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Synthesis, Characterization, Molecular docking and Anti-anxiety Evaluation of Some Novel Phenothiazine Derivatives

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ABSTRACT

The phenothiazine derivatives 1-(10H-phenothiazin-10-yl)-2-(4-(1-(phenylimino)ethyl) phenoxy)ethan-1-one (4a-4j) are produced from 2-(4-acetylphenoxy)-1-(10H-phenothiazin-10-yl) ethan-1-one (3) and after that, condensing them with various carbonyl compounds. Acetonitrile was used as solvent. The purity of the analogues and reaction progress were identified through their retention factor value and melting point. Characterization of the prepared analogues was completed via performing their Infra-red, proton-nuclear magnetic resonance spectroscopy with their elemental analysis. The set of molecular docking parameters of the compounds were assessed to check to their potentiality. Autodock Vina 1.2.0 was used to dock the derivatives and the docking score of all the synthesized derivatives ranges from -8.7 to -10.2. Investigation of anti-anxiety activity on albino wistar rat, was executed for all the prepared phenothiazine analogues. EPM model was approached for performing anti-anxiety study, taking Diazepam as standard drug. The compounds 2-(4-(1-((3-nitrophenyl) imino)ethyl)phenoxy)-1-(10H-phenothiazin-10-yl)ethan-1-one (4e) and 2-(4-(1-((3,4-dinitrophenyl)imino)ethyl)phenoxy)-1-(10H-phenothiazin-10-yl)ethan-1-one (4g) were showed maximum potency among all the prepared derivatives as compared to Diazepam.

Keywords: Computational Studies, Elevated Plus Maze model, Molecular docking, Phenothiazine, Schiff Bases.

INTRODUCTION

Present scenario of the world reveals that the anxiety is a communal human emotion that includes behavioral, affective, and cognitive reactions to perceived threat. Occasionally, anxiety encourages a proactive and receptive reaction to stressful situations. When anxiety is strong, it destabilizes the person and interferes with his daily tasks. When anxiety is out of proportion to the difficulty and manifests itself in the absence of stress, it is deemed unhealthy. Obsessive-compulsive disorder, generalized anxiety disorder, post-traumatic stress disorder, anxiety disorders and frights are a few examples of the numerous types of anxiety disorders[1]. Due to its great prevalence, anxiety affects one-eighth of the world's population and has grown to be a very important topic of

research focus in psychopharmacology[2]. Despite having negative side effects such skeletal muscle relaxation, drowsiness, physical dependence, and cognitive impairment, benzodiazepines are still the most commonly used medications to treat generalized anxiety disorder[3].

Heterocyclic compounds having nitrogen and sulphur atom shows various biological activities because the presence of these functional groups makes these compounds more active and potent[4]. Phenothiazine comes under the category of thiazine class of heterocyclic compound and its structure comprised of two benzene rings linked together in a tricyclic system with a sulphur and a nitrogen atom[5]. Various substitutions on phenothiazine nucleus produced a variety of derivatives with potential therapeutic effects[6]. The neuroleptic effects of phenothiazine derivatives are their most intriguing pharmacological feature[7]. Phenothiazine provides a wide range of medicinal benefits like anti-inflammatory activity[8], bactericidal activity[9], anti-depressant activity[10], anti-psychotropic activity[11], anti-tumour activity[12], anti-viral activity[13], anti-cancer activity[14], anti-tubercular activity[15] and utilized extensively throughout the globe today due to its potential therapeutic activity. The basic nucleus of phenothiazine is shown in Figure 1.

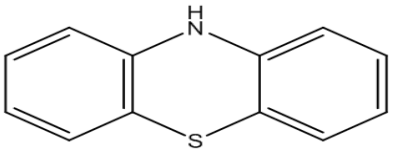


Fig. 1. Basic Nucleus of Phenothiazine

Hence from the above information, phenothiazine as heterocyclic moiety and evaluating them for anxiety disorder was selected for this research work. The new phenothiazine derivatives as Schiff bases was synthesized and characterized by spectroscopic methods. Molecular docking studies were done to assess the potentiality of the compounds.

