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Design, Synthesis and Evaluation of Anti-Depressant Activity of Some New Oxadiazol derivatives of Phenothiazine

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A B S T R A C T

There has been considerable interest in the development of novel compounds of some new oxadiazole fused Phenothiazine derivatives containing heterocyclic moiety. Many researchers have synthesized these compounds as target structures and evaluated their biological activities. These observations have been guiding for the development of new phenothiazines that possess varied biological activities. The synthesized compounds were tested for their preliminary tests, physical constants, TLC, IR, ^{1}H-NMR Spectra and CHN analysis confirmed the structures of the final compounds. 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 despair swim test, all the synthesized derivatives showed antidepressant activity. Among them three Compounds (A_4, A_5, A_7) showed significant antidepressant activity comparing with control drug imipramine. These results are useful for the further investigation in the future.

Keywords: Antidepressant activities, Despair swim test, Phenothiazine and *Sprague Dawley Rats.*

A R T I C L E I N F O

CONTENTS

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1. Introduction

Depression is a common but serious illness [1]. It is common among women and people with other chronic conditions. Left untreated, depression may disrupt work, family, and personal life [2]. According to World Health Organization (WHO) it is

one of the top 10 cause of morbidity and mortality. Even after vast research in this field, the therapeutic remedy remains unsatisfactory [3]. This is because the only 65% of the depressive patient responds to the current anti-depressant therapeutics [4] and to achieve clinical benefit it takes several weeks which could be a reason to worry as depression tends to increase the risk of suicide in advanced stage [5]. 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) in the rat is widely used for the initial screening of antidepressants. This test has good predictive validity and allows rapid and economical detection of substances with potential antidepressant like activity. The tests are based on the same principle: measurement of the duration of immobility when rodents are exposed to an inescapable situation. The majority of clinically used antidepressants decreases the duration of immobility [6].

2. Materials and Methods

Chemistry: The chemicals which are used in this study were supplied by E. Merck and LOBA Co. All the reactions were monitored by TLC using silica gel G. The melting point determinations were done by using in open glass capillary using Kjeldahl flask containing liquid paraffin. IR spectra were recorded on the (JASCO) FTIR-Spectrophotometer using KBr pellets. ^{1}HNMR spectra were recorded on BRUKERAVANCE II 400 NMR spectrometer in DMSO using tetra methyl silane (TMS) as internal reference.

Synthesis Of Phenothiazine derivatives

Step I: General procedure for the preparation of 7, 8 or 9 substituted aniline Benzoic acid derivatives:

Equimolar amount of substituted aniline was added to a chloro benzoic acid in 20 mL of DMF and 0.1 percent of potassium hydroxide solution and the reaction mixture was heated under refluxed at about 80°C temperature, for 2 h. TLC indicated the end of reaction. The mixture was cooled by addition of a water/ice mixture. The solid was filtered in excellent yield. [I]

Step II: General procedure for the preparation of 7, 8 or 9 substituted 10H-phenothiazine 1 carboxylic acid derivatives: Equimolar amount of 7, 8 or 9 substituted Anilino Benzoic acid was added to a solution of sulfur powder and iodine in 5 ml of ethanol. Reaction mixture was heated under reflux with stirring for about 2h and poured into ice/water mixture. The precipitate was filtered and washed with cold water. [II]

Step III] Synthesis of derivatives of ethyl 10H-phenothiazine-1-carboxylate:

0.01 mole of 10H-phenothiazine-1-carboxylicacid was reflux with conc. H_2SO_4 using ethanol as solvent for 1 hour in 250ml RBF. After which the resulting reaction mixture was kept in ice cold water.[III]

Step IV: Synthesis of derivatives of 10H-phenothiazine-1-carbohydrazide: 0.01mole of compound A was reflux with 3-4 ml of hydrazine hydrate for 1 hour. After which the resulting reaction mixture was allow to cool in ice bath. The resulting precipitate was collected and dried. (IV)

Step V: Synthesis of derivatives of N'-benzylidene-10H-phenothiazine-1-carbohydrazide:

0.01mole of compound B was reflux with 0.01mole of substituted aromatic aldehyde for 1 hour. After which the resulting reaction mixture was allow to cool in ice bath. The resulting precipitate was collected and dried and recrystallized from ethanol. (V)

Step VI: Synthesis of derivatives of (1,3,4-oxadiazol-3(2H)-yl)(10H-phenothiazin-1-yl)Methanone:

0.01 mole of compound C was reflux with 0.01 mole of substituted aromatic acid and phosphorous trichloride for 2 hour. After which the resulting reaction mixture was allow to cool in ice bath. The resulting precipitate was collected and dried and recrystallized from ethanol. (VI).

