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Research Article

Design, synthesis and evaluation of acute toxicity studies and anti-depressant activities of some new derivatives of 1, 3-benzoxazin- 4-one

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Abstract

This study was designed to synthesize, characterize and to evaluate the pharmacological activity of 1, 3-benzoxazin- 4-one derivatives. Totally thirteen compounds, were synthesized by conventional method. Purity of the synthesized compounds was ascertained by TLC and melting point determination by open capillary tube method and they were characterized by IR and NMR spectroscopic methods. Antidepressant activity of all the synthesized compounds was evaluated by despair swim test by using Sprague Dawley Rats. Standard drug Imipramine was used as the control. In the results of the spectral study, all the compounds showed characteristic peak in IR and NMR spectroscopy. In the despair swim test, all the synthesized derivatives showed antidepressant activity. Among them three Compounds (A_4, A_5 and A_7) showed significant antidepressant activity comparing with control drug imipramine. These results are useful for the further investigation in the future.

Keywords: Antidepressant activity, 1, 3-benzoxazin- 4-one, Despair swim test and Sprague Dawley Rat.

1. Introduction

Depression is a serious medical issue characterized by a variety of debilitating symptoms, such as persistent sadness and anxiety, chronic fatigue, feelings of worthlessness, disturbances in cognitive functioning and thoughts and attempts of suicide[1]. Depression has been determined to be the leading cause of disability and the 4th leading contributor to the global burden of disease and is characterized by relapse, recurrence and chronicity[2]. Antidepressants are the drugs used to treat depression thereby elevates mood and modifies the behavior. Half a century ago, antidepressants were discovered by serendipity[3]. Current treatments for depression either fail to produce recovery or induce unwanted side effects. So there is still a large unmet clinical need[4]-[6]. The main aims in the development of new antidepressants were greater efficacy, absence of side effects, lack of toxicity in over dose and earlier onset of action[7]. Elaborate research work has been carried out in the past and continuing in the present to synthesize new compounds to meet this depression. The forced swim test (behavioral despair test) and tail suspension test in the rat are widely used for the initial screening of antidepressants. These tests have good predictive validity and allow rapid and economical detection of substances with potential antidepressant like activity. The tests are base on the same principle: measurement of the duration of immobility when rodents are exposed to an inescapable situation. The majority of clinically used antidepressants decrease the duration of immobility[8].

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1, 3-benzoxazin- 4-one derivatives are considered to be important chemical synthons of various physiological significances and pharmaceutical utilities. They possess a variety of biological activities including antibacterial activities[9], antiplatelet aggregation activity[10], human protease inhibitor activity *in vitro*[11], human leukocyte elastase (HLE) inhibitory activity[12] and acyl-enzyme inhibitors of human chymase[13].

2. Materials and methods

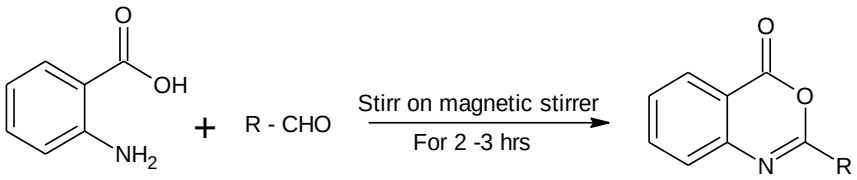
2.1 Chemistry

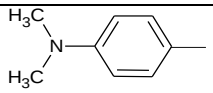
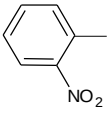
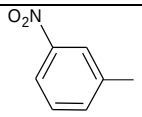
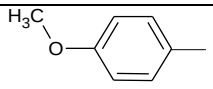
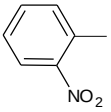
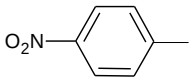
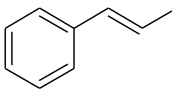
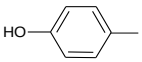
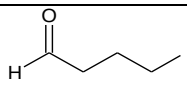
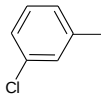
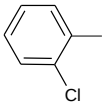
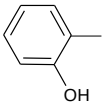
Melting points were determined in open capillary method and are uncorrected. The ^{1}H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spec-trometer model Advance-II (Bruker) using dimethylsulfoxide-*d*6 as solvent and tetramethylsilane as internal standard. IR spectra were recorded on Jasco FT-IR-spectrophotometer using KBr disc method. Antidepressant activity of all the synthesized compounds was evaluated by despair swim test using *Sprague Dawley Rats*. Pharmacological screening values therein were converted into Log (% Inh) were used for multiple correlation analysis with descriptors generated using TSAR 3.3 software.

