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Synthesis and Biological Evaluation of *N*-(Substituted Phenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamides as Antimicrobial, Antidepressant, and Anticonvulsant Agents[1]

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Abstract—A new series of *N*-Aryl-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamides were synthesized by condensation of tricyclic compound 2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione with chloro *N*-phenylacetamides. The tricyclic compound was obtained by condensation of Isatin with thiosemicarbazide. Chloro *N*-phenylacetamides were obtained from different substituted anilines. Their structures were characterized by IR, ^{1}H NMR, LC-MS and elemental analyses. Newly synthesized compounds were screened for antimicrobial, antidepressant and anticonvulsant activities. Preliminary results indicated that most of the compounds showed lesser MIC value than the standard drug used when tested for antimicrobial activity. Some of the compounds were endowed with very good antidepressant and anticonvulsant activity.

Keywords: [1,2,4]triazino[5,6-b]indoles, antimicrobial activities, antidepressant activities, anticonvulsant activities

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INTRODUCTION

Most of the antidepressants exert its actions on the metabolism of monoamine neurotransmitters particularly on nor-adrenaline and serotonin [1]. They are effective and tolerated well, but noncompliance due to the slow action, low response and a plethora of side effects are generally observed [2–7]. Also, they inhibit sexual behavior [8]. Epilepsy is another common neurological disorder characterized by unprovoked seizures. Phenobarbital and mephobarbital are well-known drugs used for the treatment of epilepsy [9]. Available antiepileptic drugs are effective in controlling seizures of only about 70–80% of patients. But they suffer from major side effects like sedation and hypnosis along with drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia and even life-threatening conditions [10, 11]. Hence, there is a continuing demand for the new anticonvulsant and antidepressant agents as it has not been possible to control the side effects by currently available drugs. Heterocyclic nucleus imparts an important role in the field of medicinal chemistry and serves as a key template for the development of various bioactive molecules. The triazino[5,6-*b*]indole derivatives have aroused considerable interest as a result of their high potential of biological activities such as antibacterial, antifungal [12], antihypertensive [13], antimalarial [14], antihypoxic [15], antiparasitic [16] and antidepressant [17] activities. Importantly, this tricyclic structure is comparable to β-carboline (9*H*-pyrido[3,4-*b*]indole), an endogenous monoamine oxidase inhibitor [18]. The majority of the 5*H*-as-triazino[5,6-*b*] indoles are active in vitro against a variety of viruses including several strains of rhinovirus [19]. They are used in the prophylaxis and treatment of an extensive index of viruses and have for a long time attracted attention in connection with the search for new antimicrobial and anticancer agents [20, 21]. For a search of effective small molecules with promising biological activity, it was envisaged to synthesize a series of *N*-(substituted phenyl)-2-(5*H*-[1,2,4] triazino [5,6-*b*] indol-3-ylsulfanyl)acetamide derivatives.

RESULTS AND DISCUSSIONS

In our present work, tricyclic compound 1,2,4-triazino[5,6-*b*]indole-3-thione (**II**) was prepared by referring previously reported literature procedure [22]. Isatin (**I**) was condensed with thiosemicarbazide in

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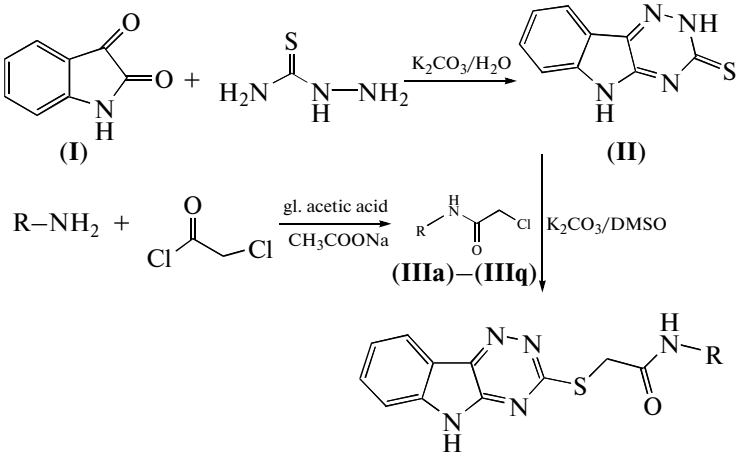
aqueous solution of potassium carbonate. Obtained clear solution was acidified with glacial acetic acid to afford the condensed product (**II**). Different chloro *N*-phenylacetamides (**III**) was prepared as per the literature procedure [23] involving the reaction of primary amines with chloro acetyl chloride in glacial acetic acid in the presence of saturated solution of sodium acetate. The title compounds (**IVa**)–(**IVq**) were accomplished by overnight stirring of tricyclic product (**II**) with appropriate chloro *N*-phenylacetamides (**III**) in dry DMSO containing anhydrous milled potassium carbonate. The synthetic route is depicted in Scheme 1. Synthesized compounds were characterized by IR, ^{1}H NMR, LC-MS and elemental analyses.



a: R = 3-CN–C_6H_4; **b**: R = 2-CF_3–C_6H_4;
c: R = 2-CN–C_6H_4; **d**: R = 4-F–C_6H_4;
e: R = 4-Br-2-pyridinyl; **f**: R = 2-thiazolyl;
g: R = 3-CF_3–C_6H_4; **h**: R = 4-OCF_3–C_6H_4;
i: R = 4-CN-2-pyridinyl; **j**: R = 3,5-Cl_2–C_6H_3;
k: R = 2,4,5-Cl_3–C_6H_2; **l**: R = 3-Cl-4-F-C_6H_3;
m: R = benzothiazolyl; **n**: R = 2,5-Cl_2–C_6H_3;
o: R = 2-Cl-6-CH_3-pyridinyl; **p**: R = 2,4-F_2–C_6H_3;
q: R = 5-CH_3-2-pyridinyl

Scheme 1. Synthetic route for the compounds (**IVa**)–(**IVq**).

