Synthesis and biological evaluation of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives as antidepressant, anxiolytics and anticonvulsant agents

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives as antidepressant, anxiolytics and anticonvulsant agents

Rajesh Sharma · Ganesh Prasad Misra · Jitendra Sainy · Subhash Chandra Chaturvedi

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Abstract A new series of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives has been synthesized. The newly synthesized compounds were characterized by analytical and spectral methods. Compounds were screened for central nervous system activity. Compounds **1**, **5**, **7**, **10**, **14** exhibited significant antidepressant, anxiolytic and anticonvulsant activity in comparison to the reference drugs.

Keywords 2-Amino-5-sulfanyl-1,3,4-thiadiazole · Antidepressant · Anxiolytic and anticonvulsant activity

Introduction

Substituted thiadiazole derivatives have been found to show diverse pharmacological activities such as analgesic (Schenone *et al*., 2006), antidepressant and anxiolytic (Clerici and Pocar, 2001), anticonvulsant (Stillings *et al*., 1986; Chapleo *et al*., 1986), anti-inflammatory (Mullican *et al*., 1993), antimicrobial (Servi *et al*., 2005), antitubercular (Oruç *et al*., 2004), antitumor (Matysiak, 2006) and antiviral (Jones *et al*., 1965) activity. A series of 2-amino-5-sulfanyl-1,3,4-thiadiazoles originally been synthesized and evaluated for antidepressant and anxiolytic activity by Clerici and Pocar (2001). Due to their effect on central nervous system (CNS) it was considered worthwhile to synthesize some new derivatives to evaluate for antidepressant, anxiolytic and anticonvulsant activity. The synthetic pathway is given in Scheme 1.

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Materials and methods

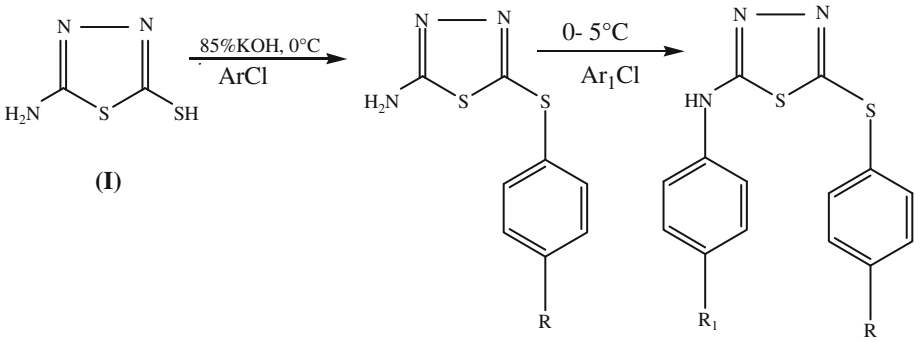
Chemistry

Melting points were determined in an open capillary tube and uncorrected. Purity of the compounds was checked on pre-coated silica gel G plates (0.2 mm thickness, Merck, India) using iodine vapor as visualizing agent. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 IR spectrometer (USA) and ^{1}H-NMR spectra were obtained on a Bruker DRX-300 MHz FT NMR spectrometer (USA) in $CDCl_3$. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on a Jeol SX-102 mass spectrometer (Japan). Elemental analyses were performed on an Elemental Vario EL II/Carlo N 1108 (Italy) and satisfactory results $\pm 0.4\%$ of calculated values (C, H, N) were obtained. All the reagents used in the present work were of synthetic grade (Aldrich, Germany, Lancaster, UK). The various substitution was made on sulfanyl and amino group of 2-amino-5-sulfanyl-1,3,4-thiadiazole nucleus. The various substituents are presented in Table 1.

Synthesis of compounds

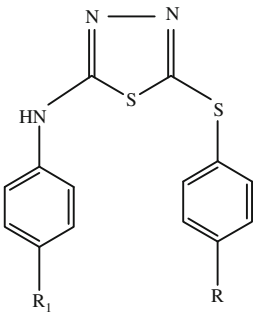
Synthesis of 4-[5-chlorophenylamino)-1,3,4-thiadiazole-2-yl-sulphanyl]-benzenesulphonamide (**1**)

2-Amino-5-sulfanyl-1,3,4-thiadiazole (**I**) (0.01 mol, 1.31 g) was suspended in a minimum quantity of water and a



Scheme 1 General synthetic pathway for compounds (**1**–**15**). ArCl: 4-chlorobenzene sulfonamide (**1**, **3**, **5**, **7**, **9**, **11**, **14**, **15**) and 4-chlorobenzene sulfonylchloride (**2**, **4**, **6**, **8**, **10**, **12**, **13**). Ar_1Cl: 1,4-disubstitutedbenzene[1,4-dichlorobenzene (**1**,**2**), 1-bromo-4-chlorobenzene (**3**, **4**), 1-chloro-4-fluorobenzene (**5**, **6**), 1-chloro-4-methylbenzene (**7**, **8**), 1-chloro-4-trifluoromethylbenzene (**9**, **10**), 1-chloro-4-trichloromethylbenzene (**11**, **12**), 4-chlorobenzene sulfonylchloride (**13**, **14**), 4-chlorobenzene sulfonamide (**15**)