2.2 Synthesis of 2-substituted 1, 3-benzoxazin- 4-one [A_1 To A_{13}]

Appropriate mixture of anthranilic acid (0.01 mol) and substituted aldehydes (0.01 mol) and 5 ml of ethanol were stirred for 2-3 h (Scheme 1). The completion of reaction was confirmed by TLC. The reaction mixture was transferred into a 500 ml conical flask containing 250 ml ice cold water. The resulting precipitate was collected, dried and recrystallized from hot ethanol. The purity of known compounds was compared with physical constants and spectral data. The characterization data of all chalcones are presented in Table 1 and the spectral data are shown in Table 2.

Scheme 1:



Comp. Code	R	Comp. code	R	Comp. code	R
A_1		A_6		A_{11}	
A_2		A_7		A_{12}	
A_3		A_8		A_{13}	
A_4		A_9			
A_5		A_{10}			

2.3 Pharmacology

Rat-Sprague Dawley (220-255 gm), 8-12 weeks old, was obtained from National Institute of Bioscience, Pune. They were housed in autoclaved polypropylene cages in groups of 2-3 rats per cage and kept in a room maintained at 19 to 25 ^{0} C and humidity 45 to 65 % with a 12-h light/dark cycle. They were allowed to acclimatize for four days before the experiments and were given free access to Standard sterilized extruded rodent diet was

provided *ad libitum*, Reverse Osmosis water treated with UV light was provided *ad libitum* in autoclaved polypropylene bottles and Autoclaved corn cob was used as bedding material.

All procedures of the present study was in accordance with the standard operating procedures of the Prado Pvt. Ltd. guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) as published in The Gazette of India, December 15, 1998. Prior approval of the Institutional Animal Ethics Committee (IAEC) was obtained before initiation of the study (IAEC-13-002).

2.4 Acute oral toxicity study

In the present study acute oral toxicity of the synthesized compounds were performed by OECD guideline for testing of chemicals, No. 423, 'Acute Oral Toxicity – Acute Toxic Class Method'[14-15]. In this method the toxicity of synthesized compounds were tested using a step wise procedure, each step using three rats of single sex (female). The rats were fasted prior to dosing (food but water should be with held) for three to four hours. Following the period of fasting the animal should be weighted and synthesized compounds were administered initially at a dose of 1000 mg/kg (b. w.) and 0.5 % Na CMC and were observed for 14 days for acute toxicity.

2.5 Antidepressant activity (forced swim test in rat)

Behavioral despair or forced swim test (FST) was proposed as a model to test antidepressant activity by Porsolt *et al*[16][17]. It was suggested that mice or rats when forced to swim in restricted space from where they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The behavioral despair test is employed to assess the antidepressant activity of synthesized derivatives. Sprague-Dawley rats of 200-270 gm in a group of two each were used and on the first day of the experiment (pretest session), rats were individually placed in a cylindrical recipient (Plexiglass cylinder) of dimensions (diameter, 10 cm; height, 25 cm) containing 10 cm of water 25°C. The animals were left to swim for 6 min before being removed, dried and returned to their cages. The procedure was repeated 24 h later, in 5 min swim session (test session). The synthesized compounds (25 mg kg-1), and imipramine, as a reference antidepressant drug (25 mg kg-1) were suspended in a 0.5 % aqueous solution of Na CMC (Corboxy Methyl Cellulose). The drugs were given by gavage in a standard volume of 10ml/kg body weight, 1 h prior to the test. Control animals received 0.5 % aqueous solution of Na CMC (Corboxy Methyl Cellulose). This test was performed after 1 hr, 5 hrs and 24 hrs of dose administration. For individual animal video recording was made.Then, the rats were dropped individually into the Plexiglass cylinder and left in the water for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. An immobility time is the time spent by rat floating in water without struggling, making only those moment necessary to keep the head above the water. The total duration of immobility was recorded during the last 5 min of the 6 min test session.