IR spectrum of prototype compound (**IVa**) showed prominent absorption bands corresponding to N–H stretching of secondary amide near 3323 cm^{-1} and an amide carbonyl at 1670 cm^{-1}. Presence of aromatic skeleton is confirmed by peak at 3057 cm^{-1} corresponding to aromatic C–H stretching. ^{1}H NMR of (**IVa**) displayed singlet at δ 4.28 for methylene protons alpha to carbonyl group. Presence of two doublets and two triplets confirmed the presence of triazino indole moiety. A singlet at δ 8.08 and multiplet at δ 7.47–7.57 integrating for one and three aromatic protons respectively affirmed the presence of 3-cyanophenyl ring in the title compound. Further, broad singlet at δ 10.74 revealed the presence of an amide NH proton. The downfield broad singlet at δ 12.52 corresponds to indole NH proton. Formation of the compound is further confirmed by the presence of M^+ and $M^+ + 2$ peaks at *m/z* values 360 and 362 respectively.

The newly synthesized compounds (**IVa**)–(**IVq**) were screened for in vitro antimicrobial activity (minimum inhibitory concentration) against gram-positive bacterial strains *Stephylococcus aureus*, *Enterococcus faecalis*, gram-negative bacterial strains *Escherichia coli*, *Klebsiella pneumonia* and two fungal strains *Aspergillus niger* and *Candida albicans* by tube dilution method. Results of antimicrobial study are shown in Table 1 and Table 2. Antibacterial studies revealed that most of the compounds have shown good inhibition against gram-positive bacterial strains when compared with reference drug used, ciprofloxacin (MIC: 2 μg/mL against *S. aureus* and MIC: 1.0 μg/mL against *E. feacalis*). Compounds (**IVa**), (**IVb**), (**IVc**), (**IVd**), (**IVe**), (**IVf**), (**IVh**), (**IVi**), (**IVj**), (**IVk**), (**IVl**), (**IVn**), (**IVo**), (**IVp**) have shown more inhibition against gram-positive bacterial strains as compared to ciprofloxacin where as compounds (**IVg**), (**IVm**) and (**IVg**) found to be less effective. Against gram-negative bacterial strains, compounds (**IVe**) and (**IVi**) were showed

Table 1. MIC values of in vitro antibacterial study for the analogs **(IVa)**–**(IVq)**

Compound	MIC values (μg/mL)			
	gram-positive		gram-negative	
	Staphylococcus aureus	*Enterococcus faecalis*	*Escherichia coli*	*Klebsiella*
(IVa)	0.4	0.4	6.25	12.5
(IVb)	0.8	0.8	12.5	6.25
(IVc)	0.4	0.2	3.12	3.12
(IVd)	0.2	0.2	3.12	1.6
(IVe)	0.4	0.8	0.2	0.8
(IVf)	0.4	0.8	3.12	6.25
(IVg)	12.5	6.25	3.12	12.5
(IVh)	0.8	0.4	3.12	12.5
(IVi)	0.2	0.2	0.2	0.2
(IVj)	0.4	0.2	6.25	6.25
(IVk)	0.2	0.2	12.5	25
(IVl)	0.2	0.2	12.5	6.25
(IVm)	12.5	6.25	25	25
(IVn)	0.8	3.12	25	12.5
(IVo)	0.2	0.2	50	25
(IVp)	0.8	1.6	50	12.5
(IVq)	6.25	6.25	50	25
Ciprofloxacin	**2.0**	**1.0**	**2.0**	**1.0**

good inhibition than standard drug used; whereas rests of the compounds were shown weak inhibition. From structural activity relationship it was clear that electron withdrawing group and heterocyclic substituted compounds have shown good inhibition against *S. aureus* and *E. faecalis* bacterial strains. Against *E. coli* and *K. pneumonia*, Halo-pyridine substituted derivatives were found to be potent inhibitors.

The reference drug flucanazole is selected as positive control for antifungal activity assay. All the compounds have shown excellent inhibition (MIC < 16 μg/mL) against *C. albicans* when compared with standard drug Flucanozole (MIC: 16 μg/mL). Against *A. niger*, except **(IVg)** and **(IVm)** rest of the compounds showed MIC value less than standard used (MIC: 8 μg/mL). It was observed that all the compounds are potent against *C. albicans* and *A. niger,* except electron donating heterocyclic substituted derivatives against *A. niger* when compared with Flucanazole.

