching and 800–720 cm^{-1} due to aromatic C—H deformation. The NMR peaks in

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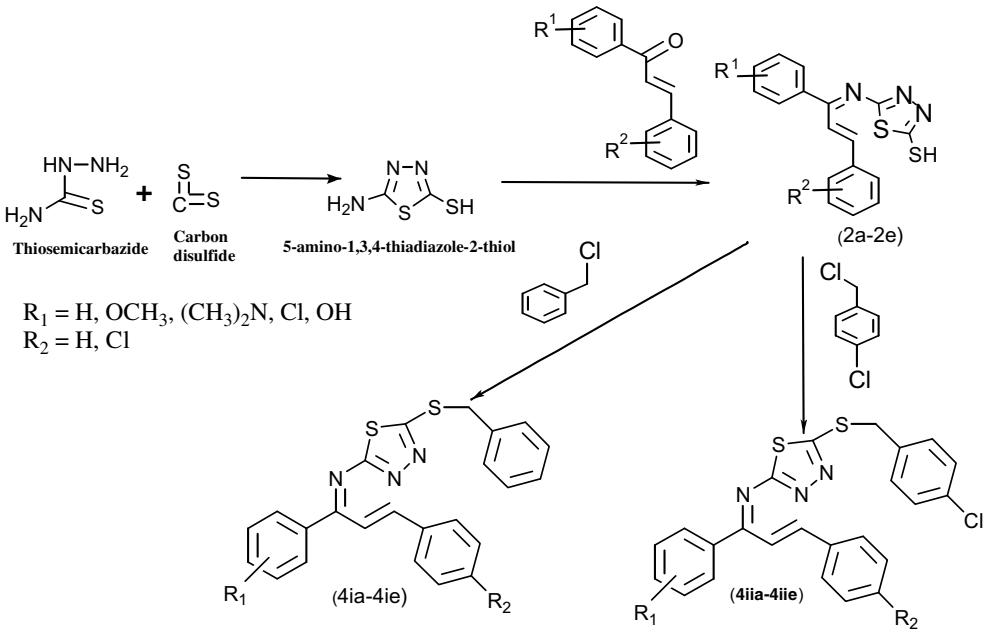

Figure 1. Synthesis of 5-amino-1,3,4-thiadiazole-2-thiol imine and imino-thiobenzyl derivatives.

the range of 6.5–8.00 ppm were due to different aromatic protons. 8–10 ppm was characteristic to imino proton; and 10–13.1 ppm was assigned to thiol-proton. The peaks in the range of 3.8–4.9 ppm were also found due to methylene group. Two doublets in the range of 7.5–8 ppm having coupling constant 15.6 Hz were very characteristic due to *trans*-proton of the ethylene group.

The conventional tricyclic anti-depressants inhibit the neuronal reuptake of neurotransmitters and metabolize to effective inhibitors of both norepinephrine and serotonin by dealkylation. The tricyclics have been analyzed for their lack of co-planarity in the molecular structure and effects of substitutions in side chains primarily responsible for alternating activity profile. However, the *str*uctural similarities among non-tricyclic antidepressants are less obvious. The aryl, aryloxy-alkylamines, flouxetin and viloxazine antidepressants have their own *str*uctural parameters with less interrelation. However, a broader look suggested a spatial minimality, wherein the contraction (or expansion) of available *str*uctural entities pointed out toward a spatial dimension occupation similar to that of tricyclic compounds with lesser considerations for the substitutions. The observation of minimum *str*uctural feature as presence of an aromatic ring and an aliphatic nitrogen with aliphatic chain of 4 atoms does not distinguish between inhibitors of dopamine, norepinephrine and serotonin uptakes, but does have interaction with other uptake receptor sites.