2.6 QSAR methodology

All molecules were drawn in Chem draw ultra 8.0 module in Chemoffice 2004 software and imported into TSAR software. Charges were derived using Charge 2-Derive charges option and optimized by using Cosmic-optimize 3 D option in the structure menu of the project table. Substituents were defined and descriptors were calculated for whole molecule as well as for the Substituents. Several equations were generated correlating both Log (% Immobility) with physicochemical parameters (descriptors) by multiple linear regression analysis (MLR) method. Data was standardized by range and leave one out method was used for cross validation. Models were excluded if correlation was exceeding 0.9 for more rigorous analysis. Correlation matrix was generated to find any Intercorrelation between the descriptors. Intercorrelation between the descriptors in the final equation is less than 0.2.[18]

3. Result and Discussion

3.1 Chemistry

The structures, yields and melting points of the compounds have been given in the (Table 1). Melting points of the synthesized compounds were sharp indicating that the compounds were pure; the yield value of the compounds also suggested that the chemical methods were reliable for the synthesis of the compound. All spectral data were in accordance with assumed structures (Table 2).

Table no. 01: Analytical & physicochemical data of the synthesized compounds (A_1-A_{13})

Comp.	Mol. Formula	Mol. Wt.	M.P. ° C	Yield %	Elemental analyses Calculated		
					C	H	N
A_1	$C_{14}H_{14}N_2O_2$	266	257-258	80	72.11	5.25	10.48
A_2	$C_{15}H_{11}NO_3$	253	235-237	75	71.10	4.32	5.50
A_3	$C_{16}H_{11}NO_2$	249	205-207	67	77.05	4.45	5.62
A_4	$C_{14}H_8ClNO_2$	257	230-232	80	65.26	3.13	5.40
A_5	$C_{14}H_8ClNO_2$	257	232-235	78	65.26	3.13	5.40
A_6	$C_{14}H_8N_2O_4$	268	265-267	69	62.65	3.01	10.40
A_7	$C_{14}H_8ClNO_2$	257	230-233	72	65.26	3.13	5.40
A_8	$C_{14}H_9NO_3$	239	234-238	65	70.25	3.75	5.86
A_9	$C_9H_7NO_2$	161	110-112	71	67.01	4.32	8.65
A_{10}	$C_{14}H_9NO_3$	239	234-236	69	70.25	3.75	5.86
A_{11}	$C_{14}H_8N_2O_4$	268	240-242	62	62.65	3.01	10.40
A_{12}	$C_{14}H_8N_2O_4$	268	262-264	70	62.65	3.01	10.40
A_{13}	$C_{12}H_{11}NO_3$	217	185-187	69	66.30	5.05	6.40

Table no.02 spectral data of the synthesized compounds (A_1-A_{13})