Table 1 Substituents on 2-amino-5-sulfanyl-1,3,4-thiadiazole nucleus



Compounds	R	R_1
1	$-SO_2NH_2$	–Cl
2	$-SO_2Cl$	–Cl
3	$-SO_2NH_2$	–Br
4	$-SO_2Cl$	–Br
5	$-SO_2NH_2$	–F
6	$-SO_2Cl$	–F
7	$-SO_2NH_2$	$-CH_3$
8	$-SO_2Cl$	$-CH_3$
9	$-SO_2NH_2$	$-CF_3$
10	$-SO_2Cl$	$-CF_3$
11	$-SO_2NH_2$	$-CCl_3$
12	$-SO_2Cl$	$-CCl_3$
13	$-SO_2Cl$	$-SO_2Cl$
14	$-SO_2NH_2$	$-SO_2Cl$
15	$-SO_2NH_2$	$-SO_2NH_2$

sufficient quantity of 85% KOH solution was added under stirring at room temperature. After a few minutes (5–10), the solution was brought to 0°C in an ice bath and 4-chlorobenzene sulfonamide (ArCl) (0.01 mol, 1.92 g) was added under vigorous stirring. The reaction mixture was checked by thin layer chromatography.

The solution was neutralized upon completion of the reaction. The precipitate of 4-[5-amino-(1,3,4)thiadiazole-2-yl-sulphanyl]benzene sulphonamide formed slowly. It was filtered, washed with distilled water and crystallized using ether as a solvent. The purity of the synthesized intermediate was ascertained by thin layer chromatography using a methanol and xylene mixture (4:6, V/V). The intermediate (0.01 mol; 2.88 g) was dissolved in ether and 1,4-dichlorobenzene (0.01 mol, 1.48 g) was added under stirring while maintaining the temperature between 0 and 5°C. After 30 min, water was added and a precipitate was formed. Filtration of the compound and recrystallization from ether yielded compound **1**. Yield 75%, m.p: 141–142°C. Spectroscopic analysis: IR (cm^{-1}, KBr) 3334 (NH asym.), 3285 (NH sec.), 3241 (NH sym.), 3100 (C–H, sp^2),1637 (C=C/C=N),1606, 1572, 1475(C=C ring str.),1331 (S (=O_2) asym.),1165 (S (=O_2) sym.), 627 (C–S), 540 (C–Cl), ^{1}H NMR (δ ppm): 2.51 (s, 2H, NH_2),4.40 (s, NH),7.47 (s, 4H, Ar-H), 7.64–7.67 (d, 2H, Ar-H_a), 7.82–7.85 (d, 2H, Ar-H_b), MS (*m*/*z*): 398 (M^+, 15), 400 (M^+ +2,5), 274 (100),156 (55), 111 (25). Anal. calcd. for $C_{14}H_{11}ClN_4O_2S_3$: C, H, N in %: Calc: 42.15, 2.78, 14.04. Obs: 42.05, 2.68, 14.00.

Compounds **2**–**15** were synthesized using procedure described for compound **1**.

4-[5-(4-Chloro-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonylchloride (**2**)

Yield: 78%; mp: 136–37°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3285 (NH asym.), 3240 (NH sec.), 3118 (NH sym.), 3096 (C–H, sp^2), 1656 (C=C/C=N), 1634, 1572, 1435 (C=C ring str.), 1331 (S (=O_2) asym.), 1176 (S (=O_2) sym.), 626 (C–S), 560 (C–Cl). ^{1}H NMR (δ ppm): 3.42 (s,

NH), 7.48 (Cl, 2H, Ar-H_a), 7.65 (Cl, 2H, Ar-H_b), 7.75 (Cl, 2H, Ar-H_c), 7.85 (Cl, 2H, Ar-H_d). MS (*m/z*): 418 (M^+, 5), 420 (M^+ +2, 10), 422 (M^+ +4,5), 274 (100), 111 (18). Anal. calcd. for $C_{14}H_{11}ClN_4O_2$: C, H, N in %: Calc: 40.19, 2.17, 10.04, Obs: 40.19, 2.11, 10.01.