As 'the amine hypothesis of mood' postulates, the brain amines, particularly norepinephrine (NE) and serotonin (5-HT), are neuro-

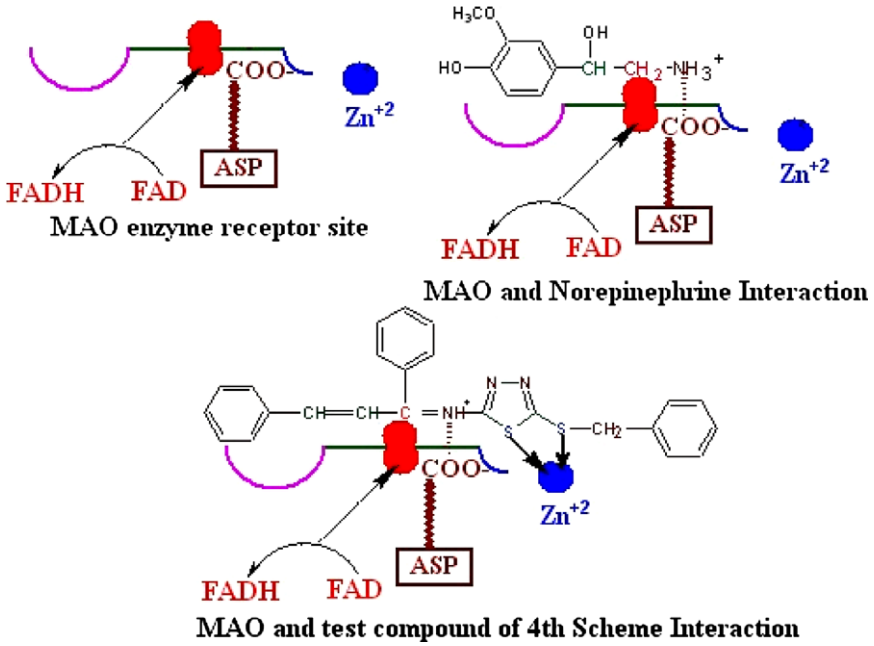

Figure 2. Possible interaction of norepinephrine and the test drug pharmacophore with MAO Enzyme.[5–14]

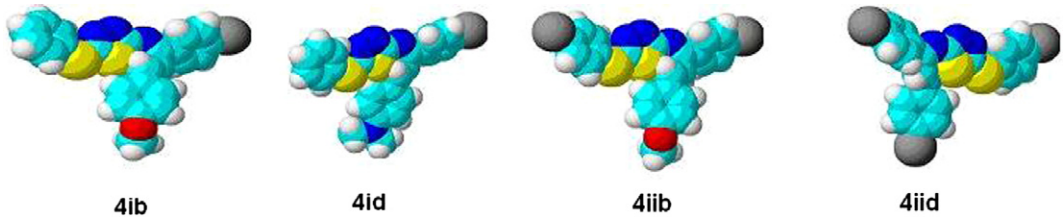

Figure 3. Space-filling models for **4i** & **4ii** series compounds.

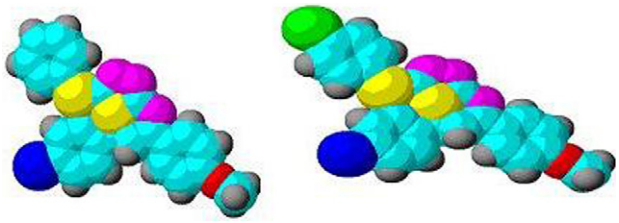

Figure 4. A Space-filling model for **4i** & **4ii** series compounds substituted with OCH_3 and Cl. The extra second Cl is colored green.

transmitters in pathways and function in the expression of mood. A functional decrease in the activity of such amines would result in depression and a functional increase of activity would result in mood elevation.[4]

MAO oxidizes the amines having hydrogen at α-position against the nitrogen, which are bonded with the amino acid carboxy (C) terminus, where the oxidation of α-carbon takes place by FAD cofactor. FAD itself reduces to FADH and oxidizes the α-H against the nitrogen to Schiff (imine) compound after being hydrolyzed to give aldehyde or ketone and an amine.[5–9]

We have synthesized a number of MAO inhibitors, which abolished the enzyme activity by one electron mechanism,[10] chelating the Zn^{2+} ions to interact with enzyme, creating non-availability of α-hydrogen against the nitrogen,[11,12]or by oxidation of trans ene proton and attachment to cysteine residue[13,14] as shown in Fig. 2.