Antidepressant activity of the synthesized compounds **(IVa)**–**(IVq)** was done by using tail suspension test. This test is effectively used to predict the activity of a wide variety of antidepressants such as Mao inhibitors. During the pharmacological evaluation, only a few of the synthesized compounds exhibited moderate antidepressant activity in the tail suspension test, with many exhibiting weak activity as shown in Table 3. However, in the series, mono and dihalo substituted

Table 2. Results of in vitro antifungal activity for the compounds **(IVa)**–**(IVq)**

Compound	MIC values (μg/mL)	
	Aspergillus niger	*Candida albicans*
(IVa)	0.4	0.4
(IVb)	0.8	0.8
(IVc)	0.4	0.2
(IVd)	0.2	0.2
(IVe)	0.4	0.8
(IVf)	0.4	0.8
(IVg)	12.5	6.25
(IVh)	0.4	0.4
(IVi)	0.8	0.2
(IVj)	0.2	0.2
(IVk)	0.4	0.2
(IVl)	0.2	6.25
(IVm)	12.5	0.2
(IVn)	0.2	3.12
(IVo)	1.6	0.2
(IVp)	0.2	1.6
(IVq)	6.25	6.25
Flucanazole	8.0	16.0

Table 3. Results of antidepressant activity for the compounds (**IVa**)–(**IVq**): tail suspension test

Compound	Duration of immobility (s) (mean ± SEM)	% decrease in immobility duration (%DID)
(IVa)	136.35 ± 9.73	26.81
(IVb)	128.76 ± 7.71	30.88
(IVc)	147.57 ± 8.38	20.76
(IVd)	**97.58 ± 8.67**	**47.62**
(IVe)	140.66 ± 8.54	24.49
(IVf)	132.41 ± 7.93	28.92
(IVg)	147.26 ± 8.55	20.86
(IVh)	133.51 ± 7.98	28.33
(IVi)	139.13 ± 9.38	25.31
(IVj)	104.26 ± 9.51	44.03
(IVk)	114.26 ± 7.48	38.66
(IVl)	118.68 ± 8.43	36.29
(IVm)	131.91 ± 8.77	29.19
(IVn)	101.73 ± 7.55	45.39
(IVo)	133.66 ± 9.81	28.25
(IVp)	104.55 ± 6.93	43.88
(IVq)	147.26 ± 8.55	20.86
Control	**186.30 ± 9.28**	–
Standard	**79.81 ± 8.76**	**57.16**

Values represent the mean ± S.E.M. ($n = 6$).

derivatives showed a favorable influence on the activity as seen in the compounds (**IVd**), (**IVj**), (**IVk**), (**IVl**), (**IVn**) and (**IVp**). Among the tested compounds, 4-F substituted derivative (**IVd**) is found to be more active as it showed lesser decrease in immobility duration than the other compounds in the series. Derivatives with heterocyclic substitution ((**IVe**), (**IVg**), (**IVi**) and (**IVm**)) exhibited decreased activity, where as those with electron withdrawing group substitution showed intermediate activity. So it can be said that, the compounds with halogen substitution enhances the activity.

Maximal electroshock (MES) test was used to screen the synthesized compounds (**IVa**)–(**IVq**) for anticonvulsant activity. The results are displayed in Table 4. The compound (**IVb**) with o-CF_3 substitution showed very good activity with more reduced duration of extensor tonus than the standard drug used Phenyltoin. Compounds (**IVa**) and (**IVp**) with m-CN and 2,4-difluoro substitutions respectively showed good activity, whereas (**IVc**) and (**IVf**) exhibited medium activity. Rest of the compounds showed least activity. Electron withdrawing and halo substituted phenyl ring compounds showed better activity than heterocyclic substituted compounds and electron releasing group substituted compounds.

EXPERIMENTAL

Melting points were determined in open capillaries and uncorrected (melting point apparatus: Sewell instruments Inc., India). All solvents used were of analytical grade and the reagents used were purchased from commercial vendors. The purity of the compounds was checked by thin layer chromatography on a silica coated aluminum sheet (silica gel F_{254}). IR spectra (KBr pellets) were recorded on a Shimadzu FT-IR 157-spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker Avance II 400 (400 MHz) spectrometer using TMS as internal standard. Mass spectra were determined on a Joel SX 102/Da-600 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. Elemental analyses were carried out using a CHNS elemental analyzer. The progress and completion of the reaction was confirmed by TLC analysis in Ethyl acetate–hexane mixture (4 : 6).

Synthetic procedure for the preparation *N*-(sub phenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamides (IV). To a solution of 2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione (5 mmol) in dry DMSO (25 mL) containing anhydrous milled potassium carbonate (10 mmol), appropriate 2-chloro-*N*-substituted acetamide (5 mmol) was added. The reaction mixture was kept for stirring 16 h at room temperature. Then it was poured into the water with stirring. The formed product was filtered, washed with water, dried, and recrystallized from DMF–water mixture to get pure product.

***N*-(3-Cyanophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVa).** Yield: 86%, melting point: 237–239°C; IR: 3323 (–NH), 3057 (Ar-H), 2210 (C≡N), 1670 (amide C=O), 656 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.28 (s, 2H, -CH_2), 7.40 (t, 1H, Ar-H, $J = 7.6$ Hz), 7.47–7.57 (m, 3H, Ar-H), 7.66 (t, 1H, Ar-H, $J = 8$), 7.84 (d, 1H, Ar-H, $J = 7.6$ Hz), 8.08 (s, 1H, Ar-H), 8.26 (d, 1H, Ar-H, $J = 7.6$ Hz), 10.74 (bs, 1H, NH), 12.52 (bs, 1H, NH); MS (*m/z*): 360 (M^+), 362 ($M^+ + 2$); Found for $C_{18}H_{12}N_6OS$ (360.39): C, 59.92; H, 3.32; N, 23.30; S, 8.88; Anal. calc.: C, 59.99; H, 3.36; N, 23.32; S, 8.90.