4-[5-(4-Bromo-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonamide (**3**)

Yield: 67%. m.p: 125–126°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3500 (NH asym.), 3390 (NH sym.), 3280 (NH sec.), 3050 (C–H, sp^2), 1640 (C=C/C=N), 1600, 1500, 1395 (C=C ring str.), 1300 (S (=O_2) asym.), 1120 (S (=O_2) sym.), 680 (C–S), 560 (C–Br).^{1}H NMR (δ ppm): 2.01 (s, 2H, NH_2), 3.41 (s, NH), 7.48 (s, 4H, Ar-H), 7.65–7.68 (Cl, 2H, Ar-H_a), 7.83–7.86 (Cl, 2H, Ar-H_b). MS (*m/z*): 443 (M^+, 24), 445 (M^+ +2, 8), 274 (100). Anal. calcd. for $C_{14}H_{11}BrN_4O_2S_3$: C, H, N in %: Calc: 37.93, 2.50,12.64, Obs: 37.53, 2.42, 12.34.

4-[5-(4-Bromo-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonylchloride (**4**)

Yield: 69%. m.p: 192–93°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3350 (NH asym.), 3210 (NH sec.), 3185 (NH sym.), 3050 (C–H, sp^2), 1590 (C=C/C=N), 1600, 1500, 1495 (C=C ring str.), 670 (C–S), 550 (C–Br). ^{1}H NMR (δ ppm): 3.42 (s, NH), 6.85 (Cl, 2H, Ar-H_a), 7.18 (Cl, 2H, Ar-H_b), 7.41 (Cl, 2H, Ar-H_c), 7.78 (Cl, 2H, Ar-H_d). MS (*m/z*): 462 (M^+, 10), 464 (M^+ +2, 20), 466 (M^+ +4, 70). Anal. calcd. for $C_{14}H_9BrClN_3O_2S_3$: C, H, N in %: Calc: 36.33, 1.96, 9.08, Obs: 36.23, 1.89, 9.01.

4-[5-(4-Fluoro-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonamide (**5**)

Yield: 80%. m.p: 101–102°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3289 (NH asym.), 3241 (NH sec.), 3150 (NH sym.), 3100 (C–H, sp^2), 1656 (C=C/C=N), 1572, 1506, 1475 (C=C ring str.), 1386 (S (=O_2) asym.), 1176 (S (=O_2) sym.), 1022 (C-F), 626 (C-S). ^{1}H NMR (δ ppm): 2.50 (S, 2H, NH_2), 3.49 (S, NH), 7.48 (S, 4H, Ar-H), 7.65–7.68 (Cl, 2H, Ar-H_a), 7.82–7.85 (Cl, 2H, Ar-H_b). MS (*m/z*): 382 (M^+, 27), 384 (M^+ +2, 9), 274 (100). Anal. calcd. for $C_{14}H_{11}FN_4O_2S_3$: C, H, N in %: Calc: 43.97, 2.90, 14.65, Obs: 43.57, 2.70, 14.55.

4-[5-(4-Fluoro-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonylchloride (**6**)

Yield: 72%. m.p: 220–222°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3340 (N–H, asym.), 3215 (N–H, sym.), 1660 (C=C/C=N), 1600, 1490, 1405 (C=C ring str.), 980 (C-F), 910, 840, 720 (sub. phenyl), 620 (C-S). ^{1}H NMR (δ ppm): 4.0 (s, NH), 6.44 (d, 2H, Ar-H_a), 6.72 (d, 2H, Ar-H_b), 7.41 (d, 2H, Ar-H_c), 7.78 (d, 2H, Ar-H_d). MS (*m/z*): 401 (M^+, 87), 403 (M^+ +2, 16), 405 (M^+ +4, 8). Anal. calcd. for $C_{14}H_9ClFN_3O_2S_3$: C, H, N in %: Calc: 41.84, 2.26, 10.46, Obs: 41.75, 2.19, 10.42.

4-(5-*p*-Tolylamino-[1,3,4]thiadiazol-2-ylsulfanyl)-benzenesulfonamide (**7**)

Yield: 70%. m.p: 244–245°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3333 (NH asym.), 3286 (NH sec.), 3240 (NH sym.), 3090 (C–H, sp^2), 2850 (C–H, sp^3), 1634 (C=C/C=N), 1572, 1506, 1435 (C=C ring str.), 1329 (S (=O_2) asym.), 1167 (S (=O_2) sym.), 627 (C-S). ^{1}H NMR (δ ppm): 2.35 (s, 3H, CH_3), 3.43 (s, NH), 7.48 (s, H H, Ar-H), 7.65–7.69 (Cl, 2H, Ar-H_a), 7.83–7.86 (Cl, 2H, Ar-H_b). MS (*m/z*): 378 (M^+, 25), 274 (100), 91 (45). Anal. calcd. for $C_{15}H_{14}N_4O_2S_3$: C, H, N in %: Calc: 47.60, 3.73, 14.80, Obs: 47.44, 3.66, 14.66.

