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Synthesis, antidepressant evaluation and QSAR studies of novel 2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylthio)-*N*-(substituted phenyl)acetamides

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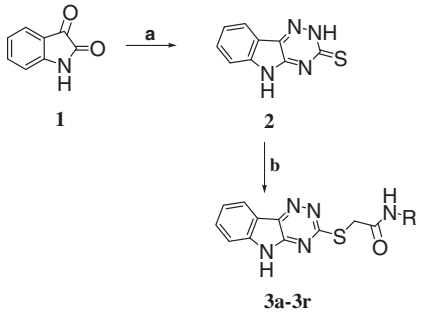
ABSTRACT

In search for novel antidepressants, a series of 2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylthio)-*N*-(substituted phenyl)acetamides was synthesized and screened for potential antidepressant activity by tail suspension test (TST) in mice. Number of synthesized compounds exhibited impressive antidepressant activity, measured in terms of percentage decrease in immobility duration (%DID). QSAR analysis was also undertaken which correlated three parameters FOSA, PISA, and glob with biological activity.

Major depressive disorder (MDD) is highly rampant psychological illness with 9–18% prevalence and ranking fourth among the causes of death or injury worldwide.[1,2] Monoamine hypothesis of depression, the most widely accepted one, emphasizes the dysfunction of the norepinephrine (NE), serotonin (5-HT), and dopamine (DA) neurotransmitters in the corticolimbic synaptic cleft as responsible for depression.[3] Although clinically used antidepressants have varying mechanism of actions; they all however eventually increase brain monoamine levels. Clinical limitations and adverse effects of currently used antidepressants necessitate continuous development of novel, efficient and safe drugs for treatment of depression.[4] Additional reason for encouraging research in this field comes from the prediction that by 2020 MDD is expected to be the second leading cause of disease or injury worldwide.[5]

Derivatives of 1,2,4-triazino[5,6-*b*]indole-3-thione are known to possess diverse biological activities like actoprotector,[6] antiviral,[7] antihypoxic, anti-inflammatory,[8] antimalarial,[9] antimicrobial,[10] antitumor,[11] and hepatoprotective.[12] Importantly, this tricyclic structure is comparable to *β*-carboline (9*H*-pyrido[3,4-*b*]indole), an endogenous monoamine oxidase (MAO) inhibitor.[13] So we conceived it interesting to evaluate derivatives of 1,2,4-triazino[5,6-*b*]indole-3-thione for antidepressant activity. In an attempt to explore novel antidepressant compounds, we designed the present series of 2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylthio)-*N*-(substituted phenyl)acetamides. While designing present series, cognizance was taken of central nervous system activity of various heterocyclic phenylacetamides.[14–18] Herein we report synthesis, antidepressant evaluation and QSAR studies of title compounds (**3a**–**3r**).

Substituted acetamides were prepared as per routine[18] procedure which involves reaction of primary amines with chloroacetyl chloride in glacial acetic acid containing saturated solution of sodium acetate. The tricyclic compound 1,2,4-triazino[5,6-*b*]indole-3-thione **2** was prepared referring to a previously reported method.[7] Isatin **1** was condensed with thiosemicarbazide by refluxing in aqueous solution of potassium carbonate. The solution so formed was filtered and acidified with glacial acetic acid to yield condensed product **2**. Synthesis of title compounds **3a**–**3r** was accomplished by stirring overnight solution of **2** in dry dimethyl sulfoxide (DMSO) containing anhydrous milled potassium carbonate with appropriate acetamides (Scheme 1).[19] Yields of final compounds were in the range of 66–78% after recrystallization



Scheme 1. Reagents and conditions: (a) $NH_2C(S)NHNH_2$, K_2CO_3, H_2O, reflux, 4 h; (b) $RNHCOCH_2Cl$, K_2CO_3, DMSO, rt, 16 h. (For R, refer Table 1.)

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from *N*,*N*-dimethylformamide–water. Structure conformation of synthesized compounds was done by IR, ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT, MS, and elemental analysis.