MATERIAL AND METHODS

Reagents and Materials

Reagents were obtained from CDH & S.D. Fine Chemicals of laboratory grade (India). The melting point was calculated using open tube capillary method and was uncorrected. For calculating Rf value, silica gel G was employed for coating on glass slide and performing the chromatography in TLC chamber. While refluxing, the progress of the reactions was tracked on the basis of TLC and iodine chamber was employed for visualization of the spots. Solubility was checked in different solvents benzene, ethanol, methanol, chloroform, dimethyl sulfoxide, dimethyl formamide, acetone and acetonitrile. FT-IR spectrophotometer was used for FT-IR analysis of the derivatives. ^{1}HNMR spectroscopy was performed on Bruker Advance Neo NMR spectrometer at 500MHz frequency from SAIF, Punjab University, India.

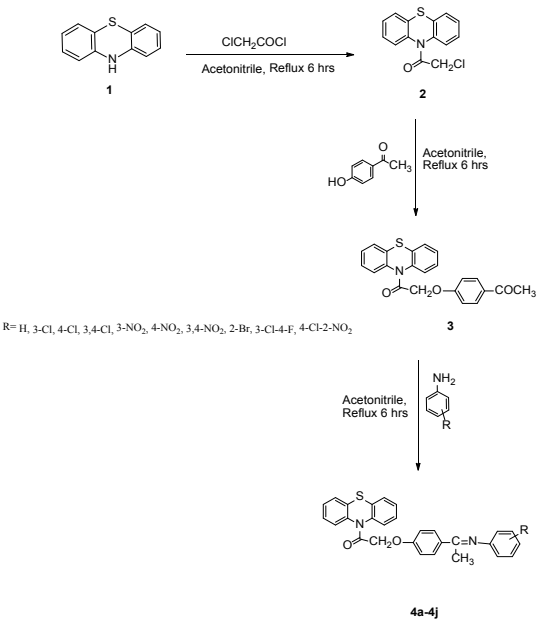
General procedure

Synthesis of Compound 2

In 100 mL anhydrous acetonitrile, equal moles of phenothiazine and chloroacetyl chloride were dissolved and transferred to a 250 mL RBF (round bottom flask). Further anhydrous K_2CO_3 (0.02 mol) were added to the flask and refluxed for 6 hours. The flask's content was cooled and filtered. Solvent was drained away by applying decreased pressure to obtain the end product. End product was recrystallized by ethanol[16].

Synthesis of Compound (3)-Compound 2 (0.01 mol) and p-hydroxy acetophenone (0.01 mol) were dissolved in 100 mL anhydrous acetonitrile and (0.02 mol) anhydrous potassium carbonate were placed in a RBF & refluxed for 7 hours. The flask's content was cooled and filtered. Solvent was drained away by applying decreased compression to get the end product. Ethanol was used to recrystallize the end product[17,18].

Methods for the preparation of Compounds (4a-4j).-In a 250 mL RBF, (0.01 mol) compound 3 and substituted anilines (0.01 mol) were taken and dissolved in 100 mL anhydrous acetonitrile and to this flask, (0.02 mol) anhydrous K_2CO_3 was added and refluxed for 7-8 hours. After 8 h the flask's content was cooled and filtered. The solvent was drained away by applying decreased pressure to get the end product. Ethanol was used to recrystallize the end product[19,20].



Scheme 1. Formation of Phenothiazine Derivatives

Spectral Characterization

Compound (4a)-IR (KBr) cm^{-1}: 3235 (stretch, N-H amide), 3036 (stretch, C-CH, Ar), 3026 (stretch, C-H Ar), 2562 (stretch C-S Ar), 1739 (stretch, C=O Amide), 1637 (stretch, nitrile), 1508 (stretch, C-C, Ar), 1441 (stretch, C=C Ar), 1274 (stretch, C-N Ar), 1086 (stretch, C-O), 713 (bend, C-H Ar) 617 (stretch, C-Cl Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.5 (s, 1H, C=CH), 7.8-6.4 (t, 7H, Ar-H), 4.3 (s, 2H, CH_3). m.p. 150°C, R_f value 0.52.

Compound (4b)-IR (KBr, cm^{-1}): 3434 (stretch, N-H amide), 2923 (stretch, C-H Ar), 2592 (stretch, C-S Ar), 1632 (stretch, C=O Amide), 1463 (stretch, C=C Ar), 1412 (stretch, C-C), 1255 (stretch, C-N Ar), 1093 (stretch, C-O),672 (bend, C-H Ar), 648 (stretch, C-Cl Ar). ^{1}H-NMR (500 MHz; DMSO d_6),δ (ppm): 8.5 (s, 1H, C=CH), 7.0-5.3 (t, 7H, Ar-H), 4.5 (s, 1H, CH_3). M.P. 102°C, Rf value 0.81.