4-(5-*p*-Tolylamino-[1,3,4]thiadiazol-2-ylsulfanyl)-benzenesulfonylchloride (**8**)

Yield: 68%. m.p: 111–112°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3270 (N–H, sec.), 3070 (C–H, sp^2), 2850 (C–H, sp^3), 1630 (C=C/C=N), 1595, 1500, 1400 (C=C ring str.), 1350 (S (=O_2) asym.), 1140 (S (=O_2) sym.), 610 (C-S). ^{1}H NMR (δ ppm): 2.35 (s, 3H, CH_3), 3.43 (s, NH), 6.34 (d, 2H, Ar-H_a), 6.81 (d, 2H, Ar-H_b), 7.41 (d, 2H, Ar-H_c), 7.78 (d, 2H, Ar-H_d). MS (*m/z*): 397 (M^+, 18), 399 (M^+ +2, 6), 91(40). Anal. calcd. for $C_{15}H_{12}ClN_3O_2S_3$: C, H, N in %: Calc: 45.27, 3.04, 10.56. Obs: 45.11, 2.99, 10.49.

4-[5-(4-Trifluoromethyl-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonamide (**9**)

Yield: 65%. m.p: 134–36°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3333 (NH asym.), 3241 (NH sec.), 3151 (NH sym.), 3070 (C–H, sp^2), 1633 (C=C/C=N), 1572, 1506, 1498 (C=C ring str.), 1151 (C–F), 627 (C–S). ^{1}H NMR (δ ppm): 2.51 (s, 2H, NH_2), 3.41 (s, NH), 6.39 (d, 2H, Ar-H_a), 7.20 (d, 2H, Ar-H_b), 7.46 (d, 2H, Ar-H_c), 7.77 (d, 2H, Ar-H_d). MS (*m/z*): 432 (M^+, 4), 434 (M^+ +2, 12), 436 (M^+ +4, 12), 430 (M, +6, 4). Anal. calcd. for $C_{15}H_{11}F_3N_4O_2S_3$: C, H, N in %: Calc: 41.66, 2.56, 12.96. Obs: 41.64, 2.35, 12.77.

4-[5-(4-Trifluoromethyl-phenylamino)-[1,3,4] thiadiazol-2-ylsulfanyl]-benzenesulfonylchloride (**10**)

Yield: 78%, m.p: 116–18°C, IR (cm^{-1}, KBr): 3245 (N–H, sec.), 3075 (C–H, sp^2), 1600, 1500, 1400 (C=C ring str.), 1640 (C=C/C=N), 1336 (S (=O_2) asym.), 1152 (S (=O_2)

sym.), 1090 (C–F). ^{1}H NMR (δ ppm): 4.0 (s, NH), 6.39 (d, 2H, Ar-H_a), 7.20 (d, 2H, Ar-H_b), 7.41 (d, 2H, Ar-H_c), 7.78 (d, 2H, Ar-H_d). MS (*m/z*): 452 (M^+, 8). Anal. calcd. for $C_{15}H_9ClF_3N_3O_2S_3$: C, H, N in %: Calc: 39.87, 2.01, 9.30, Obs: 39.68, 1.99, 9.22.

4-[5-(4-Trichloromethyl-phenylamino)-[1,3,4]thiadiazol -2-ylsulfanyl]-benzenesulfonamide (**11**)

Yield: 58%. m.p: 137–38°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3334 (NH asym.), 3240 (NH sec.), 3151 (NH sym.), 3050 (C–H, sp^2), 1651 (C=C/C=N), 1614, 1505, 1409 (C=C ring str.), 1328 (S (=O_2) asym.), 1176 (S (=O_2) sym.), 1014 (C–Cl), 627 (C–S). ^{1}H NMR (δ ppm): 2.51 (s, 2H, NH_2), 3.49 (s, NH), 7.55–7.58 (d, 2H, Ar-H_a), 7.64–7.67 (d, 2H, Ar-H_b), 7.82–7.85 (d, 2H, Ar-H_c), 7.93–7.96 (d, 2H, Ar-H_d). MS (*m/z*): 482 (M^+, 8). Anal. calcd. for $C_{15}H_{11}Cl_3N_4O_2S_3$: C, H, N in %: Calc: 37.39, 2.30, 11.63. Obs: 37.28, 2.19, 11.54.

4-[5-(4-Trichloromethyl-phenylamino)-[1,3,4]thiadiazol-2-ylsulfanyl]-benzenesulfonylchloride (**12**)

Yield: 71%. m.p: 125–127°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3250 (N–H, sec.), 3040 (C–H, sp^2), 1650 (C=C/C=N), 590, 1505, 1400 (C=C ring str.), 1333 (S (=O_2) asym.), 1150 (S (=O_2) sym.), 550 (C–Cl). ^{1}H NMR (δ ppm): 3.49 (s, NH), 6.39 (d, 2H, Ar-H_a) 7.56 (d, 2H, Ar-H_b), 7.41 (d, 2H, Ar-H_c), 7.75 (d, 2H, Ar-H_d). MS (*m/z*): 501 (M^+, 12). Anal. calcd. for $C_{15}H_9Cl_4N_3O_2S_3$: C, H, N in %: Calc: 35.94, 1.81, 8.38. Obs: 35.78, 1.75, 8.16.