Pharmacology:

Rat-*Sprague Dawley* (220-255 gm), 8-12 weeks old, were 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-13-003).

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. (1977)[7-10]. 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 (Plexiglas scylinder) 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 (Carboxy Methyl Cellulose).The drugs were given by gavagein a standard volume of 10 ml/kg body weight, 1 h prior to the test. Control animals received 0.5 % aqueous solution of Na CMC (Carboxy 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 mice 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 [12,13].

QSAR methodology

All molecules were drawn in Chem draw ultra 8.0 module in Chem office 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 Inter-correlation between the descriptors. Inter-correlation between the descriptors in the final equation is less than 0.2

3. Results and Discussions

The structures, yields and melting points of the compounds have been given in the (Table 2). 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 structure. 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. (Table 3).

Spectral Data of the Synthesized Compounds (A_1-A_{12})

A_1: IR :3023.10 (Ar-CH str.), 1611.15 (-C=O str.),1532.11 (-C=N str.), 1236.60 (-C-N str.), 3223.50 (-N-H str.), 1321.21 (-C-O str.).

^{1}HNMR:CH 6.61 1.50 methine, CH 7.41 6.91 phenothiazine,CH 7.95 7.62 benzylidenimin, CH 7.38 7.26 1-benzene

A_2: IR : 3033.50 (Ar-CH str.),1615.11 (-C=O str.),1532.20 (-C=N str.), 1236.40 (-C-N str.), 3225.40 (-N-H str.), 1325.42 (-C-O str.).

^{1}HNMR: CH 6.61 1.50 methine, CH 7.06 7.29 benzylidenimin, CH 7.11 7.26 1-benzene, CH3 3.83 0.86 methyl

A_3: IR :822.50 (-C-Cl str.), 3023.15 (Ar-CH str.), 1678.11 (-C=O str.), 1518.32 (-C=N str.), 1256.36 (-C-N str.), 3255.23 (-N-H str.), 1360.32 (-C-O str.).

^{1}HNMR:CH 6.6 1.50 methine, CH 7.41 6.91 phenothiazine, 0.25 1 -C(=O)N from 1-benzene, CH 7.02 7.29 benzylidenimin

A_4: IR : 3110.25 (Ar-CH str.), 848.23(-C-Cl str.), 1625.12 (-C=O str.), 1492.2 (-C=N str.), 1260.16 (-C-N str), 3280.23 (-N-H str.), 1340.33 (-C-O str.).

^{1}HNMR:CH 6.61 1.50 methine, CH 7.00 6.99 phenothiazine, CH_2 2.56 1.37 methylene, CH 7.40 7.26 1-benzene

A_5: IR:1255.36 (-N-O str.),3110.23 (Ar-CH str.),1615.11 (-C=O str.),1510.32 (-C=N str.), 1260.36 (-C-N str.),3250.23 (-N-H str.), 1360 (-N-O str.),1320.32 (-C-O str.).

^{1}HNMR: CH 6.61 1.50 methine, CH 8.19 7.26 1-benzene, CH 7.52 7.29 benzylidenimin, CH 7.42 6.91 phenothiazine

A_6: IR:3056.23 (Ar-CH str.),1615.11 (-C=O str.),1520.32 (-C=N str.), 1254.36 (-C-N str.),
3260.23 (-N-H str.), 1329.32 (-C-O str.),965.20(-C-Cl str.).

^{1}HNMR: CH 7.06 7.29 benzylidenimin, CH 7.73 7.26 1-benzene, CH 7.41 6.91 phenothiazine, OH 9.68 5.00 aromatic C-OH

A_7: IR:3658.21 (-OH str.), 3010.23 (Ar-CH str.), 1615.11 (-C=O str.), 1520.32 (-C=N str.), 1255.36 (-C-N str.), 3250.23 (-N-H str.), 1320.32 (-C-O str.).