Compound (4c)-IR (KBr) cm^{-1}: 3420 (stretch, N-H Amide), 1737 (stretch, C=O), 1634 (stretch, nitrile), 1519 (stretch, C-C Ar), 1467 (stretch, C=C Ar), 1355 (stretch, C-C Ar), 1061 (stretch, C-O), 833 (bend, C-H Ar), 738 (stretch, C-Cl Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.7 -8.4 (d, 3H C=CH), 8.1- 6.5 (m, 8H, Ar-H) 2.4 (s, 2H, Cl-CH) m.p. 180°C, R_f value 0.66.

Compound (4d)-IR (KBr) cm^{-1}: 3433 (stretch, N-H amide), 2927 (stretch, C-H), 1634 (stretch, C=N), 1630 (stretch, C=O amide), 1473 (stretch, C-C Ar), 1438 (stretch, C=C), 1297 (stretch, C-N Ar), 1030 (stretch, C-O), 812 (stretch, C-Cl Ar), 734 (bend, C-H Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.5 (s, 1H, C=CH), 7.1 -6.4 (d, 11H, Ar-H), 5.5 (s, 4H, O=CNH) M.P. 142 oC, Rf value 0.57.

Compound (4e)-IR (KBr) cm^{-1}: 3235 (stretch, N-H amide), 687 (bend, C-H Ar), 1032 (stretch, C-O Ar), 1230 (stretch, C-N Ar), 1574 (stretch, NO_2), 1618(stretch, C=C Ar), 1638 (stretch, C=O), 2596 (stretch, C-S Ar), 1509 (stretch, C-C Ar), 3015 (stretch, C-H Ar),1740 (stretch, C=N Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.6 (s, 2H, C=CH), 7.6-6.0 (m, 17H, Ar-H) 2.5 (s, 3H, C=CH). m.p. 160°C, R_f value 0.61.

Compound (4f)-IR (KBr) cm^{-1}: 3435 (stretch, N-H amide), 3029 (stretch, C-H Ar), 2588 (stretch, C-S Ar), 1638 (stretch, C=O), 1620 (stretch, C=C Ar), 1575 (stretch, NO_2), 1475 (stretch, C-C Ar), 1238 (stretch, C-N), 1038 (stretch, C-O Ar), 690 (bend, C-H Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.4 (s, 2H, C=CH), 7.5- 6.0 (m, 17H, Ar-OH) 2.4 (s, 2H, Ar-CH) M.P. 150°C, Rf value 0.91.

Compound (4g)-IR (KBr) cm^{-1}: 3427 (stretch, N-H amide), 3040 (stretch, C-H Ar), 2592 (stretch, C-S Ar), 2140 (stretch, C-CH), 1640 (stretch, C=O amide), 1530 (stretch, NO_2), 1460 (stretch, C=C Ar),1240 (stretch, C-N), 797 (bend, C-H Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.5 (s, 2H, C=CH). 6.9-6.5 (m, 4H, Ar-H), 4.4 (s, 3H, C-NH) M.P. 180 oC, Rf value 0.68.

Compound (4h)-IR (KBr) cm^{-1}: 3235 (stretch, N-H amide), 3094 (stretch, C-H Ar), 2581(stretch, C-S Ar), 1740 (stretch, C=N), 1640 (stretch, C=O), 1568(stretch, C-C Ar), 1305 (stretch, C-N), 1029 (stretch, C-O), 892 (bend, C-H), 622 (stretch, C-Br). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.6 (s, 1H, C=CH), 7.7- 6.7 (m, 14H, Ar-H), 2.3 (s, 3H, Br-CH) m.p. 165°C, R_f value 0.82.

Compound (4i)-IR (KBr) cm^{-1}: 3434 (stretch, N-H amide), 2923 (stretch, C-H Ar), 2592 (stretch, C-S Ar), 1738 (stretch, C=N), 1632 (stretch, C=O Amide), 1512 (stretch, C-C), 1463 (stretch, C=C Ar),1255 (stretch, C-N Ar), 1234 (stretch C-O Ar), 1009 (stretch, C-F), 681 (bend, C-H Ar), 648 (stretch, C-Cl). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.5 (s, 1H, C=CH), 7.9- 6.5 (m, 4H, Ar-H), 5.2 (s, 4H, O-C-H) 4.4 (s, 1H, C-NH) m.p. 170°C, R_f value 0.72.