The SAR has also been suggested for the aromatic ring and side chain amine. The aromatic substitution with electron-withdrawing groups and heterocyclic of 2-thienyl, 2-pyridyl, 2-furyl, and 2-pyrazinyl nature increases the potency. Thus, the minimum structure requirement includes an electron-rich moiety separated in the plane of aromatic ring by a distance of 5.25° A from its center. A QSAR molecular attribute estimate and 3D spatial orientation as well as electronic arrangements in the molecules obtained by the geometrical optimizations could not be interrelated with the activity profile of products in **4i** and **4ii** series. The products in **2a**-**e** and **4i** and **4ii** series were found to be non-planer in their stereo-orientation.

These observations suggested the structural correlation of conjugated methoxy-benzyl and chloro-benzyl groups as the larger substituents for aliphatic amine side chain in relation to the tricyclic and aryl-oxy antidepressant group of compounds. The structural entity minus the conjugated benzyl functionality is distinct in its observance of non-co-planarity. The remarkable difference was the extended spatial dimensions with electron-withdrawing groups as nuclear substitution in both the aromatic rings in the rest of the structure. These spatial and molecular attributes correlation suggested a different biological activity receptor site. (Figs. 3–6).

The observations that the products did not show any side effects with the neurological deficit have indicated the non-involvement of cross-over activity through the specific receptor sites.

3. Conclusion

Compounds of **4i** and **4ii** series were tested for their anti-depressant activity using imipramine as a standard drug. The parameter '% immobility' was calculated through forced swimming test in albino mice. All the test and standard compounds were administered at dose of 10 mg/kg intraperitoneally. Compound **4ib** and **4id** have decreased the immobility time by 77.99% and 76.26% compared to the standard drug (imipramine), which showed 82% decrease in immobility time. Hence, these compounds have superior anti-depressant activity as compared to standard drug (imipramine). Although **4iib** and **4iid** also decreased the immobility time by 84.20% and 87.57%, it was less than that of the standard drug imipramine.

4. Experimental

4.1. Synthesis of chalcones[15,16]

A solution of 2 g of sodium hydroxide in 20 mL of water and 10 g (12.5 mL) of rectified spirit was refluxed under stirring. It was cooled to room temperature and poured in freshly distilled acetophenone (0.04 mol). Benzaldehyde was added slowly under stirring at 25 °C for 2 h and it was kept in refrigerator overnight between 0 and 5 °C. The crystals obtained were filtered, washed with cold water until they were neutral and again were washed with ice-cold *n*-hexane. The product was recrystallized from *n*-hexane. TLC, (silica gel), R_f 0.5 in benzene: acetone (9:1).

4.2. Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol[17]

Thiosemicarbazide 45.5 g (0.25 mol) was suspended in absolute ethanol and anhydrous sodium carbonate (24 g) and carbon disulphide 0.25 mol (46 g) were added slowly. The mixture was stirred

Figure 5. Brufani et al. JMC, 2001, 44, 931.936, the most potent thiadizole, **3k** and **5g**, **3d**.

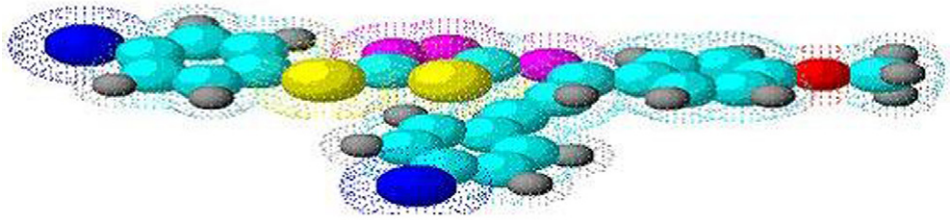

Figure 6. 3D Optimized di-chloro and methoxy substituted **4i** series compound.

under reflux for 1 h and later heated at 75–80 °C for 4 h. Solvent was removed and the residue was dissolved in water (200 mL), acidified with conc. HCl to give the product as hydrochloride salt, TLC (silica gel), TEF 5:4:1, mp 232 °C, yield 84% (54 g). ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm 7.99 (2H, s, NH_2), 12.93 (H, s, SH). FTIR (KBr) ν_{max} cm^{-1} 2553 (S—H$_{str}$, thiol).