***N*-(2-Trifluoromethyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVb).** Yield: 78%, melting point: 226–228°C; IR: 3265 (–NH), 3059 (Ar-H), 1662 (amide C=O), 650 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.30 (s, 2H, –CH_2), 7.27 (t, 1H, Ar-H; $J = 7.6$ Hz), 7.37 (d, 1H, Ar-H, $J = 7.6$ Hz), 7.55 (t, 1H, Ar-H, $J = 7.6$ Hz), 7.61–7.71 (m, 4H, Ar-H), 7.89 (d, 1H, $J = 8$ Hz), 7.92 (d, 1H, Ar-H, $J = 7.6$ Hz), 8.10 (d, 1H, $J = 8$ Hz), 10.5 (s, 1H, NH), 12.50 (bs, 1H, NH); MS (*m/z*): 403 (M^+), 405 ($M^+ + 2$); Found for

$C_{18}H_{12}F_3N_5OS$ (403.38): C, 53.56; H, 2.98; N, 17.30; S, 7.92; Anal. calc.: C, 53.60; H, 3.00; N, 17.36; S, 7.95.

***N*-(2-Cyanophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVc).** Yield: 80%, melting point: 247–249°C; IR: 3320 (–NH), 3065 (Ar-H), 2219 (C≡N), 1663 (amide C=O), 658 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.27 (s, 2H, -CH_2), 7.22 (t, 1H, Ar-H, J = 7.6 Hz), 7.30 (d, 1H, Ar-H, J = 7.6 Hz), 7.46 (m, 1H), 7.60 (t, 1H, Ar-H, J = 7.6 Hz), 7.59 – 7.68 (m, 2H), 7.89 (d, 2H, J = 8 Hz), 7.88 (d, 1H, Ar-H, J = 7.6 Hz), 8.0 (d, 1H, J = 8 Hz), 10.30 (s, 1H, NH), 12.52 (bs, 1H, NH); MS (*m/z*): 360 (M^+), 362 (M^+ + 2); Found for $C_{18}H_{12}N_6OS$ (360.39): C, 59.94; H, 3.34; N, 23.31; S, 8.85. Anal. calc.: C, 59.99; H, 3.36; N, 23.32; S, 8.90.

***N*-(4-Fluorophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVd).** Yield: 82%, melting point: 233–235°C; IR: 3450 (-NH), 3059 (Ar-H), 1656 (amide C=O), 1178 (Ar-F), 653 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.30 (s, 2H, –CH_2), 7.17 (t, 2H, J = 8.4 Hz), 7.20 (t, 1H, Ar-H, J = 7.6 Hz), 7.32 (d, 1H, Ar-H, J = 7.6 Hz), 7.62 (t, 1H, Ar-H, J = 7.6 Hz), 7.67–7.63 (m, 2H), 7.90 (d, 2H, J = 7.6), 10.10 (s, 1H, NH), 12.50 (s, 1H, NH); MS (*m/z*): 353 (M^+), 365 (M^+ + 2); Found for $C_{17}H_{12}FN_5OS$ (353.37): C, 57.74; H, 3.40; N, 19.80; S, 9.05; Anal. calc.: C, 57.78; H, 3.42; N, 19.82; S, 9.07.

***N*-(5-Bromopyridin-2-yl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVe).** Yield: 69%, melting point: 213–215°C; IR: 3455 (–NH), 3050 (Ar-H), 1658 (amide C=O), 653 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.32 (s, 2H, –CH_2), 7.20 (t, 1H, Ar-H, J = 7.6 Hz), 7.32 (d, 1H, Ar-H, J = 7.6 Hz), 7.62 (t, 1H, Ar-H, J = 7.6 Hz), 7.84 (dd, J = 9 Hz, 1H), 7.90 (d, 2H, J = 7.6 Hz), 8.11 (d, 1H, Ar-H, J = 9.0 Hz), 8.37 (d, 1H, Ar-H, J = 9.0 Hz), 10.17 (s, 1H, NH), 12.49 (br, 1H, NH);); MS (*m/z*): 415 (M^+), 417 (M^+ + 2); Found for $C_{17}H_{12}BrN_5OS$ (415.27): C, 49.25; H, 2.90; N, 16.87; S, 7.72; Anal. calc.: C, 49.29; H, 2.92; N, 16.90; S, 7.74.

***N*-(3-Trifluoromethylphenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVf).** Yield: 72%, melting point: 238–240°C; IR: 3265 (-NH), 3059 (Ar-H), 1662 (amide C=O), 650 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.29 (s, 2H, –CH_2), 7.17 (m, 2H, Ar-H), 7.20 (t, 1H, Ar-H, J = 7.6 Hz), 7.32 (d, 1H, Ar-H, J = 7.6 Hz), 7.62 (t, 1H, Ar-H, J = 7.6 Hz), 7.64 (d, 1H, Ar-H, J = 8 Hz) 7.83 (s, 1H, Ar-H), 7.90 (d, 2H, J = 7.6 Hz), 10.10 (s, 1H, NH), 12.43 (bs, 1H, NH); MS (*m/z*): 403 (M^+), 405 (M^+ + 2); Found for $C_{18}H_{12}F_3N_5OS$ (403.38): C, 53.54; H, 2.96; N, 17.29; S, 7.91; Anal. calc.: C, 53.60; H, 3.00; N, 17.36; S, 7.95.