Compound (4j)-IR (KBr) cm^{-1}: 3435 (stretch, N-H amide), 1741 (stretch C=O amide), 1634 (C=C), 1506 (stretch, nitrile), 1442 (stretch C-C Ar), 1375 (stretch, NO_2), 1283 (stretch C-N), 1116 (stretch, C-O), 954 (bend C-H Ar), 741 (stretch, C-Cl Ar). ^{1}H-NMR (500 MHz; DMSO d_6), δ (ppm): 8.5 (s, 1H, C=CH) 8.3 (s, 1H, C=CH), 7.9- 6.4 (m, 11H, Ar-H), 4.4 (s, 1H, Cl- CH), m.p. 120°C, R_f value 0.71.

Molecular docking

Investigation of molecular docking was performed on Autodock Vina v.1.2.0 (The Scripps Research Institute) docking software[21,22]. Samson platform by OneAngstrom, 2022 was utilized for visualizing and computing protein ligand interaction. For predicting receptor site on the ligand, MOE (Molecular Operating Environment) site finder[23] which works on geometric technique to compute possible binding site in a protein with the help of their 3D structure. This MOE model runs on the basis of alpha spheres which is a simplification of convex keels[24]. The structure of proteins was prepared on default parameters of MOE Quick prep's. All the ligands were converted into mol2 extension file using Chem3D 12.0 software. For performing docking experiment in Autodock Vina v.1.2.0, all the ligands were set to lessen the pre-set of 1000 steps (N=1000, M=25 and Et=0.05 kcal/mol), where N is the maximum number of minimization steps, M is consecutive minimization steps, and Et is the energy difference between steps is less than the threshold. For docking study[26], crystal structure of Human synaptic GABAA receptor (PDB ID: 6D6U)[25] was acquainted from protein data bank and used. Active binding site predicted by MOE, a search domain box and center coordinates were prepared with the dimension of 147.0x141.0x138.7 and 73.5x 34.7x 66.9, respectively. Unit for all these dimensions was Angstrom. While search parameters were used, the binding modes was set to 10, exhaustiveness was set to 32 and average energy difference was 3 kcal/mol. The results of docking were saved for further computational analysis.