4.3. Syntheses of various imines (2a-2e)

2-Amino-5-mercapto-1, 3, 4-thiadiazole (0.02 mol) was suspended in 25 mL absolute ethanol and 0.02 mol of different chalcones were added, refluxed for 5–8 h, and then left overnight. The solvent was evaporated in vacuum and the residue was recrystallized from methanol.

4.3.1. 5-{[1, 3-Diphenylprop-2-en-1-ylidene] amino}-1,3,4-thiadiazole-2-thiol (2a)

Yield 70%, mp 58–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 7.73 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.94 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 12.37 (1H, s, SH) 6.97–7.76 (m, 10H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3018 (C—H$_{str}$, *trans*-ene), 2545 (S—H$_{str}$, thiol), 1680 (imine, C=N$_{str}$), 968 (C—H$_{def}$, *trans*-ene), 706 (C—H$_{def}$, monosubst. Bz). FABMS *m*/*z* 325, 210, 131. Anal. Calcd. for $C_{17}H_{13}N_3S_2$: C, 63.13; H, 4.05; N, 12.99. Found: C, 63.10; H, 4.06; N, 12.97.

4.3.2. 5-{[1-(4-Chlorophenyl)-3-[4-(methoxyphenyl)-prop-2-en-1-ylidene]amino}- 1,3,4-thiadiazole-2-thiol (2b)

Yield 62%, mp 81–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 7.44 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.57 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 12.17 (1H, s, SH) 7.02–7.82 (m, 8H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3015 (C—H$_{str}$, *trans*-ene), 2543 (S—H$_{str}$, thiol), 1677 (imine, C=N$_{str}$), 964 (C—H$_{def}$, *trans*-ene), 808 (C—H$_{def,}$ p-disubst. Bz), 698 (C—H$_{def,}$ monosubst. Bz), 612 (Ar C—Cl$_{str}$, Bz). FABMS *m*/*z* 390, 273, 131. Anal. Calcd. for $C_{18}H_{14}ClN_3OS_2$: C, 55.73; H, 3.64; N, 10.83. Found: C, 55.71; H, 3.63; N, 10.84.

4.3.3. 5-{[1-(4-Chlorophenyl)-3-[4-(dimethylamino)phenyl-prop- 2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2c)

Yield 73%, mp 130–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 7.36 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.49 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 12.15 (1H, s, SH) 7.04–7.86 (m, 8H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3016 (C—H$_{str}$, *trans*-ene), 2544 (S—H$_{str}$, thiol), 1680 (imine, C=N$_{str}$), 967 (C—H$_{def}$, *trans*-ene), 810 (C—H$_{def}$, p-disubst. Bz), 703 (C—H$_{def}$, monosubst. Bz), 615 (Ar C—Cl$_{str}$, Bz). FABMS *m*/*z* 402, 272, 131. Anal. Calcd. for $C_{19}H_{17}ClN_4S_2$: C, 56.92; H, 4.27; N, 13.97. Found: C, 56.87; H, 4.26; N, 13.94.

4.3.4. 5-{[1,3-bis-(4-Chlorophenyl)-prop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol (2d)

Yield 69%, mp 75–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 7.68 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.91 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 12.21 (1H, s, SH) 7.10–7.92 (m, 8H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3020 (C—H$_{str}$, *trans*-ene), 2547 (S—H$_{str}$, thiol), 1679 (imine, C=N$_{str}$), 965 (C—H$_{def}$, *trans*-ene), 805 (C—H$_{def}$, p-disubst. Bz), 696 (C—H$_{def}$, monosubst. Bz), 618 (Ar C—Cl$_{str}$, Bz). FABMS *m*/*z* 409, 265, 131. Anal. Calcd. for $C_{17}H_{11}Cl_2N_3S_2$: C, 52.04; H, 2.83; N, 10.16. Found: C, 52.08; H, 2.81; N, 10.13.