***N*-(1,3-Thiazol-2-yl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVg).** Yield: 66%, melting point: 237–239°C; IR: 3268 (–NH), 3056 (Ar-H), 1668 (amide C=O), 656 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.30 (s, 2H, –CH_2), 6.56 (d, 1H, thiazole ring, J = 8.4 Hz), 7.23 (t, 1H, Ar-H, J = 8 Hz), 7.30 (d, 1H, Ar-H, J = 8 Hz), 7.60 (t, 1H, Ar-H, J = 8 Hz), 7.68 (d,1H, Ar-H, J = 8 Hz) 7.73 (s, 1H, Ar-H), 7.90 (d, 2H, J = 7.6 Hz), 10.09 (s, 1H, NH), 12.40 (bs, 1H, NH); MS (*m/z*): 342 (M^+), 344 (M^+ + 2); Found for $C_{14}H_{10}N_6OS_2$ (342.4): C, 49.08; H, 2.92; N, 24.52; S, 18.71; Anal. calc. C, 49.11; H, 2.94; N, 24.54; S, 18.73.

Table 4. Results of anticonvulsant activity for the compounds (**IVa**)–(**IVq**): maximal electroshock test

Compound	Duration of tonic hind limb extensor in seconds (mean ± SEM)
(IVa)	**2.71 ± 0.61**
(IVb)	**1.18 ± 0.53**
(IVc)	5.22 ± 0.47
(IVd)	11.52 ± 0.54
(IVe)	8.42 ± 0.41
(IVf)	6.34 ± 0.38
(IVg)	11.74 ± 0.52
(IVh)	10.61 ± 0.48
(IVi)	7.60 ± 0.55
(IVj)	7.56 ± 0.47
(IVk)	9.60 ± 0.43
(IVl)	11.37 ± 0.41
(IVm)	8.32 ± 0.29
(IVn)	10.72 ± 0.34
(IVo)	8.60 ± 0.52
(IVp)	**2.51 ± 0.44**
(IVq)	9.60 ± 0.43
Control	**12.41 ± 0.48**
Standard	**1.38 ± 0.34**

***N*-(4-Trifluoromethoxyphenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVh).** Yield: 85%, melting point: 242–244°C; IR: 3265 (–NH), 3056 (Ar-H), 1669 (amide C=O), 658 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.34 (s, 2H, –CH_2), 6.75 (d, 2H, Ar-H, J = 8.6 Hz), 7.28 (t, 1H, Ar-H, J = 8 Hz), 7.37 (d, 1H, Ar-H, J = 8 Hz), 7.63 (t, 1H, Ar-H, J = 8 Hz), 7.70 (d,1H, Ar-H, J = 8 Hz), 7.79 (d, 2H, J = 8.6 Hz), 10.2 (s, 1H, NH), 12.42 (bs, 1H, NH); MS (*m/z*): 419 (M^+), 421(M^+ + 2); Found for $C_{18}H_{12}F_3N_5O_2S$ (419.38): C, 51.54; H, 2.86; N, 16.69; S, 7.61; Anal. calc.: C, 51.55; H, 2.88; N, 16.70; S, 7.65.

***N*-(5-Chloropyridin-2-yl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVi).** Yield: 79%, melting point: 232–234°C; IR: 3268 (–NH), 3056 (Ar-H), 1668 (amide C=O), 656 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.32 (s, 2H, –CH_2), 7.20 (t, 1H, Ar-H,

J = 7.6 Hz), 7.32 (d, 1H, Ar-H, J = 7.6 Hz), 7.62 (t, 1H, Ar-H, J = 7.6 Hz), 7.86 (dd, 1H, J = 9 Hz), 7.94 (d, 2H, J = 7.6Hz), 8.14 (d, 1H, Ar-H, J = 9.0 Hz), 8.32 (d, 1H, Ar-H, J = 9.0 Hz), 10.15 (s, 1H, NH), 12.35 (bs, 1H, NH); MS (m/z): 370 (M^+); Found for $C_{16}H_{11}ClN_6OS$ (370.82): C, 51.54; H, 2.96; N, 22.65; S, 8.63; Anal. calc.: C, 51.58; H, 2.99; N, 22.66; S, 8.65.

***N*-(3,5-Dichlorophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVj).** Yield: 88%, melting point: 235–237°C; IR: 3256 (-NH), 3043 (Ar-H), 1664 (amide C=O), 657 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.28 (s, 2H, -CH_2), 7.26 (t, 1H, Ar-H, J = 8 Hz), 7.33 (d, 2H, J = 8.4 Hz), 7.39 (d, 1H, Ar-H, J = 8 Hz), 7.63 (t, 1H, Ar-H, J = 8 Hz), 7.69 (d,1H, Ar-H, J = 8 Hz), 7.73 (t, 1H, J = 8.4 Hz), 7.79 (d, 2H, J = 8.0 Hz), 10.23 (s, 1H, NH), 12.38 (bs, 1H, NH); MS (m/z): 404 (M^+); Found for $C_{17}H_{11}Cl_2N_5OS$ (404.27): C, 50.54; H, 2.76; N, 17.30; S, 7.91; Anal. calc.: C, 50.51; H, 2.74; N, 17.32; S, 7.93.