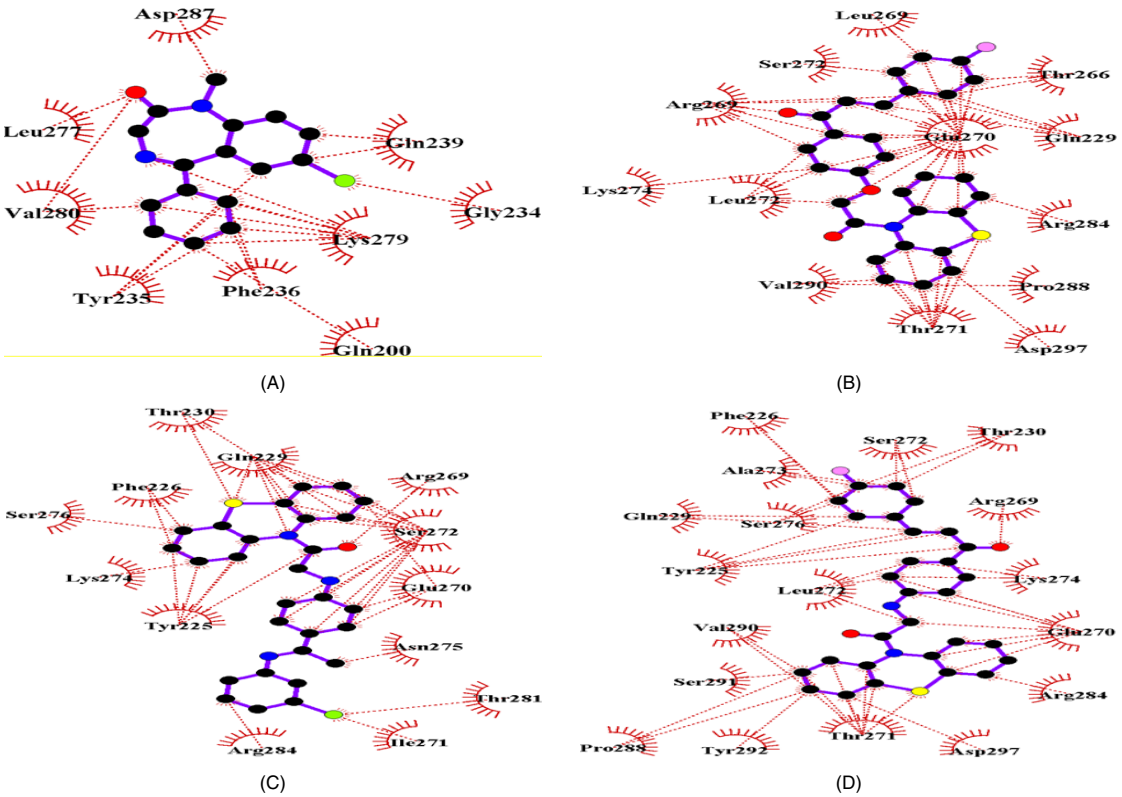


Fig. 2. Ligplot showing the interaction of (A) Diazepam and (B,C,D) Phenothiazine Derivatives (4b, 4e, 4g) with GABAA receptor (PDB: 6D6U). Purple lines-phenothiazine structure ligand bond, Black circles-carbon atoms, Blue circle-nitrogen atoms, Pink circle-fluorine atoms, Red circle-oxygen atoms, Green circles-chlorine atom, Yellow circles-sulphur atom, Red dotted lines-hydrophobic interactions, Radial lines-non-ligand residues involved in hydrophobic contacts

RESULTS AND DISCUSSION

A series of novel phenothiazine analogues (Scheme I) 1-(10H-phenothiazin-10-yl)-2-((4-(1-(phenylimino)ethyl) phenyl)amino)ethan-1-one (4a-4j) were prepared. The prepared derivatives, 4a-4j were synthesized in the form of Schiff bases. Formation of Schiff bases includes the reaction between 2-(4-acetylphenoxy)-1-(10H-phenothiazin-10-yl)ethan-1-one (3) and substituted aniline using acetonitrile as solvent. All these derivatives monitored by checking their melting point, solubility, Rf value, colour, IR, and NMR spectroscopy. In computational studies, a set of physicochemical parameters like Log P, M. W., HBD, HBA, TPSA and MTI were calculated. Prepared derivatives has TPSA value within the range of 41.9-145.52 which exhibits that these are potent effectively. The analytical data correlates and confirms the structures of the derivatives. The log P value showed lipophilicity of the test compounds within the range of 5.83-7.28 which represented that these derivatives have a good potency. The docking study was performed on Autodock Vina software and receptor configuration was procured from protein data bank PDB: 6D6U was employed to dock the prepared derivatives for evaluating their potency as anxiolytic agent. The docking score and physicochemical parameters of prepared derivatives and Diazepam are listed in Table 2. The lowest docking score suggested the highest binding affinity towards the receptor. Compound 4e and 4g was exhibited the lowest docking score and hence they are better analogues to inhibit anxiety among all the prepared analogues. While the moderate active analogues are 4c, 4d and 4i that shows binding energy -9.8, -9.7, -9.5 respectively. The docking image of the potent derivatives is shown in figure 2. Ligplot Fig. 2 showed hydrogen bond interactions of diazepam and the prepared derivatives (4b, 4e, 4g) with Asp287, Leu277, Val280, Gln239, Tyr235, Lys279, Phe236, Gly234, Leu269, Ser272, Arg269, Lys274, Leu272, Arg284, Thr266, Gln270, Gln299, Asp297, Pro288, Thr230, Phe226, Ser276, Gln229, Arg269, Ser272, Glu270, Tyr225, Lys274, Asp275, Arg284, Ile271, Thr281, Val290 and Ser291 amino acid residues.

Anti-Anxiety Activity

Wistar albino rats were taken from the animal house of IFTM University. The rats have free access to food and water, with an average weight of 150–200 g. Animals were boarded in a temperature -controlled room at 25±2°C. Each compound's concentration (i.p., 5 mg/kg) was employed in freshly made suspensions in 1% tween 80. On test day, each solution was freshly made and administered intra-peritoneally in a dose of 0.5 mL of rat body's mass. The test drugs (i.p., 5 mg/kg) and Diazepam (2 mg/kg) were administered to experimental animals 60 minutes before to their evaluation. Normal saline (1% tween 80) was given to the control group (n=6). The elevated plus maze device had an open canopy facing each other, two arms are open and two are closed[27]. At 25 cm height the instrument is raised, evaluation time for each test group treated rat is five minutes at once and they are kept in the center of the platform facing towards the open arm. During this five minutes period, the total no. of entries in open & closed arms and total time spent in the open arm was noted. The proportion of entries of each mouse in open arms was calculated by (open arm entries/ total time spent) x 100. The summary of the Elevated plus maze (EPM) results is depicted in Table 1.