Comp. Code	FT-IR (KBr, cm^{-1})	H^1 NMR ($CDCl_3$,ppm)
A_1	3010.23 (Ar-CH str.),1715.11 (-C=O str.),1500.32 (-C=N str), 1234.36 (-C-N str),	6.4-7.2 (8H phenyl), 3.06 (3H –CH_3),
A_2	3050.23 (Ar-CH str.), 1750.12 (-C=O str.), 1550.32 (-C=N str), 1260.02 (-C-O str).	6.5-7.2 (8H phenyl), 2.82(3H –CH_3).
A_3	1700.11 (-C=O str.), 1640.32 (-C=C str), 3020.23 (Ar-CH str.), 1546.32 (-C=N str),	6.42-7.67 (9H –phenyl), 6.79 (1H ethylene).
A_4	3010.23 (Ar-CH str.), 1710.11 (-C=O str.), 1550.32 (-C=N str), 850.22 (-C-Cl str),	6.6-7.67 (8H –phenyl).
A_5	3020.23 (Ar-CH str.), 1682.11 (-C=O str.), 1555.22 (-C=N str), 830.23(-C-Cl str).	6.6-7.2 (8H –phenyl).
A_6	3012.25 (Ar-CH str.), 1702.21 (-C=O str), 1505.32 (-C=N str), 1255.36 (-N-O str).	6.4-7.2 (8H –phenyl).
A_7	945.20 (-C-Cl str), 3110.23 (Ar-CH str.), 1682.11 (-C=O str.), 1525.32 (-C=N str),	6.6-7.2 (8H –phenyl).
A_8	3650 (-OH str.), 3010.23 (Ar-CH str.), 1750.11 (-C=O str.), 1530.32 (-C=N str),1245.36 (-C-N str).	6.85-8.21(8H phenyl), 9.43 (1H OH)
A_9	3015.23 (Ar-CH str.), 1700.11 (-C=O str.), 1510.32 (-C=N str), 1245.36 (-C-N str),	7.6-8.2 (4H phenyl), 1.16 (3H CH_3),
A_{10}	3570 (-OH str.) 3110.23 (Ar-CH str.), 1650.78 (-C=O str), 1525.32 (-C=N str), 1235.36 (-C-N str)	6.8-7.2 (8H phenyl), 11.82 (1H – OH).
A_{11}	3310.23 (-CH=CH str.), 1515.36 (-N-O str). 3010.23 (Ar-CH str.), 1710.78 (-C=O str),1250.32 (- C-N str), 1525.32 (-C=N str).	6.4-7.2 (8H –phenyl).
A_{12}	3210.23 (-CH=CH str.), 3020.23 (Ar-CH str.), 1750.78 (-C=O str), 1535.32 (-C=N str), 1245.36 (-C-N str),	6.5-8.2 (8H –phenyl).
A_{13}	3110.23 (Ar-CH str.), 2810.23 (-CH_3 str.), 1689.78 (-C=O str), 1525.32 (-C=N str), 1245.36 (-C-N str),	6.8-8.21 (4 H phenyl), 9.72 (1H –CHO), 1.84(2H of CH_2)

3.2 Acute Oral Toxicity Studies

No sign of toxicity observed at 1000 mg/kg b.w. in the experimental animals, the LD50 value of the title compounds expected to exceed 1000 mg/kg b. w. Thus, 25

mg/k.g. b.w. was considered as the dose for the further studies.

3.3 Antidepressant Activity

All the synthesized compounds were subjected to antidepressant activity study on Sprague-Dawley rats by despair swim test. Imipramine was used as standard control. The animals show more stable levels of immobility during the last four minutes of the session. The results showed that all the compounds showed antidepressant activity. Among them three Compounds (A_4, A_5 and A_7) showed significant antidepressant activity comparing with standard control imipramine (Table 03).

Table 03: Antidepressant activities of the compounds

Compound code.	Immobility time			% Immobility		
	1 Hr	5 Hr	24 Hr	1 Hr	5 Hr	24 Hr
A_1	161.5	161.5	171	94.15	88.56	87.82
A_2	157.5	161	168.5	91.56	88.21	86.41
A_3	175	174.5	183.5	101.84	95.58	94.01
A_4	142.5	156	163.5	82.84	85.47	83.84
A_5	144	158.5	163.5	83.72	86.84	83.84
A_6	164	164	173	95.34	89.86	88.71
A_7	145.5	158	167.5	84.59	86.57	85.89
A_8	160.5	163.5	173	93.34	89.90	88.74
A_9	169.5	178.5	184	98.54	97.80	94.35
A_{10}	151.5	156	165	88.08	85.47	84.61
A_{11}	168	169.5	177	97.68	92.09	90.88
A_{12}	166	167.5	174	96.51	91.78	89.23
A_{13}	169	173.5	181	98.25	95.06	92.82
Control	172	182.5	195	100	100	100
Imipramine (std.)	136.5	150.5	154.5	79.41	82.49	79.26