4.3.5. 2-{-3-(4-Chlorophenyl)-3-[(5-mercapto-1,3,4-thiadiazol-2-yl)imino]-prop-1-en-1-yl} phenol (2e)

Yield 45%, mp 126–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.92 (1H, s, OH), 7.52 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.77 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 12.19 (1H, s, SH) 7.05–7.84(m, 8H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3019 (C—H$_{str}$, *trans*-ene), 2548 (S—H$_{str}$, thiol), 1672 (imine, C=N$_{str}$), 960 (C—H$_{def}$, *trans*-ene), 739 (C—H$_{def}$, o-disubst. Bz), 693 (C—H$_{def}$, monosubst. Bz), 614 (Ar C—Cl$_{str}$, Bz). FABMS *m*/*z* 372, 246, 131. Anal. Calcd. for $C_{17}H_{12}ClN_3OS_2$: C, 54.61; H, 3.24; N, 11.24. Found: C, 54.62; H, 3.21; N, 11.23.

4.4. Thio-thiadiazole imine derivatives of aromatic chalcones (4a–4e)

A mixture of Fig. 2 compounds namely **2a**–**2e** (0.005 mol) and 0.005 mol of benzyl chloride/4-chloro-benzyl chloride in ethanolic alkali (0.08 g KOH in 20 mL EtOH) was refluxed till the completion of reaction (as indicated by TLC using silica gel as stationary phase and TEF 5:4:1 as mobile phase). On cooling, the reaction mixture was poured into crushed ice, and a crude precipitate was obtained, which was filtered and then recrystallized from acetone.

4.4.1. 5-(Benzylthio)-*N*-[1,3-diphenylprop-2-en-1-ylidene]-1,3,4-thiadiazol-2-amine (4ia)

Yield 47%, mp 46 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.81 (2H, s, CH_2), 7.89 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.65 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 6.98–7.81 (m, 15H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3021 (C—H$_{str}$, *trans*-ene) 1687 (C=N$_{str}$), 1462 (methylene, C—H$_{str}$), 976 (C—H$_{def}$, *trans*-ene), 709 (C—H$_{def,}$ monosubst. Bz). FABMS *m*/*z* 415, 274, 209. Anal. Calcd. for $C_{24}H_{19}N_3S_2$: C, 69.70; H, 4.63; N, 10.16. Found: C, 69.71; H, 4.62; N, 10.14.

4.4.2. 5-{[1-(4-Chloro phenyl)-3-(4-methoxyphenyl)prop-2-en-1-ylidene]amino}-5-benzylthio-1,3, 4-thiadiazole (4ib)

Yield 50%, mp 80 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.83 (2H, s, CH_2), 7.66 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.86 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.91–7.68 (m, 13H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3027 (C—H$_{str,}$ *trans*-ene), 1689 (C=N$_{str}$), 1466 (methylene, C—H$_{str}$), 976 (C—H$_{def}$, *trans*-ene), 815 (C—H$_{def,}$ p-disubst. Bz), 712 (C—H$_{def,}$ monosubst. Bz), 610 (Ar C—Cl$_{str}$, Bz). FABMS *m*/*z* 478, 271, 208. Anal. Calcd. for $C_{25}H_{20}ClN_3OS_2$: C, 62.81; H, 4.22; N, 8.79. Found: C, 62.79; H, 4.21; N, 8.78.

4.4.3. 5-{[1-(4-Chlorophenyl)-3-(4-dimethylaminophenyl)-prop-2-en-1- ylidene]amino}-5-benzylthio-1,3,4-thiadiazole (4ic)

Yield 48%, mp 95 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.79 (2H, s, CH_2) 7.65 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.89 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.93-7.72 (m, 13H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3023 (C—H$_{str,}$ *trans*-ene), 1682 (C=N$_{str}$), 1461 (methylene, C—H$_{str}$), 973 (C—H$_{def}$, *trans*-ene), 812 (C—H$_{def,}$ p-disubst. Bz), 709

(C—H_{def}, monosubst. Bz), 605 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 491, 285, 209. Anal. Calcd. for $C_{26}H_{23}ClN_4S_2$: C, 63.59; H, 4.72; N, 8.43. Found: C, 63.56; H, 4.71; N, 8.43.