4-[5-(4-Chlorosulfonyl-phenylsulfanyl)-[1,3,4]thiadiazol-2-ylamino]-benzenesulfonylchloride (**13**)

Yield: 82%. m.p: 152–153°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3285 (N–H, sec.), 3070 (C–H, sp^2), 1640 (C=C/C=N), 1610, 1595, 1405 (C=C ring str.), 1340 (S (=O_2) asym.), 1145 (S (=O_2) sym.). ^{1}H NMR (δ ppm): 3.53 (s, NH), 6.69 (d, 2H, Ar-H_a), 7.69 (d, 2H, Ar-H_b), 7.41 (d, 2H, Ar-H_c), 7.75 (d, 2H, Ar-H_d). MS (*m/z*): 482 (M^+, 5). Anal. calcd. for $C_{14}H_9Cl_2N_3O_4S_4$: C, H, N in %: Calc: 34.86,1.88,8.71. Obs: 34.71, 1.76, 8.52.

4-[5-(4-Sulfamoyl-phenylsulfanyl)-[1,3,4]thiadiazol-2-ylamino]-benzenesulfonylchloride (**14**)

Yield: 68%. m.p: 105–106°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3271 (N–H, asym.), 3185 (N–H, sym.), 3087 (C–H, sp^2), 1638 (C=C/C=N), 1539, 1504, 1400 (C=C ring str.), 1336 (S (=O_2) asym.), 1139 (S (=O_2) sym.), 846, 824, 774 (sub. phenyl), 610 (C–S). ^{1}H NMR (δ ppm): 2.51 (s, 2H, NH_2), 3.53 (s, NH), 7.48 (d, 2H, Ar-H_a), 7.65 (d, 2H, Ar-H_c), 7.68 (d, 2H, Ar-H_b), 7.83 (d, 2H, Ar-H_d). MS (*m/z*): 463 (M^+, 24) 465 (M^+ +2, 8), 274 (100), 175 (40). Anal. calcd. for $C_{14}H_{11}ClN_4O_4S_4$: C, H, N in %: Calc: 36.32, 2.39, 2.10. Obs: 36.28, 2.38, 12.06.

4-[5-(4-Sulfamoyl-phenylsulfanyl)-[1,3,4]thiadiazol-2-ylamino]-benzenesulfonamide (**15**)

Yield: 60%. m.p: 133–134°C. Spectroscopic analysis: IR (cm^{-1}, KBr): 3310 (–NH, asymmetric), 3250 (N–H, sym.), 1640 (C=C/C=N), 1595, 1500, 1390 (C=C ring str.), 1345 (S (=O_2) asym.), 1150 (S (=O_2) sym.), 810,790,740 (sub. phenyl), 590 (C–S). ^{1}H NMR (δ ppm): 2.52 (s, 2H, NH_2), 3.46 (s, NH), 7.36–7.39 (d, 2H, Ar-H_a), 7.50–7.59 (d, 2H, Ar-H_c), 7.64–7.67 (d, 2H, Ar-H_b), 7.85–7.88 (d, 2H, Ar-H_d). MS (*m/z*): 443 (M^+, 25), 274 (100), 156 (45). Anal. calcd. for $C_{14}H_{13}N_5O_4S_4$: C, H, N in %: Calc: 37.91, 2.95, 15.79. Obs: 37.88, 2.91, 15.62.

Biological activity

Antidepressant activity was determined by the tail suspension test in Steru *et al*. (1985) and despair swim test in mice (Porsolt *et al*., 1977), anxiolytic activity was determined by elevated plus maze test (Moser, 1989; Rabbani *et al*., 2004) and light and dark box model (Crawley and Goodwin, 1980) by using balb/cj mice and Swiss albino mice respectively. Balb/cj mice and Swiss albino mice (20–25 g of body mass) of both sexes were divided into different groups (control, test and standard) containing six animals each. The animals were housed in standard polypropylene cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 3°C).They had free access to standard commercial diet and water. The ethical guidelines for the investigations of animals used in experiments were followed in all tests.

Tail suspension test

In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 15 mg kg^{-1} (body mass) dose of standard drug fluoxetine hydrochloride. The test and standard compounds were suspended in 10% tween-20 suspension and administered intraperitoneally 30 min prior to testing. The control group animals, however, received the same volume of vehicle (10% tween-20 suspension). Test mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of tail. The duration of immobility is reported for a period of 5 min and this time were divided into 20 phases and each phase consist of 15 s mice were considered immobile when they hang passively

and completely motionless for at least 10–12 s out of 15 s. The results are reported in Table 2 and were analyzed for statistical significance using Students *t*-test and significant was set at $P < 0.05$.