3.4 QSAR

Intercorrelation between the descriptors in the final equations is less than 0.2. Best Equations correlating Log (% Immobility) with descriptors for series (A_1-A_{13}) generated are presented in Table no. 04

Table 04: Equations generated between Log (% Immobility) and descriptors

Sr. No.	Equation	N	S	R	r^2	r^2_{cv}	F
series (A_1-A_{13})	Y = -0.199 *X3 - 0.229 * X1 - 1.553 * X2 - 12.575	13	0.361	0.838	0.702	0.538	14.17

Where

Y = Log (% Immobility)

X1: C log P -

X2 = VAMP HOMO (Whole Molecule)

X3 = Dipole Moment Z Component (Whole Molecule)

X4 = Inertia Moment 2 Length (Whole Molecule)

Significance of the terms –

N= No. of Molecules

s = standard error --- less is better

r = correlation coefficient – higher is better > 0.7,

r^2cv = cross validated r^2 - higher is better > 0.5,

F Value = higher is better

Observed and predicted data and graphs are presented in Table no. 05 and Graph I for Series

Table 05: Observed and predicted log (% Immobility) value data for 13 compounds

Comp. No.	Observed Value	Predicted Value	Residual Value	Residual Variance
A_1	1.97382	1.9695203	0.00069	0.0043
A_2	1.96171	1.9653058	-0.0078	0.0027
A_3	2.00792	1.9989184	0.0009	0.0090
A_4	1.91824	1.9143401	0.0098	0.0009
A_5	1.92283	1.9277292	0.007264	0.0049
A_6	1.97928	1.9869751	-0.0044	0.0070
A_7	1.92732	1.912919	0.001696	0.0044
A_8	1.97007	1.9640678	0.0094	0.0060
A_9	1.99361	1.9949126	-0.0091	0.0313
A_{10}	1.94488	1.9467773	-0.00752	0.0019
A_{11}	1.98981	1.9863057	0.0004	0.0035
A_{12}	1.98457	1.9851723	0.0078	0.0006
A_{13}	1.99233	1.9966326	-0.0042	0.0403

Fig. No 01: Antidepressant-like effects of 1, 3-benzoxazin- 4-one derivatives test compounds in FST Data are presented as compared to control group.(Compounds code Vs Immobility time)

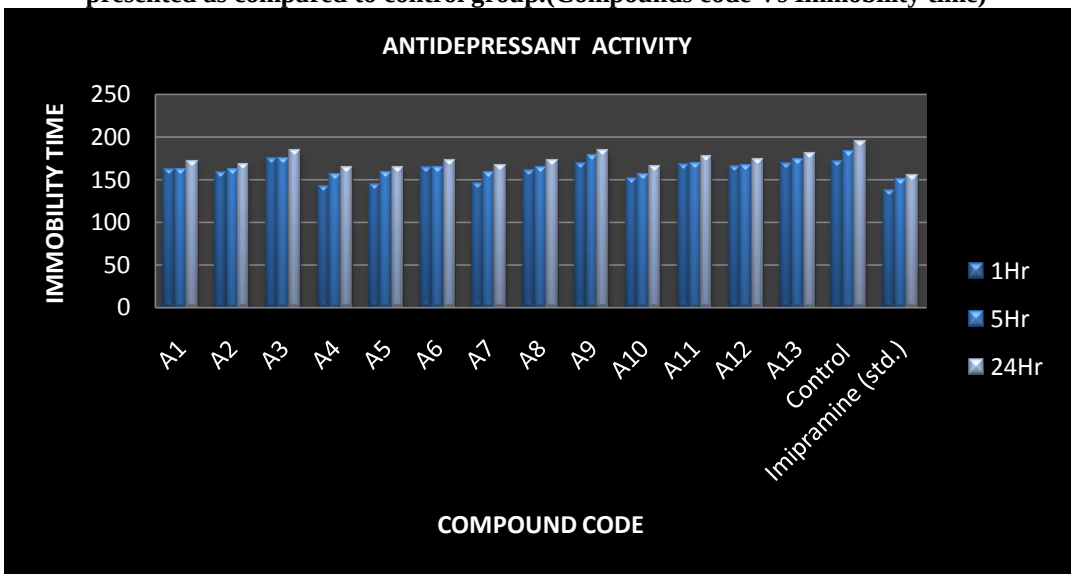


Fig. No 02: Antidepressant-like effects of 1, 3-benzoxazin- 4-one derivatives test compounds in FST Data are presented as compared to control group.(Compounds code Vs % Immobility)