IR spectrum of prototype compound **3l** showed prominent absorption bands of N–H stretching in secondary amides near 3289 cm^{-1} and an amide carbonyl at 1660 cm^{-1}. Presence of aromatic skeleton is confirmed by peak at 3050 cm^{-1} corresponding to aromatic C–H stretching. ^{1}H NMR of **3l** displayed singlets at δ 2.24 for methyl protons and δ 4.25 for methylene protons alpha to carbonyl group. Multiplets at δ 7.1–8.28 affirmed the presence of eight aromatic protons. Further, a broad singlet at δ 10.28 revealed the presence of –NH– proton. ^{13}C NMR of **3l** indicated presence of methyl carbon with peak at δ 20.67, methylene carbon with a peak at δ 35.58 and carbonyl carbon with a peak at 166.73. Peaks for fifteen carbons in the aromatic region δ 112.99–166.02 underlined the presence of required aromatic skeleton. Mass spectra (ESI) of **3l** showed a molecular ion peak at *m*/*z* 372.1 ($M+Na^{+}$).[19]

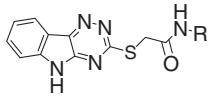
For the purpose of biological evaluation of synthesized compounds, adult male Swiss Albino mice (22 ± 2 g) were used. Animals were maintained in humidity and temperature controlled rooms with day–night cycle. They were allowed to acclimatize with the environment for one week before commencement of the experiments. Free access to food and water was permitted. Title compounds were screened for antidepressant activity employing tail suspension test (TST).[20] In TST, after 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. Duration of immobility was measured for a period of 6 min.[21] In this established behavioral model, ability of a compound to decrease immobility duration is taken as a measure of its antidepressant activity. Solutions of the synthesized compounds as well as of standard drugs (moclobemide, fluoxetine, and imipramine) were prepared in DMSO and administered intraperitoneally (ip) to mice. To control group, DMSO was injected ip at a constant volume of 5 mL/kg. Synthesized compounds (30 mg/kg; ip) were administered thrice in 24 h duration (sub-chronic) at t = 0, 18, and 24 h. Similar dosing regimen was followed for standard drugs. Test was performed 1 h after the administration of last dose. Percentage decrease in immobility duration (%DID) for test and standard drugs was calculated using following formula:

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

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

Majority of the synthesized compounds exhibited considerable antidepressant potential as evident from their high %DID values (Table 1). Any substituent on the phenyl ring diminished the activity of parent compound **3a** from small to considerable extent depending upon its type and position. By and large for all the substituents, biological activity increased as we went from *ortho*- to *para*-position. Presence of bulky non-polar groups on phenyl ring as in **3b** and **3c** tended to lessen the activity drastically. Compounds with polar substituents like **3d**, **3j**, and **3p** were all effective in appreciably reducing immobility duration though not better than their un-substituted counterpart **3a**. Replacement of phenyl by benzyl as in **3q** shrunk the activity to some extent. Again, decline in activity was noted on replacing phenyl by cyclohexyl

Table 1
Antidepressant evaluation of synthesized compounds with observed and predicted values of log (%DID) for training and test set compounds