Despair swim test

The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape directed behavior. The apparatus consisted of a clear plexi glass cylinder ($25 \times 12 \times 25$ cm^3) filled to 15 cm depth with water (24 ± 1°C). Two session were conducted: an initial 15-min training session (pretest session) followed 24 h later by a 6-min test session following the training session mice were removed from the cylinder, towel dried and placed under a lamp for 5 min then returned to the home cage for testing the next day. A mouse was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4-min of a total 6-min test. In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 15 mg kg^{-1} (body mass) dose of standard drug fluoxetine hydrochloride. The test and standard compounds were suspended in 10% tween-20 suspension and administered intraperitoneally 30 min prior to testing. The control group animals, however, received the same volume of vehicle (10% tween-20). The results

Table 3 Antidepressant activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: despair swim test

Compounds	Duration of immobility (mean ± SEM)	Percentage decrease in immobility compared to negative control
Positive control	100.01 ± 0.01	–
Negative control	98.86 ± 0.02	–
1	69.98 ± 0.01*	29.21
2	75.22 ± 0.03	23.91
3	71.93 ± 0.03	27.24
4	68.52 ± 0.01	30.68
5	48.95 ± 0.01**	50.48
6	76.32 ± 0.02	22.79
7	47.90 ± 0.03**	51.54
8	69.99 ± 0.02	29.20
9	71.25 ± 0.03**	27.92
10	45.96 ± 0.01*	53.51
11	74.14 ± 0.02*	25.00
12	68.96 ± 0.01*	30.24
13	73.24 ± 0.02	25.91
14	48.99 ± 0.01**	50.44
15	73.92 ± 0.02	25.22
Fluoxetine HCl	40.93 ± 0.01**	58.59
One-way ANOVA *f*	609.80	
df	17,90	
P	<0.0001	

$n = 6$ in each group

** $p < 0.0001$, * $p < 0.001$ compared against control group

Table 2 Antidepressant activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: tail suspension test

Compounds	Number of mobile phase in pretreatment period (mean ± SEM)	Number of mobile phase in post-treatment period (mean ± SEM)	% increase in mobile phase as compare to pretreatment
Positive control	12.30 ± 1.20	13.00 ± 0.50	5.69
Negative control	11.50 ± 0.20	14.50 ± 0.80	26.08
1	10.50 ± 1.38	14.00 ± 1.57	33.33
2	11.67 ± 0.80	13.50 ± 0.84	15.68
3	11.67 ± 0.98	13.50 ± 1.17	15.68
4	10.50 ± 0.67	12.66 ± 0.61*	20.57
5	9.50 ± 0.42	13.33 ± 0.66**	40.31
6	10.83 ± 0.60	13.00 ± 0.85*	21.51
7	10.66 ± 0.88	15.16 ± 1.00*	42.21
8	13.16 ± 0.87	15.16 ± 0.67	15.19
9	8.50 ± 0.76	11.16 ± 0.90*	31.29
10	8.33 ± 0.33	12.16 ± 0.30**	45.97
11	11.16 ± 0.60	13.00 ± 0.77*	16.39
12	10.50 ± 0.50	12.50 ± 0.67*	19.16
13	13.17 ± 1.04	14.50 ± 1.08	10.09
14	10.67 ± 1.28	15.00 ± 1.43*	40.58
15	16.50 ± 0.42	18.16 ± 0.47*	10.06
Fluoxetine HCl	7.00 ± 0.73	10.50 ± 0.99*	50.00

$n = 6$ in each group

** $p < 0.05$, * $p < 0.001$ compared against control group

are reported in Table 3 and were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test and significant was set at $P < 0.05$.

Elevated plus maze test

In elevated plus maze model 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 for a 5-min period. In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 2 mg kg^{-1} (body mass) dose of standard drug diazepam. The test and standard compounds were suspended in 1% carboxymethyl cellulose and administered intraperitoneally 30 min prior to testing. The control group animals, however, received the same volume of vehicle (1% carboxymethyl cellulose). The percentage preference of animals to open arm, increases in number of entries and time spent in the open arms were calculated for each mouse. The results are reported in Table 4 and were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test and significant was set at $P < 0.05$.

Light and dark box model

In this model each mouse was placed individually in the centre of the white area and behavioral parameters were recorded over a 5-min period. In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 2 mg kg^{-1} (body mass) dose of standard drug diazepam. The test and standard compounds were suspended in 1% carboxymethyl cellulose and administered intraperitoneally 30 min prior to testing. The control group animals, however, received the same volume of vehicle (1% carboxymethyl cellulose). Two behavioral parameters were measured in the white area: the number of transition between the two chambers (n) and time (seconds) spent in the light chamber. The results are reported in Table 5 and were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test and significant was set at $P < 0.05$.