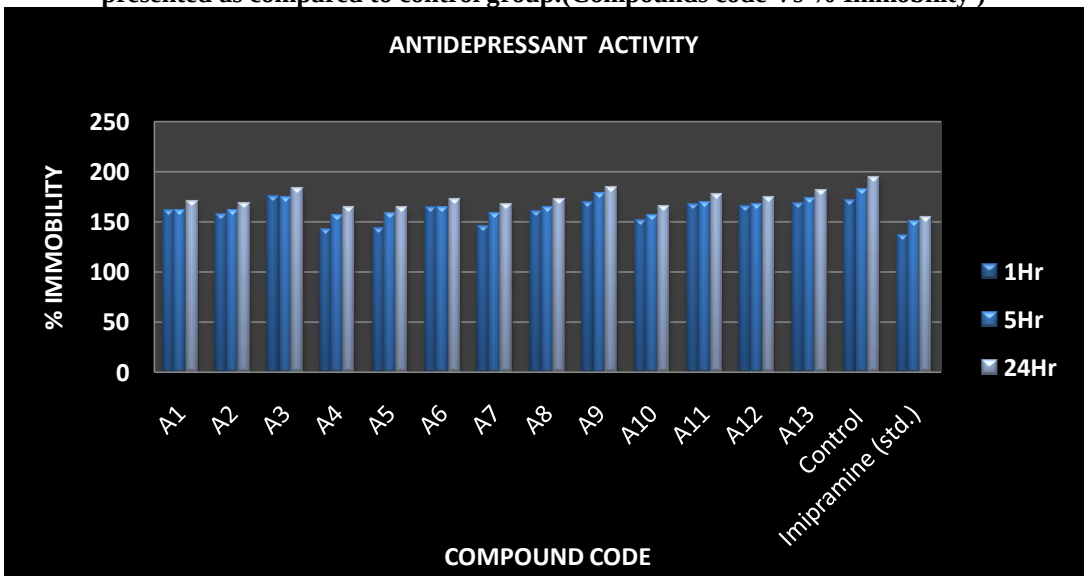
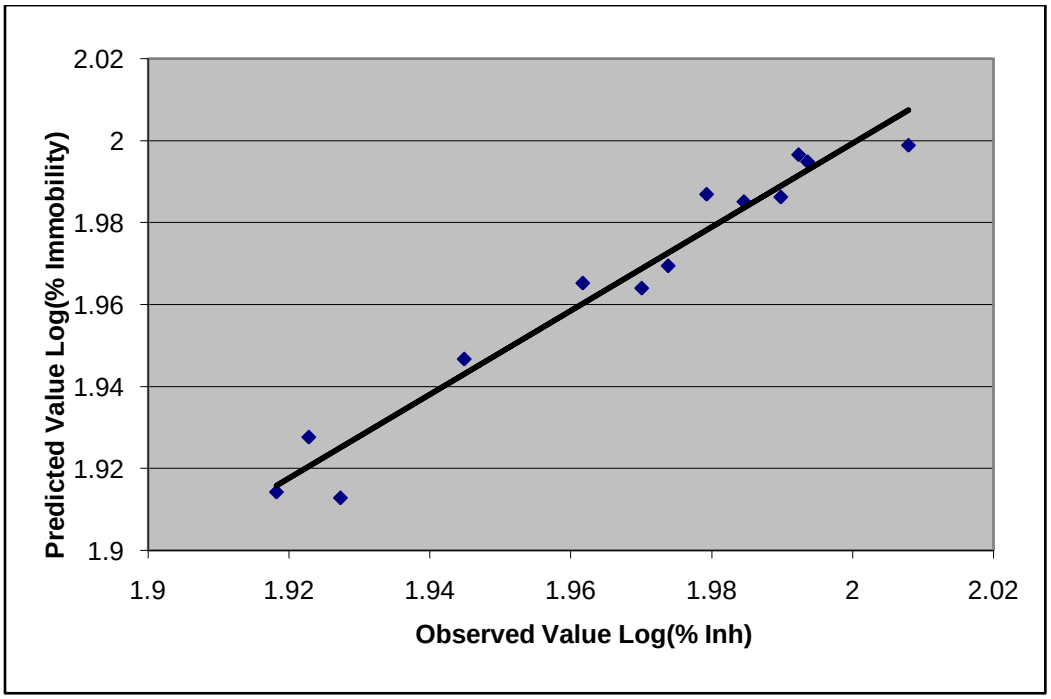
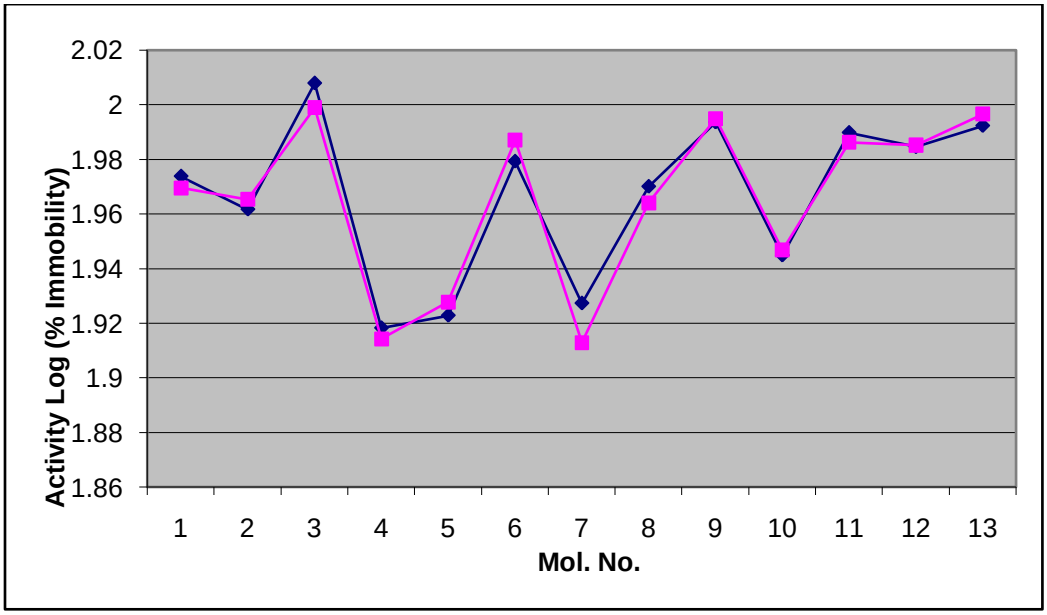


Fig. No. 03: a) Correlation graph and b) Histogram of observed and predicted log (% Immobility) data for 13 compounds

a)



b)



4. Discussion

Statistical evaluation of the equations is in accepted range. The correlation coefficient is high with less standard error. The residual value and residual variance for each series also is less indicating good predictive power of models. From equation it is observed that two electronic parameters Dipole Moment Z Component (Whole Molecule) and VAMP HOMO (Whole Molecule) as well as one steric parameter Inertia Moment 2 Length (Whole Molecule) contribute (-0.227, – 1.469 and – 0.414 respectively) negatively for the activity so electron withdrawing and less bulky groups may enhance the activity (%1 Immobility).

5. Conclusion

We investigated the importance of functional group substitutions, in the structural framework of the compounds for their antidepressant activity. All compounds showed significant antidepressant activity at dose (25 mg/kg). The compounds A_4, A_5 and A_7) showed better activity. Finally, the encouraging result of the antidepressant activity displayed by these compounds may be of interest for further structural modifications to the lead compound and next level studies in the hope of finding a new potent antidepressant prescription.

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