***N*-(2,4,5-Trichlorophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVk).** Yield: 88%, melting point: 217–219°C; IR: 3268 (–NH), 3054 (Ar-H), 1660 (amide C=O), 647 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.26 (s, 2H, –CH_2), 7.27 (t, 1H, Ar-H, J = 8 Hz), 7.39 (d, 1H, Ar-H, J = 8 Hz), 7.48 (s, 1H), 7.60 (t, 1H, Ar-H, J = 8 Hz), 7.70 (d,1H, Ar-H, J = 8 Hz), 7.89 (s, 1H), 10.33 (s, 1H, NH), 12.28 (bs, 1H, NH); MS (m/z): 438.72 (M^+), 440.21 (M^+ + 2); Found for $C_{17}H_{10}Cl_3N_5OS$: C, 46.53; H, 2.32; N, 15.95; S, 7.33; Anal. calc.: C, 46.54; H, 2.30; N, 15.96; S, 7.31.

***N*-(3-Chloro-4-fluorophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVl).** Yield: 83%, melting point: 232–234°C; IR: 3266 (–NH), 3053 (Ar-H), 1663 (amide C=O), 654 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.26 (s, 2H, -CH_2), 7.13 (t, 1H, Ar–H, J = 9.0 Hz), 7.27 (t, 1H, Ar-H, J = 8 Hz), 7.31–7.37 (m, 1H, Ar–H), 7.39 (d, 1H, Ar-H, J = 8 Hz), 7.60 (t, 1H, Ar-H, J = 8 Hz), 7.70 (d,1H, Ar-H, J = 8 Hz), 7.72–7.75 (m, 1H, Ar–H), 7.89 (s, 1H), 10.33(s, 1H, NH), 12.28 (bs, 1H, NH); MS (m/z): 387 (M^+); Found for $C_{17}H_{11}ClFN_5OS$ (387.82): C, 52.64; H, 2.85; N, 18.05; S, 8.26; Anal. calc.: C, 52.65; H, 2.86; N, 18.06; S, 8.27.

***N*-(1,3-Benzothiazol-2-yl))-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVm).** Yield: 80%, melting point: 233–235°C IR: 3265 (–NH), 3052 (Ar-H), 1666 (amide C=O), 651 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.44 (s, 2H, –CH_2), 7.29 (t, 1H, Ar-H, J = 8 Hz), 7.38–7.50 (m, 2H, benzothiazole–H),7.58 (d, 1H, Ar-H, J = 8 Hz), 7.67 (t, 1H, Ar-H, J = 8 Hz), 7.77 (d,1H, Ar-H, J = 8 Hz), 7.95 (d, 1H, J = 8 Hz), 8.27 (d, 1H, J = 8 Hz), 10.63 (s, 1H, NH), 12.78 (bs, 1H, NH); MS (m/z): 394 (M^+); Found for $C_{18}H_{14}N_6OS_2$ (394.47): C, 54.84; H, 3.59; N, 21.28; S, 16.27; Anal. calc.: C, 54.81; H, 3.58; N, 21.30; S, 16.26.

***N*-(2,5-Dichlorophenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVn).** Yield: 82%, melting point: 237–239°C; IR: 3261 (–NH), 3052 (Ar-H), 1663 (amide C=O), 650 (C–S); ^{1}H NMR ($CDCl_3$): δ 4.26 (s, 2H, –CH_2), 6.83 (s, 1H), 7.32 (t, 1H, Ar-H, J = 8 Hz), 7.37 (dd, 1H, J = 8.6 Hz), 7.40 (d, 1H, Ar-H; J = 8 Hz), 7.60 (d, 1H, J = 8.7 Hz), 7.65 (t, 1H, Ar-H, J = 8 Hz), 7.70 (d, 1H, Ar-H, J = 8 Hz), 10.33 (s, 1H, NH), 12.28 (bs, 1H, NH); MS (m/z): 404 (M^+); Found for $C_{17}H_{11}N_5OS$ (404.27): C, 50.50; H, 2.73; N, 17.31; S, 7.92; Anal. calc.: C, 50.51; H, 2.74; N, 17.32; S, 7.93.

***N*-(2-Chloro-4-methylpyridin-3-yl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVo).** Yield: 79%, melting point: 246–248°C; IR: 3263 (-NH), 3059 (Ar-H), 1661 (amide C=O), 650 (C–S); ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H), 4.36 (s, 2H, –CH_2), 7.36 (t, 1H, Ar-H, J = 8 Hz), 7.45 (d, 1H, Ar-H, J = 8 Hz), 7.66 (t, 1H, Ar-H, J = 8 Hz), 7.76 (d,1H, Ar-H, J = 8 Hz), 7.83 (d, 1H, Ar-H, J = 8.4 Hz), 7.90 (d, 1H, J = 8.4 Hz), 10.33 (s, 1H, NH), 12.29 (bs, 1H, NH); MS (m/z): 384 (M^+); Found for $C_{17}H_{13}ClN_6OS$ (384.84): C, 53.05; H, 3.39; N, 21.82; S, 8.32; Anal. calc.: C, 53.06; H, 3.40; N, 21.84; S, 8.33.