^{1}HNMR: CH 6.01 1.50 methine, CH 8.09 7.62 benzylidenimin, CH 7.41 6.91 phenothiazine, CH 7.24 7.26 1-benzene

A_8: IR:3050.23 (Ar-CH str.), 3608.23 (-OH str.), 1715.11 (-C=O str.), 1510.32 (-C=N str.), 1258.36 (-C-N str.), 3275.23 (-N-H str.), 1320.32 (-C-O str.).

^{1}HNMR:CH_3 3.83 methyl, 7.62 1-benzene, 6.61 methine, 7.00 phenothiazine,

A_9: IR :3017.23 (Ar-CH str.), 1690.11 (-C=O str.), 1540.32 (-C=N str.), 1260.36 (-C-N str.), 1340 (-N-O str.), 3259.23 (-N-H str.), 1335.32 (-C-O str.).

^{1}HNMR:CH_3 2.34 methyl, 8.54 pyridine,6.70 phenothiazine, 7.76 benzylidenimin, 7.06 benzylidenimin

A_{10}: IR: 3018.23 (Ar-CH str.),1685.11 (-C=O str.),1510.32 (-C=N str.), 1320 (-N-O str.), 1250.36 (-C-N str.),3250.23 (-N-H str.), 1320.32 (-C-O str.).

^{1}HNMR: 9.43 aromatic C-OH, 6.68 1-benzene, CH 7.41 phenothiazine, 7.62 benzylidenimin

A_{11}:IR :3120.28 (Ar-CH str.),830.24(-C-Cl str.),1635.12 (-C=O str.),1590.2 (-C=N str.), 1270.16 (-C-N str),3270.25 (-N-H str.), 1340.35 (-C-O str.).

^{1}HNMR:6.94 1-benzene, 7.96 benzylidenimin, 9.43 aromatic C-OH, 6.61 methine

A_{12}:IR: 1613.05 (-C=O str.),1533.20 (-C=N str.), 1336.40 (-C-N str.),3331.50 (-N-H str.), 1432.20 (-C-O str.),920.45 (-C-OH str.).

^{1}HNMR: 9.43 aromatic C-OH, 3.06 methyl, 7.68 phenothiazine, 7.36 1- benzene.

QSAR:

Intercorrelation between the descriptors in the final equations is less than 0.2. Best Equations correlating Log (% Inh) with descriptors for series (A_1-A_{12}) generated are presented in Table 04

Discussion:

Statistical evaluation of the equations is in accepted range. The correlation coefficient is high with less standard error. The residual value 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). Synthetic procedure and clinical applications of Phenothiazines for the treatment of several diseases were critically discussed.

Scheme:

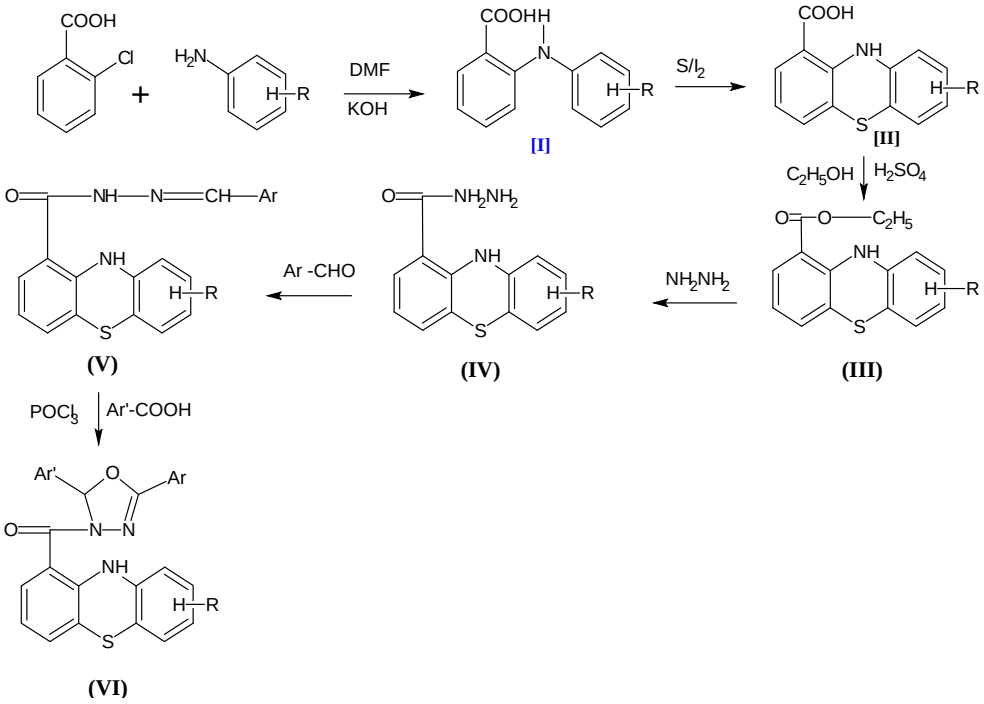
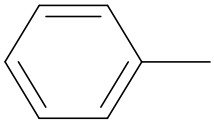
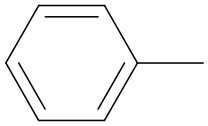
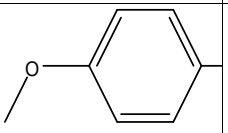
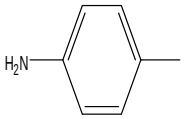
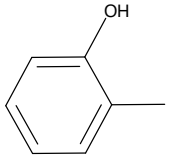
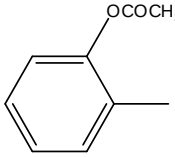
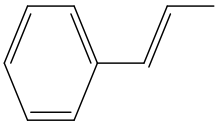
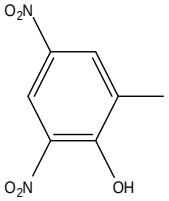
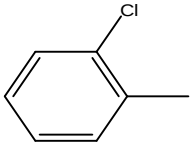
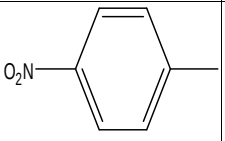
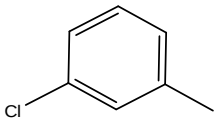
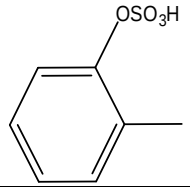
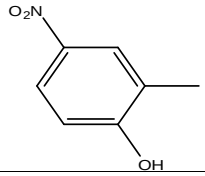
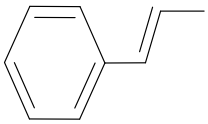
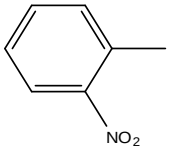
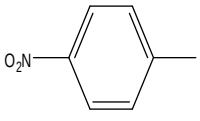
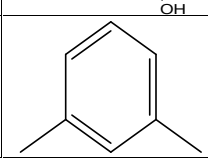
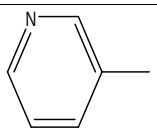
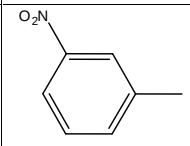
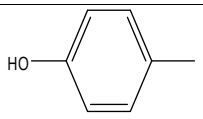


Table 1: For Compounds A_1-A_{12}

Comp. Code	Ar	Ar'	R	Comp. Code	Ar	Ar'	R
A_1			**NO_2**	**A_2**			**Cl**
A_3			**Cl**	**A_4**			**Cl**
A_5			**NO_2**	**A_6**			**NO_2**
A_7			**Cl**	**A_8**			**Br**
A_9			**Cl**	**A_{10}**			**NO_2**

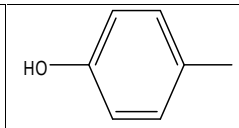
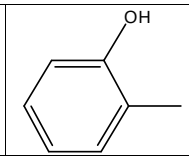
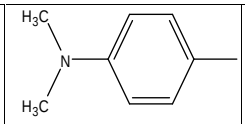
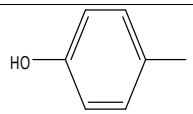
A_{11}			**NO_2**	**A_{12}**			**NO_2**