Compound[a]	R	TST		log (%DID)		
		Duration of immobility (s) (mean ± SEM)[b]	% Decrease in immobility duration (%DID)	Expt.	Pre.	Res.
Training set						
3a	Phenyl	51.5 ± 7.1	70.62	1.849	1.829	0.020
3b	2-Methylphenyl	138.7 ± 10.1[c]	20.87	1.320	1.309	0.011
3d	2-Chlorophenyl	84.3 ± 5.4	51.89	1.715	1.667	0.048
3e	2-Bromophenyl	100.2 ± 7.2	42.84	1.632	1.674	−0.042
3f	2-Nitrophenyl	100.5 ± 13.7	42.66	1.630	1.712	−0.082
3g	3-Methylphenyl	129.2 ± 8.5	26.29	1.420	1.453	−0.033
3h	3-Methoxyphenyl	120.8 ± 11.7	31.08	1.492	1.397	0.095
3i	3-Chlorophenyl	75.1 ± 6.1	57.15	1.757	1.773	−0.016
3k	3-Nitrophenyl	72.3 ± 6.0	58.75	1.769	1.784	−0.014
3m	4-Methoxyphenyl	138.8 ± 8.1[c]	20.82	1.318	1.381	−0.063
3n	4-Chlorophenyl	55.5 ± 7.7	68.33	1.835	1.773	0.062
3o	4-Bromophenyl	56.8 ± 12.1	67.59	1.830	1.793	0.037
3q	Benzyl	75.8 ± 4.7	56.75	1.754	1.777	−0.023
Test set						
3c	2-Methoxyphenyl	132.7 ± 9.2	24.30	1.386	1.435	−0.049
3j	3-Bromophenyl	58.6 ± 4.1	66.57	1.823	1.793	0.030
3l	4-Methylphenyl	121.7 ± 10.2	30.57	1.485	1.446	0.040
3p	4-Nitrophenyl	66 ± 7.6	62.35	1.795	1.782	0.013
3r[d]	Cyclohexyl	92.0 ± 11.41	47.51	–	–	–
Moclobemide		55.8 ± 9.6	68.16	–	–	–
Imipramine		69.1 ± 8.4	60.58	–	–	–
Fluoxetine		53.5 ± 4.3	69.48	–	–	–
Control		175.3 ± 8.5	–	–	–	–

n = 6.
Data were analyzed by one way ANOVA followed by Dunnett's test.
[a] Dose: 30 mg/kg.
[b] $p < 0.01$ versus control except **3b** and **3m**.
[c] $p < 0.05$ versus control.
[d] Detected as an outlier in QSAR analysis by MLR.

Table 2
Descriptors used in final MLR analysis

Descriptor	Description[25]
Dipole	Computed dipole moment of the molecule
SASA	Total solvent accessible surface area in square angstroms using a probe with a 1.4 Å radius
FOSA	Hydrophobic component of the solvent accessible surface area (SASA) (saturated carbon and attached hydrogen)
FISA	Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms)
PISA	π (carbon and attached hydrogen) component of the SASA
WPSA	Weakly polar component of the SASA (halogens, P, and S)
glob	Globularity descriptor, $(4\pi r^2)/(\text{SASA})$ where r is the radius of a sphere with a volume equal to the molecular volume. Globularity is 1.0 for a spherical molecule
Volume	Total solvent accessible volume in cubic angstroms using a probe with a 1.4 Å radius
dip^2/V	Square of the dipole moment divided by the molecular volume
QP log BB	Predicted brain/blood partition coefficient
IP	PM3 calculated ionization potential
EA	PM3 calculated electron affinity

as in **3r**. In TST, many of the synthesized compounds like **3a**, **3n**, **3o**, **3j**, and **3p** exhibited activity very comparable to standard antidepressant drugs moclobemide, imipramine and fluoxetine.

The quantitative structure–activity relationship (QSAR) analysis was undertaken to establish the relationship between antidepressant activity of the synthesized and their molecular descriptors. For the purpose of QSAR, log (%DID) was taken as an activity parameter. Structures were built and cleaned-up (Universal Force Field) in Maestro.[22] Next, the structures were processed using Ligprep[23] and minimized using MacroModel[24] with OPLS_2005 force field. The OPLS (Optimized Potentials for Liquid Simulations) force field was initially developed by Jorgensen.[25] OPLS force fields are reputed for successful computation of liquid state thermodynamic properties[26] and protein/protein–ligand modeling.[27] OPLS_2005 is an enhanced version of this force field developed by Schrödinger to provide a larger coverage of organic functionality. In OPLS_2005 all torsional parameters have been refit to reproduce the conformational energetics derived at a higher level of quantum theory and additional charges have been fit to support additional organic functionality.[24]