Anticonvulsant activity were determined by Maximal-electro-shock (MES)-induced convulsion (Woodbury and Davenport, 1952) and Pentylenetetrazole-induced-clonic convulsion (Ahmadiani *et al.*, 2003; Ambawade *et al.*, 2002) using albino rats of Wistar strain (150–200 g of body mass) of both sexes were divided into different groups

Table 4 Anxiolytic activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: elevated plus maze test

Compounds	% preference to open arm	Open arm	
		No. of entries (mean ± SEM)	Average time spent (mean ± SEM)
Positive control	4.80	2.50 ± 0.01	12.25 ± 0.02
Negative control	5.01	2.20 ± 0.01	14.00 ± 0.01
1	16.35	4.01 ± 0.01**	45.51 ± 0.02*
2	9.75	2.23 ± 0.02	30.01 ± 0.01
3	8.01	3.01 ± 0.02*	29.26 ± 0.02
4	9.25	2.99 ± 0.01*	25.20 ± 0.01
5	18.01	4.00 ± 0.02*	46.31 ± 0.02**
6	10.54	3.06 ± 0.01	25.26 ± 0.01
7	18.25	4.20 ± 0.01*	45.69 ± 0.01**
8	7.58	2.21 ± 0.02*	22.20 ± 0.02
9	8.92	2.35 ± 0.02*	23.72 ± 0.02**
10	18.92	4.80 ± 0.01**	47.92 ± 0.01**
11	7.85	2.56 ± 0.02*	24.20 ± 0.02
12	8.75	2.54 ± 0.01	26.20 ± 0.01
13	7.90	3.09 ± 0.01	27.63 ± 0.02
14	17.20	4.06 ± 0.02**	45.93 ± 0.01**
15	6.65	2.26 ± 0.01	26.64 ± 0.01
Fluoxetine HCl	20.21	4.90 ± 0.01**	60.01 ± 0.02**
One-way ANOVA f		650.9	254.0
df		17,90	17,90
P		<0.0001	<0.0001

$n = 6$ in each group

** $p < 0.0001$, * $p < 0.001$ compared against control group

Table 5 Anxiolytic activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: light and dark box model

Compounds	Number of transition between two chambers (mean ± SEM)	Time spent in the light chamber (mean ± SEM)
Positive control	1.98 ± 0.02	130.20 ± 0.01
Negative control	1.20 ± 0.01	135.25 ± 0.01
1	2.70 ± 0.01**	178.20 ± 0.01**
2	1.66 ± 0.02	151.50 ± 0.01
3	1.98 ± 0.02	162.30 ± 0.03
4	2.01 ± 0.01*	159.30 ± 0.01
5	2.92 ± 0.02**	185.90 ± 0.03*
6	1.99 ± 0.02	166.30 ± 0.01
7	2.88 ± 0.02**	190.25 ± 0.03*
8	2.12 ± 0.01	169.25 ± 0.03
9	2.11 ± 0.01	151.26 ± 0.03
10	2.99 ± 0.01**	192.00 ± 0.02*
11	2.13 ± 0.01**	162.00 ± 0.03
12	2.00 ± 0.03	170.50 ± 0.03
13	2.20 ± 0.02*	172.50 ± 0.03
14	2.85 ± 0.03**	180.25 ± 0.03**
15	2.73 ± 0.01*	133.20 ± 0.03
Diazepam	3.10 ± 0.01**	196.25 ± 0.03
One-way ANOVA *f*	260.1	255.2
df	17,90	17,90
P	<0.0001	<0.0001

$n = 6$ in each group

** $p < 0.0001$ and * $P < 0.001$ compared against the control group

(control, test and standard) containing six animals each. The animals were housed in standard polypropylene cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 3°C). They had free access to standard commercial diet and water. The ethical guidelines for the investigations of animals used in experiments were followed in all tests.

Maximal-electro-shock (MES)-induced convulsion

In this test, the rats were subjected to 50 mA of alternating current from a convulsiometer for 0.2 s through a pair of electrodes attached to each ear. The duration of the tonic hind limb extensor phase, and the number of animals protected from convulsions was noted. In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 25 mg kg^{-1} (body mass) dose of standard drug phenytoin. The test and standard compounds were suspended in 10% tween-20 and administered intraperitoneally 30 min prior to testing. The control group animals, however, received the same volume of vehicle (1% carboxymethyl cellulose) and results are reported in Table 6 and were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test and significant was set at $P < 0.05$.

Pentylenetetrazole-induced-clonic convulsion

Pentylenetetrazol used as chemo-convulsant and dissolved in normal saline. In this study, the animals were administered 30 mg kg^{-1} (body mass) dose of the test drug and 4 mg kg^{-1} (body mass) dose of standard drug diazepam. The test and standard compounds were suspended in 1% carboxymethyl cellulose and administered intraperitoneally 30 min prior to intraperitoneal administration of pentylenetetrazole 80 mg kg^{-1} (body mass). The control group animals however received the same volume of vehicle (1% carboxymethyl cellulose) and observed the onset of convulsion. The results are reported in Table 7 and were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test and significant was set at $P < 0.05$.