Table 02: Analytical & Physicochemical data of the synthesized compounds (A_1-A_{12})

Comp.	Mol. Formula	Mol. Wt.	M.P. ° C	Yield %	Elemental analyses Calculated		
					C	H	N
A_1	$C_{27}H_{18}N_4O_4S$	494.52	242-246	61	65.58	3.67	11.37
A_2	$C_{28}H_{21}ClN_4O_3S$	529.00	230-235	56	63.57	4.00	10.59
A_3	$C_{29}H_{20}ClN_3O_5S$	558.00	263-265	62	62.42	3.61	7.53
A_4	$C_{29}H_{20}ClN_5O_7S$	618.01	265-270	59	56.36	3.26	11.33
A_5	$C_{27}H_{16}ClN_5O_6S$	573.96	252-255	63	56.50	2.81	12.20
A_6	$C_{20}H_{20}ClN_3O_7S_2$	610.05	265-270	49	55.13	3.30	6.89
A_7	$C_{29}H_{18}Cl_2N_4O_4S$	589.44	220-225	58	59.09	3.08	9.50
A_8	$C_{28}H_{19}ClN_4O_5S$	558.99	245-250	60	60.16	3.43	10.02
A_9	$C_{27}H_{18}Cl_2N_4O_2S$	533.42	252-255	57	60.79	3.40	10.50
A_{10}	$C_{27}H_{18}N_4O_5S$	510.52	260-265	63	63.52	3.55	10.97
A_{11}	$C_{27}H_{17}N_5O_8S$	571.51	253-257	55	56.74	3.00	12.25
A_{12}	$C_{29}H_{23}N_5O_5S$	553.58	235-242	51	62.92	4.19	12.62

Table 03: Antidepressant Activities of the Compounds(A_1-A_{12})

Compound code.	Immobility time (sec.)			% Immobility		
	1 Hr	5 Hr	24 Hr	1 Hr	5 Hr	24 Hr
A_1	150.5	161	163.5	86.49	86.79	84.27
A_2	164.5	170	165.5	94.54	91.64	85.30
A_3	158.5	162.5	171	91.09	87.60	88.14
A_4	161.5	162	173	92.81	87.33	89.17
A_5	159.5	160	168.5	91.66	86.25	86.85
A_6	151.5	158	164.5	87.06	85.17	84.79
A_7	140	151.5	163.5	80.45	81.67	84.27
A_8	139	146	154.5	79.88	78.70	79.63
A_9	158	161	168.5	90.80	86.79	86.85
A_{10}	147	151.5	156	84.48	81.67	80.41
A_{11}	156.5	163	174.5	89.94	87.87	89.94
A_{12}	160.5	166	172.5	92.24	89.48	88.91
Control	174	185.5	194	100	100	100
Imipramine std.	136.5	150.5	154.5	79.41	82.49	79.26

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

Sr. No.	Equation	N	S	r	r^2	r^2_{cv}	F
series (A_1-A_{12})	Y = -0.199 *X3 - 0.229 * X1 - 1.553 * X2 - 12.575	12	0.009498	0.9636	0.9285	0.9214	1.15

Table 05: Observed and predicted log (% Inh) value data for 20 compounds

Comp. No.	Observed Value	Predicted Value	Residual Value
A_1	1.936966	1.941866	-0.0049
A_2	1.975616	1.974813	0.000803
A_3	1.959471	1.962781	-0.00331
A_4	1.967595	1.962121	0.005474
A_5	1.96218	1.95981	0.00237
A_6	1.939819	1.931564	0.008255
A_7	1.905526	1.919658	-0.01413
A_8	1.902438	1.899654	0.002784
A_9	1.958086	1.951069	0.007017