Table 1: Anti-anxiety activity of the derivatives

Compound Code	Consumed time(open arm)	Number of Entry(open arm)	% No. of entrances (open arm)
4a	36.62±1.70	8.31±0.60	36.94
4b	39.11±2.23	3.17±0.66	33.66
4c	58.93±1.34	2.56±0.26	54.61
4d	59.66±2.18	3.28±0.69	55.94
4e	60.06±0.65	7.23±0.37	59.00
4f	41.17±1.25	5.00±1.76	39.61
4g	71.73±0.02	6.59± 0.28	64.94
4h	51.10±1.32	6.78±0.56	47.44
4i	42.87±0.61	11.21±0.87	37.28
4j	43.23±1.78	5.07±0.37	41.61
Diazepam	90.73±2.45	11.87±0.76	66.46
Vehicle	41.15±4.22	3.24±0.81	21.24

Table 2: Physicochemical parameters & Docking score of derivatives and diazepam

S. No.	Comp. Code	MW[a]	Log P[b]	MR[c]	ASA[d]Å^2	TPSA[e]Å^2	MTI[f]	WI[g]	Ov[h]	HBA[i]	HBD[j]	nRB[k]	Docking score
1	4a	450.55	6.04	131.51	672.57	41.9	27802	3636	1.60	3	0	6	-8.9
2	4b	484.10	6.66	136.11	702.28	41.9	29392	3972	1.60	3	0	6	-9.5
3	4c	484.10	6.66	136.10	758.30	41.9	29519	3999	1.65	3	0	6	-9.3
4	4d	519.44	7.28	140.73	722.54	41.9	31115	4338	1.62	3	0	6	-9.7
5	4e	496.13	5.93	129.06	709.10	93.71	34284	4714	1.61	4	0	7	-9.9
6	4f	495.55	5.93	131.06	717.64	93.71	34773	4795	1.62	4	0	7	-9.4
7	4g	540.55	5.83	132.67	734.31	145.52	41427	5912	1.63	5	0	8	-10.2
8	4h	528.05	6.83	139.23	685.34	41.9	29265	3945	1.59	3	0	6	-9.3
9	4i	502.99	6.82	136.52	709.45	41.9	31115	4338	1.61	4	0	6	-9.8
10	4j	529.99	6.55	135.55	714.09	93.71	35557	5010	1.61	4	0	7	-9.3
11	Diazepam	284.74	2.84	80.88	475.24	32.67	5393	726	1.421	2	0	1	-7.8

[a] Molecular weight [e]Topological Surface Area [i]Hydrogen Bond Donor [b]Log P [f]Molecular topological index [j]Hydrogen Bond Acceptor [c]Molecular refractivity [g]Wiener Index [k]No. of Rotatable Bonds [d]Accessible Surface Area [h]Ovality

CONCLUSION

The study focused on the synthesis and anti-anxiety investigation of ten phenothiazine derivatives having different substituents on different position of phenyl ring. This study provides a simple and efficient method to produce new phenothiazine derivatives. For the anti-anxiety investigation, Elevated plus maze method was used taking Diazepam as reference drug. The prepared phenothiazine derivatives showed promising activity against anxiety. Ten derivatives of phenothiazine were prepared. In these derivatives, the analogues that nitro substituent on ortho and para position showed highest potency towards the inhibition of anxiety. In conclusion of this study it is found that nitro analogues leads to more effective than other analogues. The chloro analogues at ortho & para position and fluoro at ortho position found moderate active against anxiety. The analogue that has simple aniline and methoxy group at meta position was found to be less active as compared to others. The docking score of the derivatives revealed a very good binding affinity towards the GABAA receptor. Characterization of the compounds was done by evaluating their melting point, R_f value, solubility, spectral data using infra-red spectroscopy and nuclear magnetic resonance spectroscopy. this research work conclude that, these analogues are possibly active compounds beneficial to treat fretfulness and nervousness up to some extent, which can prompt further modification to produce more efficient derivatives.

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Conflict of interest

The author declare that we have no conflict of interest.

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