4.4.4. 5-Benzylthio-*N*-[1,3-bis(4-chlorophenyl)prop-2-en-1-ylidene]-1,3,4- thiadiazol-2-amine (4id)

Yield 39%, mp 51 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.80 (2H, s, CH_2) 7.63 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.88 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.98–7.76 (m, 13H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3028 (C—H_{str}, *trans*-ene), 1688 (C=N_{str}), 1465 (methylene, C—H_{str}), 976 (C—H_{def}, *trans*-ene), 815 (C—H_{def}, p-disubst. Bz), 711 (C—H_{def}, monosubst. Bz), 607 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 525, 276, 209. Anal. Calcd. for $C_{24}H_{17}Cl_2N_3S_2$: C, 59.75; H, 3.55; N, 8.71. Found: C, 59.73; H, 3.56; N, 8.70.

4.4.5. 2-[3-{[5-(Benzylthio)-1,3,4-thiadiazol-2-yl]imino}-3-(4-chlorophenyl)prop-1-en-1-yl]phenol (4ie)

Yield 52%, mp 101 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.75 (2H, s, CH_2), 4.88 (1H, s, OH), 7.61 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.85 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 7.04–7.70 (m, 13H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3030 (C—H_{str}, *trans*-ene), 1692 (C=N_{str}), 1460 (methylene, C—H_{str}), 977 (C—H_{def}, *trans*-ene), 817 (C—H_{def}, p-disubst. Bz), 717 (C—H_{def}, monosubst. Bz), 613 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 463, 258, 208. Anal. Calcd. for $C_{24}H_{18}ClN_3OS_2$: C, 62.12; H, 3.91; N, 9.06. Found: C, 62.11; H, 3.92; N, 9.10.

4.4.6. 5-(4-Chlorobenzylthio)-N-[1,3-diphenylprop-2-en-1-ylidene]-1,3,4- thiadiazol-2-amine (4iia)

Yield 23%; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.96 (2H, s, CH_2), 7.64 (1H, d, *J* = 5.6 Hz, *trans*-ene-H), 7.9 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 7.09–7.73 (m, 14H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3014 (C—H_{str}, *trans*-ene), 1683 (C=N_{str}), 1466 (methylene, C—H_{str}), 971 (C—H_{def}, *trans*-ene), 704 (C—H_{def}, monosubst. Bz), 609 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 447, 207, 244. Anal. Calcd. for $C_{24}H_{18}ClN_3S_2$: C, 64.34; H, 4.05; N, 9.38. Found: C, 64.32; H, 4.02; N, 9.33.

4.4.7. 5-{[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-prop-2-en-1-ylidene]amino}- 5-(4-chlorobenzylthio)-1,3,4-thiadiazole (4iib)

Yield 39%, mp 72 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 5.1 (2H, s, CH_2), 7.71 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.94 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.96–7.63 (m, 12H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3027 (C—H_{str}, *trans*-ene) 1689 (C=N_{str}), 1464 (methylene, C—H_{str}), 976 (C—H_{def}, *trans*-ene), 813(C—H_{def}, p-disubst. Bz), 604 (Ar C—Cl *str*, Bz). FABMS *m*/*z* 513, 273, 243. Anal. Calcd. for $C_{25}H_{19}Cl_2N_3OS_2$: C, 58.59; H, 3.74; N, 8.20. Found: C, 58.54; H, 3.71; N, 8.21.

4.4.8. 5-{[1-(4-Chlorophenyl)-3-(4-dimethylaminophenyl)-prop-2-en-1- ylidene]amino}-5-(4- chlorobenzylthio)-1,3,4-thiadiazole (4iic)

Yield 41%, mp 81 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 4.98 (2H, s, CH_2), 7.67 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.93 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 7.01–7.78 (m, 12H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3014 (C—H_{str}, *trans*-ene) 1678 (C=N_{str}), 1454 (methylene, C—H_{str}), 969 (C—H_{def}, *trans*-ene), 603 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 527, 285, 242. Anal. Calcd. for $C_{26}H_{22}Cl_2N_4S_2$: C, 59.42; H, 4.22; N, 10.66. Found: C, 59.39; H, 4.18; N, 10.59.