***N*-(2,4-Difluorophenyl))-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVp).** Yield: 89%, melting point: 226–228°C; IR: 3268 (–NH), 3056 (Ar-H), 1662 (amide C=O), 657 (C–S); ^{1}H NMR (400 MHz, DMSO, δ ppm); 4.32 (s, 2H, –CH_2), 7.36 (t, 1H, Ar-H, J = 8 Hz), 7.39 (d, 1H, Ar-H, J = 8 Hz), 7.52–7.57 (m, 3H, Ar-H), 7.66 (t, 1H, Ar-H, J = 8 Hz), 7.76 (d,1H, Ar-H, J = 8 Hz), 10.38 (s, 1H, NH), 12.24 (s, 1H, NH); MS (m/z): 371 (M^+); Found for $C_{17}H_{11}F_2N_5OS$ (371.36): C, 54.95; H, 2.98; N, 18.85; S, 8.62. Anal. calc.: C, 54.98; H, 2.99; N, 18.86; S, 8.63.

***N*-(4-Methylpyridin-3-yl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl)acetamide (IVq).** Yield: 77%, melting point: >250°C; IR: 3266 (–NH), 3052(Ar-H), 1669 (amide C=O), 651 (C–S); ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H), 4.39 (s, 2H, –CH_2), 7.38 (t, 1H, Ar-H, J = 8 Hz), 7.45 (d, 1H, Ar-H, J = 8 Hz), 7.68 (t, 1H, Ar-H, J = 8 Hz), 7.76 (d,1H, Ar-H, J = 8 Hz), 7.83 (d, 1H, Ar-H, J = 8.4 Hz), 7.90 (d, 1H, J = 8.4 Hz), 8.54 (s, 1H, Ar-H), 10.33 (s, 1H, NH), 12.29 (bs, 1H, NH); MS (m/z): 350 (M^+); Found for $C_{17}H_{14}N_6OS$ (350.05): C, 58.05; H, 4.02; N, 23.96; S, 9.12; Anal. calc.: C, 58.07; H, 4.03; N, 23.98; S, 9.15.

Synthetic procedure for the preparation 2,5-dihydro-3*H*-[1,2,4]triazino[5,6-*b*]indole-3-thione (II). A mixture of Isatin (100 mmol), thiosemicarbazide (1 mmol) and K_2CO_3 (150 mmol) in 500 mL of water was refluxed with stirring for 3 h. On cooling the mix-

ture was filtered and precipitated by acidification with acetic acid. The solid was washed with water and dried to obtain as yellow solid. Yield: 92%, melting point: 196–298°C; IR: 3108 (–NH), 3051 (Ar-H); ^{1}H NMR ($CDCl_3$): δ 7.27 (t, 1H, Ar-H, J = 7.6 Hz), 7.37 (d, 1H, Ar-H, J = 7.6 Hz), 7.55 (t, 1H, Ar-H, J = 7.6 Hz), 7.92 (d, 1H, Ar-H, J = 7.6 Hz), 12.43 (bs, 1H, NH; exchangeable with D_2O), 14.54 (bs, 1H, NH; exchangeable with D_2O); MS (m/z): 202 (M^+); Found for $C_9H_6N_4S$ (202.02): C, 53.40; H, 2.96; N, 27.72; S, 15.86; Anal. calc.: C, 53.45; H, 2.99; N, 27.70; S, 15.86.

Antibacterial and Antifungal Activities

Determination of minimum inhibitory concentration (MIC). The MIC of the samples in DMF was determined by using different concentrations of extracts in Brain Heart Infusion Broth for bacteria and fungi by macro dilution method following NCCLS recommendations [24, 25]. The lowest concentration of the sample in DMF inhibiting the visible growth of microorganisms was considered as MIC. The Tube dilution test is used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds (**IVa**)–(**IVq**). Freshly prepared nutrient broth was used as diluents. The 24 h old cultures of the test bacteria *S. aureus, E. fecalis*, *E. coli* and *K. pneumoniae* and the test fungi *C. albicans* and *A. niger* were diluted 100 folds in nutrient broth (NB) (100 μL bacterial cultures in10 mL NB). The stock solution of the synthesized compounds with concentration 1000 μg/10 μL was prepared in DMF. Different concentrations of the test samples (0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 μg/mL) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as a control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

Tail Suspension Test (TST)

For the purpose of biological evaluation of synthesized compounds, adult male Swiss Albino mice (22 ± 2 g) were used. Animals were maintained in humidity and temperature controlled room with day–night cycle. They were allowed to acclimatize to the environment for one week before commencement of the experiments. Free access to food and water was permitted. The mice were housed in Plexiglass cages with six animals for each cage. Title compounds were screened for antidepressant activity employing tail suspension test (TST) [26]. In this test, mice were suspended on the edge of a table 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. After the initial escape-oriented movements, mice develop an immobile posture when placed in an inescapable stressful situation. This stressful situation involved haemodynamic stress of being hung in an uncontrollable fashion by their tail. The total duration of immobility induced by tail suspension was measured. Immobility time was recorded during a 6-min period. The animal was immobile only when they hung passively and completely motionless. The synthesized compounds (25 mg/kg), and fluoxetine hydrochloride, as a reference drug (30 mg/kg) were suspended in a 10% aqueous solution of Tween 80. The percentage decrease in immobility duration (%DID) for the test and standard drugs was calculated using following formula:

$$\%\mathrm{DID} = [(\mathrm{A} - \mathrm{B})/\mathrm{A}] \times 100.$$

Where A is the duration of immobility(s) in the control group and B is the duration of immobility(s) in test groups.