Molecular descriptors were calculated for minimized structures using Qikprop.[28] Qikprop predicts several physically significant descriptors and pharmaceutically relevant properties of organic molecules. The QSAR model was generated by multiple linear regression (MLR) analysis using Strike[29] as a statistical package. For final statistical analysis, a set of 12 descriptors was selected on the basis of correlation matrix, descriptor significance, and training set size. Brief description of these descriptors is given in Table 2. During initial runs, **3r** was detected as an outlier and was thus excluded from the final run. Based on structural variation and biological activity, compounds were divided into training set of thirteen compounds and test set of four compounds. Best QSAR model was selected on the basis of statistical parameters like squared correlation coefficient (R^2), standard deviation (SD), Fisher's value (F) and T-value. Generated model was cross validated by computing q^2 (R^2_{cv}) with 'Leave one out' (LOO) process and test set prediction. The final statistically valid QSAR model with three descriptors is given in Table 3. From this model it can be seen that two parameters, that is, FOSA and glob negatively correlate with log (%DID), whereas PISA correlates positively. Correlation matrix of independent variables involved in the model is given in Table 4. Experimental, predicted and residual values of log (%DID) for training and test set compounds are enumerated in Table 1. The graph of predicted log (%DID) versus experimental is depicted in Figure 1. Plot of residual log (%DID) against experimental is shown in Figure 2.

Descriptor FOSA is described as the hydrophobic component of the solvent accessible surface area (SASA). It represents aliphatic part of exposed hydrophobic surface area due to saturated carbon and attached hydrogen. As FOSA is negatively correlated with log (%DID), any aliphatic substituent in the molecule which increases FOSA, will decrease biological activity. This explains the reduced biological activity of compounds like **3b** or **3c** with methyl or methoxy substituents, respectively. Descriptor PISA quantifies the π component of SASA, that is, it reflects hydrophobicity of the molecule due to aromatic region. As PISA is positively correlated with log (%DID), any substitution which decreases aromatic hydrophobic makeup of the molecule will shrink its activity. This fact is corroborated by diminished activity of **3a** on substitution, as any substitution on phenyl ring of **3a** leads to decrease in PISA.

Table 3
Best QSAR model generated by MLR[a]

Variable	Coefficient	Standard error (±)	T-value
Intercept	1.0134×10^{1}	2.0727	4.8893
FOSA	-3.6655×10^{-3}	4.3895×10^{-4}	8.3505
PISA	3.3460×10^{-3}	1.0002×10^{-3}	3.3454
glob	-1.1865×10^{1}	2.6622	4.4567

[a] Statistical values: $N = 13$, SD = 0.0593, $R^2 = 0.9287$, $F(3.0, 9.0) = 39.1$, $P = 1.735 \times 10^{-5}$, $q^2 = 0.8713$, RMS = 0.0668.

Table 4
Correlation matrix for independent variables involved in MLR equation

	FOSA	PISA	glob
FOSA	1.0000		
PISA	−0.3138	1.0000	
glob	−0.0762	0.2512	1.0000

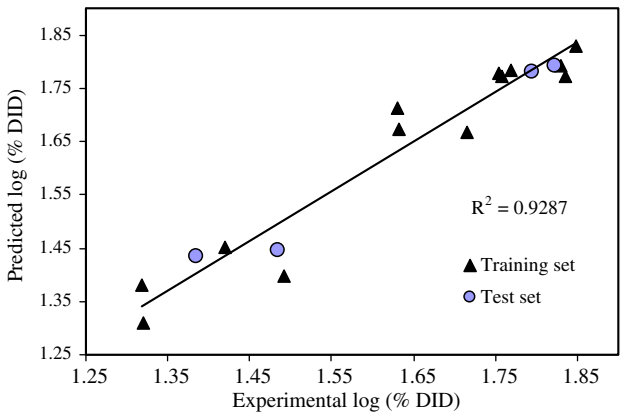


Figure 1. Plot of predicted log (%DID) against experimental log (%DID) for training and test set compounds.