Table 6 Anticonvulsant activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: maximal-electro-shock (MES)-induced convulsion

Compounds	Duration of tonic hind limb extensor (mean ± SEM)	% of animal protected
Positive control	15.71 ± 0.01	0
Negative control	15.25 ± 0.05	0
1	5.17 ± 0.02**	50
2	7.55 ± 0.05**	16.66
3	8.01 ± 0.02	33.33
4	7.63 ± 0.01*	0
5	4.50 ± 0.01*	66.66
6	8.25 ± 0.01	0
7	4.52 ± 0.03**	50
8	8.01 ± 0.01	16.66
9	7.37 ± 0.05*	33.33
10	4.42 ± 0.03	66.66
11	6.96 ± 0.01*	16.66
12	7.01 ± 0.01**	16.66
13	8.50 ± 0.02	0
14	4.69 ± 0.02**	50
15	8.25 ± 0.01**	0
Phenytoin	Absent	100
One-way ANOVA *f*	1859.1	
df	17,90	
P	<0.0001	

$n = 6$ in each group

** $p < 0.0001$ and * $P < 0.001$ compared against the control group

Table 7 Anticonvulsant activity of 2-amino-5-sulphanyl-1,3,4-thiadiazole derivatives: pentylenetetrazole-induced-clonic convulsion

Compounds	Onset of convulsions in s (mean ± SEM)	Death
Positive control	48.25 ± 0.01	4/6
Negative control	48.00 ± 0.01	6/6
1	151.30 ± 0.12*	1/6
2	63.60 ± 0.00	2/6
3	71.90 ± 0.01*	3/6
4	70.25 ± 0.02	2/6
5	171.25 ± 0.14*	1/6
6	101.26 ± 0.02**	2/6
7	170.26 ± 0.03**	1/6
8	101.26 ± 0.13	3/6
9	126.20 ± 0.14	2/6
10	180.25 ± 0.13*	0/6
11	106.70 ± 0.01	3/6
12	99.90 ± 0.13	2/6
13	125.20 ± 0.11**	3/6
14	175.20 ± 0.01**	1/6
Diazepam	No convulsion	0/6
One-way ANOVA *f*	2380.3	
df	17,90	
P	0.0001	

$n = 6$ in each group

** $p < 0.0001$, * $p < 0.001$ compared against control

Results and discussion

The synthesis of the new substituted 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives (**1–15**) is depicted in Scheme 1. The title compounds were synthesized through nucleophilic substitution reaction. In first slop step, formation of nucleophile in the presence of strong base that further react with aryl halide to form intermediate. The important feature of this intermediate is the ability of the negative charge to be delocalized into electron-withdrawing groups. In the second step, the leaving group is eliminated to regenerate the title compounds. The structure of the synthesized compounds was fully characterized by elemental analysis, ^{1}H NMR, Mass and IR findings.

The test compounds **1**, **5**, **7**, **10**, **14** have antidepressant, anxiolytic and anticonvulsant activity. Compound **1** having sulphonamide group on R position and chloride group on R_1 position having appreciable anxiolytic and anticonvulsant activity but little effect on antidepressant activity. Compound **5** having sulphonamide group on R position and fluoride group on R_1 position showed significant effects on CNS (antidepressant, anxiolytic and anticonvulsant). Compound **10** having sulphonyl chloride group on R position and trifluoro methyl group on R_1 position showed maximum activity among all compounds. Among the halogen (electron-withdrawing group) substituted compounds only compound having fluoride or trifluoromethyl group and up to some extent chloride group (compound **1** showed anxiolytic and anticonvulsant activity) on R_1 position showed significant central nervous system activity, the possible reason could be a fluorine atom are small in size, lipophilic in nature and ability to form strong hydrogen bond; moreover, they have metabolic stability under physiological condition (Shah and Westwell, 2007). Compound **7** having methyl (electron donating) group on R_1 position showed broad window of central nervous system activity (antidepressant, anxiolytic and anticonvulsant). Though the exact reason behind this abnormality is not known but the metabolic conversion of 4-methylphenyl to 4-methoxyphenyl group cannot be ignored as the possible reason (Misra *et al*., 1973: Sharma and Dandiya, 1962) and compound **14** having sulphonyl chloride group on R_1 position and sulphonamide group on R position also showed significant CNS (antidepressant, anxiolytic and anticonvulsant).

Although the exact mechanism of the title compounds on central nervous system is not known but involvement of the GABAergic system cannot be ruled out, partly may be due to its action on the HPA (hypothalamic-pituitary-adrenal) axis. However, further studies are needed to elucidate the same by observing its effect on neurotransmitter levels in brain (Shalam *et al*., 2007; Milan, 2003).

Finally, we may conclude that the diaryl substitution have made on thiadiazole nucleus that is essential for providing optimum lipophilic character for synthesized compounds showing appreciable biological activity. Compounds **1**, **5**, **7**, **10** and **14** can be selected for further studies since they showed CNS activity (antidepressant, anxiolytic and anticonvulsant) comparable to standard drug, fluoxetine HCl, phenytoin and diazepam.

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