4.4.9. 5-(4-Chlorobenzylthio)-*N*-[1,3-bis(4-chlorophenyl)prop-2-en-1-ylidene]-1, 3,4-thiadiazol-2-amine (4iid)

Yield 22%, mp 40 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm 5.12 (2H, s, CH_2), 7.69 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.97 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.98–7.65 (m, 12H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3012 (C—H_{str}, *trans*-ene). 1680 (C=N_{str}), 1457 (methylene, C—H_{str}), 964 (C—H_{def}, *trans*-ene), 601 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 518, 275, 242. Anal. Calcd. for $C_{24}H_{16}Cl_3N_3S_2$: C, 55.77; H, 3.12; N, 8.13. Found: C, 55.76; H, 3.08; N, 8.11.

4.4.10. 2-[3-{[5-(4-Chlorobenzylthio)-1,3,4-thiadiazol-2-yl]imino}- 3-(4-chlorophenyl)prop-1-en-1-yl] phenol (4iie)

Yield 25%, mp 90 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ ppm, 4.66 (1H, s, OH), 4.95 (2H, s, CH_2) 7.61 (1H, d, *J* = 15.6 Hz, *trans*-ene-H), 7.85 (1H, d, *J* = 15.6 Hz, *trans*-ene-H) 6.96-7.72 (m, 12H, Ar–Bz). FTIR (KBr) ν_{max} cm^{-1} 3031 (C—H_{str}, *trans*-ene), 1691 (C=N_{str}), 1459 (methylene, C—H_{str}), 975 (C—H_{def}, *trans*-ene), 816 (C—H_{def}, p-disubst. Bz), 715 (C—H_{def}, monosubst. Bz), 612 (Ar C—Cl_{str}, Bz). FABMS *m*/*z* 499, 258, 243. Anal. Calcd. for $C_{24}H_{17}Cl_2N_3OS_2$: C, 57.83; H, 3.44; N, 8.43. Found: C, 57.76; H, 3.43; N, 8.39.

4.5. Biological screening

4.5.1. Anti-depressant activity[18,19]

Anti-depressant activity was measured with imipramine as standard drug using the Forced Swimming Test in albino mice. Albino mice were placed in a vertical Plexiglas cylinder filled with water, maintained at 25 °C, for 15 min. Five to six minutes later immobility reached a plateau, where the mice remained immobile for approximately 80% of the time. After 15 min in the water, the mice were removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They were again placed in the cylinder for 24 h later, and the total duration of immobility was measured during a 5-min test (Table 1).

4.5.2. Neurotoxicity test

The disruptive effects on motor coordination were assessed using the Rotarod Tread[20] mill mouse test and Ethanol Potentiation[2] Test in male albino mice.

4.5.2.1. Rotarod test[20]. The animals were placed on a rotating rod (24 rpm) and then observed for 5 min. The skeletal

Table 1
Antidepressant activity of test drug compared with that of imipramine by the Forced Swimming test in albino mice

Compound	Dose (mg/kg)	Immobility time ± SEM	% Immobility
Vehicle	—	235.9 ± 5.7	100
4ib	10	184.0 ± 12.1*	77.99
4id	10	179.9 ± 5.6*	76.26
4iib	10	198.7 ± 9.9	84.23
4iid	10	206.6 ± 10.7	87.57
Vehicle	—	215.3 ± 13.1	100.00
Imipramine	10	176.3 ± 14.9	82.00

Test compounds and imipramine (reference) were administered ip 60 min before the test. Each group consisted of eight mice.
* $P < 0.01$ vs respective vehicle group.

Table 2
Neurotoxic activity of the test compounds by Rotarod an Ethanol Potentiation test in albino mice

Compound	Dose (mg/kg)	Number of mice	Result of Rotarod test[a]	Result of Ethanol Potentiation test[b]
4ib	10	6	(+)	(+)
4id	10	6	(+)	(+)
4iib	10	6	(+)	(+)
4iid	10	6	(+)	(+)

The (+) sign indicates 50% or more passed the neurotoxicity testing.
[a] Dose was administered ip and neurotoxicity was measured after 30 min.
[b] Mice were treated with the test compounds and 1 h later with ethanol 2.5 g\kg ip neurotoxicity was measured after 30 min.

muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. The dose which impairs the ability of 50% of the mice to remain on the revolving rod was considered the endpoint (Table 2).