A_{10}	1.926754	1.919258	0.007496
A_{11}	1.953953	1.949246	0.004707
A_{12}	1.964919	1.959664	0.005255

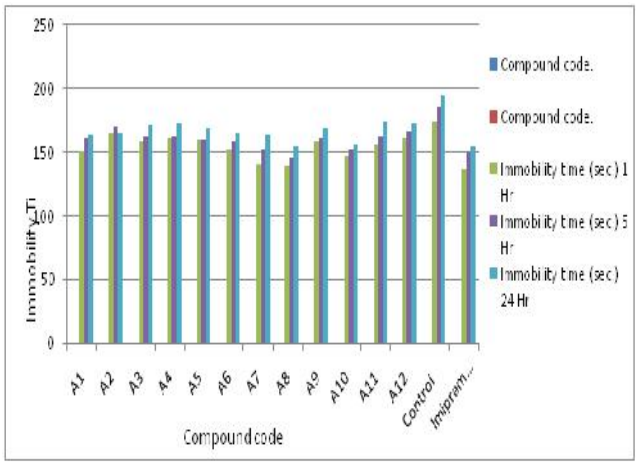


Figure 1: Antidepressant-like effects of Phenothiazine derivatives test compounds in FST Data are presented as compared to control group.(Compounds code Vs Immobility time).

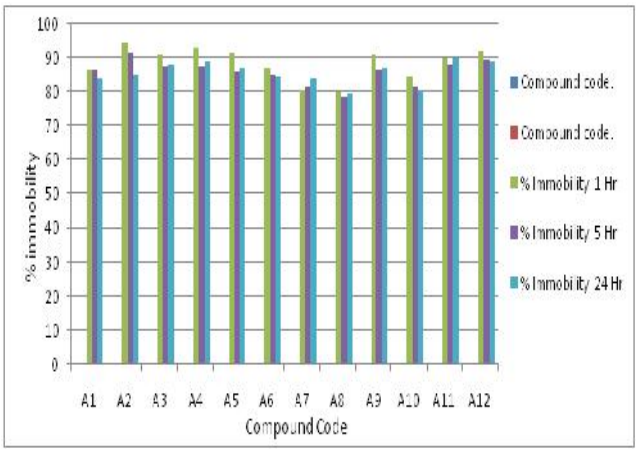


Figure 2: Antidepressant-like effects of Phenothiazine derivatives test compounds in FST Data are presented as compared to control group.(Compounds code Vs % Immobility).

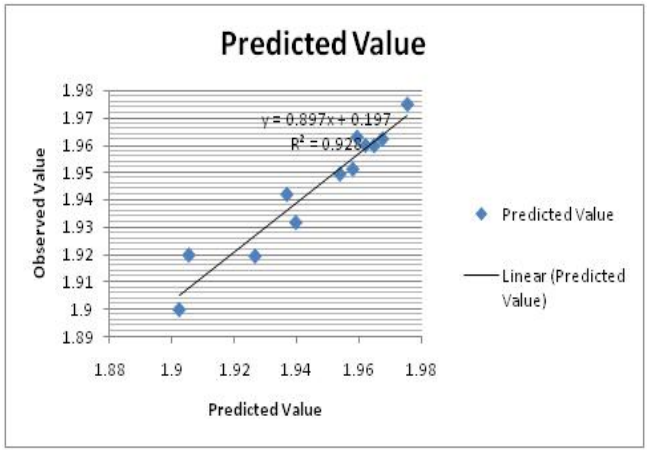


Figure 3: a) Correlation graph

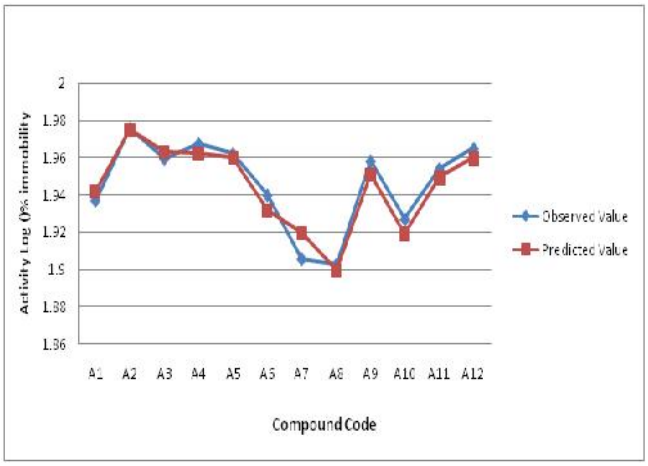


Figure 3: b) Histogram of observed and predicted log (% Immobility) data for 12 compounds

4. 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,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. From these studies, it is clear that further works needed to be done in the future for the development of clinically useful agents.

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