Maximal Electroshock (MES) Test

In the MES test [27, 28], the mice were subjected to 50 mA 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 were noted. Phenytoin (40 mg/kg) was used as standard drug.

CONCLUSION

A new series of *N*-(substituted phenyl)-2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylsulfanyl) acetamides were synthesized in good yields and characterized well. Most of the derivatives showed good inhibition against bacterial and fungal strains. Halogen substituted derivatives showed preferential increase in the both antidepressant and anticonvulsant activity. Among the synthesized newer compounds, it is conceivable that some of the derivatives showed promising biological and pharmacological activities which can be further modeled to exhibit better potency than the standard drugs.

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REFERENCES

1. Knörle, R., *J. Neural Transm.*, 2012, vol. 119, pp. 1477–1482.
2. Khawam, E.A., Laurencic, G., and Malone, D.A., *Clin. J. Med.*, 2006, vol. 73, pp. 351–353.

3. Berton, O. and Nestler, E.J., Nature, Rev. *Neurosci.*, 2006, vol. 7, pp. 137–151.
4. Dichter, G.S., Felder, J.N., Petty, C., Bizzell, J., Ernst, M., and Smoski, M.J., *Biol. Psych.*, 2009, vol. 66, pp. 886–897.
5. Fountoulakis, K.N., Grunze, H., Panagiotidis, P., and Kaprinis, G., *J. Affect. Disord.*, 2008, vol. 109, pp. 21–34.
6. Papakostas, G.I., Mischoulon, D., Shyu, I., Alpert, J.E., and Fava, M., *Am. J. Psychiatry,* 2010, vol. 167, pp. 942–948.
7. Salisbury, E.J. and Van Voorhis, P., *Crim. Justice Behav.*, 2009, vol. 36, p. 541.
8. Kaspersen, H. and Agmo, A., *Psychopharmacology*, 2012, vol. 220, pp. 269–280.
9. Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, 9th ed., New York: McGraw-Hill Company, 1996, pp. 471–472.
10. Leppik, I.E., *Epilepsia*, 1994, vol. 35, pp. 29–40.
11. Al-Soud, Y.A., Al-Masoudi, N.A., and Ferwanah, A.R.S., *Bioorg. Med. Chem.*, 2003, vol. 11, pp. 1701–1708.
12. Kinsman, O.S., Livermore, D.G., and Smith, C., *Antimicrob. Agents Chemother.*, 1993, vol. 37, pp. 1243–1246.
13. Mongel, A., Palop, J., and Ramirez, C., *Eur. J. Med. Chem.*, 1991, vol. 26, pp. 179–188.
14. Kgokong, J.L., Smith, P.P., and Matsabisa, G.M., *Bioorg. Med. Chem.*, 2005, vol. 13, pp. 2935–2942.
15. Tomchin, A.B., Uryupov, O.Y., and Smirnov, A.V., *Pharmaceut. Chem. J.*, 1997, vol. 31, pp. 632–637.
16. Ram, V.J., *Arch. Pharm.*, 1980, vol. 313, pp. 108–113.
17. Shelke, S.M. and Bhosale, S.H., *Bioorg. Med. Chem. Lett.*, 2010, vol. 20, pp. 4661–4664.
18. Aswar, U.M., Kalshetti, P.P., Shelke, S.M., Bhosale, S.H., and Bodhankar, S.L., *Asian Pac. J. Trop. Biomed.,* 2012, vol. 2, p. 992.
19. Gladych, J.M.Z., Hornby, R., Hunt, J.H., Jack, D., Boyle, J.J., Ferlauto, R.J., Haff, R.F., Kormendy, C.G., Stanfield, F.J., and Stewart, R.C., *J. Med. Chem.,* 1972, vol. 15, p. 277.
20. Holla, B.S. and Udupa, K.V., *Heterocycles*, 1991, vol. 32, pp. 1081–1088.
21. Zhang, Q., Ding, D., and Zeng, S., *PLoS One*, 2012, vol. 7, p. 46294.
22. Jan, M.Z., Gladych, R.H., Hunt, J.H., and Jack, D., *J. Med. Chem.,* 1972, vol. 15, pp. 277–281.
23. Soyer, Z., Kiliç, F.M., Erol, K., and Pabuccuog, V., *Il Farmaco*, 2003, vol. 59, pp. 595–672.
24. Richard, S., Lynn, S.M., and Avery, C.G., *Antimicrobial Susceptibility Testing Protocols*, CRC Press, Taylor and Francis Group, 2007.
25. Zgoda, J.R. and Porter, J.R., *Pharm. Biol.*, 2001, vol. 39, pp. 221–225.
26. Steru, L., Chermat, R., Thierry, B., and Simon, P., *Psychopharmacology*, 1985, vol. 85, pp. 367–370.
27. Stillings, M.R., Welbournt, A.P., and Walter, D.S., *J. Med. Chem.*, 1986, vol. 29, pp. 2280–2284.
28. Woodbury, L.A. and Davenport, V.O., *Arch. Int. Pharmacodyn. Ther.*, 1952, vol. 92, pp. 97–107.