4.5.2.2. Ethanol Potentiation test.[2] Mice were treated with the test compounds and 1 h later with ethanol (2.5 g/kg ip). This dose of ethanol did not induce lateral position in the control animals. The number of mice that were in the lateral position after receiving ethanol in each group was determined (Table 2).

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References and notes

1. Brufani, M.; Loche, A.; Perlini, V.; Pocar, D. (Laboratorio Farmaceutico C. T. S. r.l.) E.P 579, 129; *Eur. Pathol. Appl.* **1994**, *120*, 245119.
2. Clerci, F.; Pocar, D.; Guido, M.; Loche, A.; Perlini, V. *J. Med. Chem.* **2001**, *44*, 931.
3. Srivastava, K. S.; Srivastava, L. S.; Srivastava, D. S. *Indian J. Chem.* **2002**, *41B*, 2357.
4. Hollister, L. E. In *Antidepperssant agents: Antidepressant Agents: Basic and Clinical Pharmacology*; Katzung, B. G., Ed.; Lange Medical Publication: Connecticut, 1992; pp 412–413.
5. Yu, P. H. *Biochem. Cell Biol.* **1988**, *66*, 853.
6. Kopin, I. J. *J. Neural Transm. Suppl.* **1994**, *41*, 57.
7. Jonston, J. P. *Biochem. Pharmacol.* **1968**, *17*, 1285.
8. Olsson, B.; Olsson, J.; Pettersson, G. *Eur. J. Biochem.* **1976**, *64*, 327.
9. Fowler, J. S.; Logan, J.; Wang, G. J. *Nucl. Med. Biol.* **2004**, *31*, 313.
10. (a) Silverman, R. B.. In *Advances in Electron Transfer Chemistry*; Mariano, P. S., Ed.; JAI Press: Greenwich, C.T, 1992; vol. 2, pp 177–213; (b) Silverman, R. B. *Acc. Chem. Res.* **1995**, *28*, 335; (c) Lu, X.; Rodriguez, M.; Ji, H.; Silverman, R. B.; Vintém, A. P. B.; Ramsay, R. R. In *Flavins and Flavoproteins*; Chapman, S. K., Perham, R. N., Scrutton, N. S., Eds.; Rudolf Weber: Berlin, 2002; pp 817–830.
11. (a) Silverman, R. B. *Lead Modification-DrugDesign and Development: The Organic Chemistry of Drug Design and Drug Action*; Academic Press: Burlington, 2004. pp. 21–34; (b) Silverman, R. B. *Drug–Receptor Interaction: The Organic Chemistry of Drug Design and Drug Action*; Academic Press: Burlington, 2004. pp. 122–151.
12. (a) Patrick, G. L. *Protiens as Drug Targets-Receptors: An Introduction to Medicinal Chemistry*; Oxford University Press: NewYork, 2005. pp. 78–81; (b) Patrick, G. L. *Protiens as Drug Targets-Enzymes: An Introduction to Medicinal Chemistry*; Oxford University Press: NewYork, 2005. pp. 41–60.
13. (a) Silverman, R. B. *J. Biol. Chem. Soc.* **1983**, *258*, 14766; (b) Lu, X.; Rodriguez, M.; Ji, H.; Silverman, R. B.; Vintem, A. P. B.; Ramsay, R. R. In *Flavins and Flavoprotiens*; Chapman, S. K., Perham, R. N., Scrutton, N. S., Eds.; Rudolf Weber: Berlin, 2002; pp 817–830.
14. Paech, C.; Salach, J. I.; Singer, T. P. *J. Biol. Chem. Soc.* **1980**, *255*, 2700.
15. Claisen, L.; Claparede, A. *Ber* **1981**, *14*, 2460.
16. Shimidt, J. B. *Ber.* **1881**, *14*, 1459.
17. Petrow, V.; Stephenson, O.; Thomas, A. J.; Wild, A. M. *Chem. Soc.* **1952**, *2*, 1509.
18. Porsolt, R. D.; Bertin, A.; Jalfre, M. *Arch. Int. Pharmacodyn.* **1977**, *229*, 327.
19. Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. *Eur. J. Pharm.* **1978**, *47*, 379.
20. Dunham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc.* **1957**, *46*, 208.