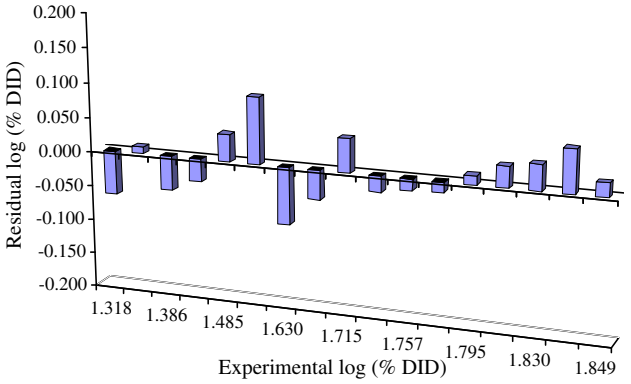


Figure 2. Plot of residual log (%DID) against experimental log (%DID).

glob is globularity descriptor, $(4\pi r^2)/(\text{SASA})$, where r is the radius of a sphere with a volume equal to the molecular volume. Thus, glob can be looked at as a measure of bulkiness. Since glob inversely correlates with log (%DID), any substituent which adds to glob will lessen the activity of the compound. This justifies decreased activity of compounds with bulky substituents.

To conclude, the present work revealed the synthesis of 2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylthio)-*N*-(substituted phenyl)acetamides. Many of the synthesized compounds significantly reduced immobility duration in TST which was comparable to standard drugs, underlying their antidepressant potential. QSAR studies of synthesized compounds indicated that increased aliphatic character and bulkiness decreased biological activity, whereas increased aromatic character enhanced biological activity. Information presented here may effectively be used for designing newer molecules with improved antidepressant potential.

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Supplementary data

Various descriptors used in QSAR analysis and physicochemical details of the synthesized compounds are mentioned. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.100.

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19. General procedure for synthesis of 2-(5*H*-[1,2,4]triazino[5,6-*b*]indol-3-ylthio)-*N*-substituted acetamides **3a**–**3r**: To a solution of **2** (0.005 mol) in dry DMSO (25 mL) containing anhydrous milled potassium carbonate (0.01 mol), appropriate 2-chloro-*N*-substituted acetamide (0.005 mol) was added. Reaction mixture was kept for stirring 16 h at room temperature. Reaction mixture was then poured into water with stirring. The precipitated product was filtered, washed with water, dried, and recrystallised from DMF–water to yield **3a**–**3r**.
Physicochemical data for **3l**: Yield 78%; mp 273–278 °C; IR (KBr, ν cm^{-1}): 3289, 3122, 3050, 2983, 1660, 1535. ^{1}H NMR (DMSO-d_6, δ): 2.24 (s, 3H, –CH_3), 4.25 (s, 2H, –CH_2–), 7.10 (d, 2H, Ar-H), 7.42 (t, 1H, Ar-H), 7.49 (d, 2H, Ar-H), 7.58 (d, 1H, Ar-H), 7.68 (t, 1H, Ar-H), 8.30 (d, 1H, Ar-H), 10.28 (br s, 1H, –NH–). ^{13}C NMR (DMSO-d_6, δ): 20.67, 35.58, 112.99, 117.81, 119.43 (2C), 121.73, 122.75, 129.39 (2C), 131.20, 132.61, 136.73, 140.69, 141.44, 146.79, 166.02, 166.73. ^{13}C DEPT (DMSO-d_6, δ): Positive peaks: 20.67, 112.98, 119.39 (2C), 121.74, 122.76, 129.39 (2C), 131.20; Inverse peaks: 35.57. MS (ESI) m/z: 372.1(M+Na$^+$, 100%). Elemental analysis $C_{18}H_{15}N_5OS$ Calcd. (Found): C, 61.87 (62.09); H, 4.33 (4.17); N, 20.04 (20.33), S, 9.18 (9.02).
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