RESEARCH ARTICLE

Computational Studies and Synthesis of New Heterocyclics as CNS Agents

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ARTICLE HISTORY

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Abstract: ***Aim*:** This research work aimed to design and synthesize some new molecules of phenothiazine. The work's emphasis was on forming new phenothiazines in two series, 1-(10H-phenothiazin-10-yl)-2-((4-(1-(phenylimino)ethyl)phenyl)amino)ethan-1-one derivatives (4a-4j) and 1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)-3-phenylprop-2-en-1-one derivatives (P1-P5).

***Methods*:** Chloroacetylation of phenothiazine was done to afford 2-chloro-1-(10H-phenothiazin-10-yl)ethan-1-one, which was further reacted with 4-amino acetophenone to produce 2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one. Then, it was treated with substituted anilines and substituted benzaldehydes to produce the final derivatives 4a-4j and P1-P5, respectively.

***Results*:** All 15 derivatives (4a-4j and P1-P5) were characterized by evaluating their R*f* value, melting point, solubility, IR spectroscopy, and ^{1}HNMR spectroscopy. Molecular docking was performed by using AutoDock Vina v.1.2.0 (The Scripps Research Institute, La Jolla, CA, USA) docking software, and the anxiolytic activity of the derivatives was assessed by using the elevated plus maze model.

***Conclusion*:** The designed scheme was executed in the departmental laboratory. The chemical structure of the compounds was confirmed on the basis of TLC, IR, and ^{1}HNMR analyses. The docking study revealed a good docking score of the compounds. The Log P value of the compounds indicated their good penetration into CNS. The compounds were also screened for anxiolytic activity. Among them, compounds 4f, 4h, and P3 showed maximum activity as anti-anxiolytic agents.

Keywords: Heterocyclic agents, anxiolytic activity, molecular docking, docking score, diazepam, aromatic molecules.

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1. INTRODUCTION

Research on the chemistry of aromatic molecules containing nitrogen-sulfur heteroatoms is gaining popularity. Phenothiazines and related substances have demonstrated a variety of biological activities, such as sedative, anti-inflammatory, antimalarial, anti-psychotropic, anti-tubercular, antimicrobial, antitumor, and stimulation of the penetration of anticancer agents through the blood-brain barrier [2]. These substances have been reported to bind to physiological targets or receptors, leading to a variety of potential action mechanisms. However, solid tumours of the stomach and brain are typically resistant to chemotherapy. Due to their accessibility and low cost, phenothiazines have also been investigated as potential anti-anxiety medications [3].

Due to their potency, compounds containing nitrogen and sulphur as heteroatoms are a wide area of investigation. The fundamental idea is to combine two or more moieties to create a novel chemical entity and discover a fresh biologically active substance [4]. These days, pharmacophores with heteroatoms in their structures are the most captivating. Sulfur and nitrogen are found in heterocyclic compounds with a wide range of biological activity, and nitrogen has a significant position among the numerous heterocyclic derivatives [5]. For instance, the tricyclic hetero aromatic chemical phenothiazine was created from the mother compound 10H-dibenzo-1,4-thiazine, which Bernthsen produced for the first time in 1883 [1].

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2. EXPERIMENTAL

2.1. Materials and Methods

All of the chemicals were purchased from CDH and Fine Chemicals, and were of laboratory grade. Thin-layer chromatography (TLC) of all the prepared derivatives was performed to check the reaction progress during the laboratory work. Silica gel G was used to make TLC plates and spots were seen in the iodine chamber. The open capillary method was performed in the melting point apparatus to determine the melting point of prepared derivatives. Benzene, ethanol, chloroform, acetone, methanol, acetonitrile, ethyl methyl ketone, and di-methyl sulfoxide were used to check the solubility. FT-IR spectroscopy of all the derivatives was performed using the FT-IR spectrophotometer of the Central Instrumentation Facility Lab, Punjab University, India. At Punjab University, 1H-NMR spectroscopy was performed using the Bruker Avance Neo Spectrophotometer at a frequency of 500MHz.

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2.1.1. Synthetic Procedure

Scheme **1** shows the synthesis of phenothiazine derivatives in two parts, *i.e*., the formation of Schiff bases and the formation of chalcones. The synthetic procedure is as follows:

2.1.1.1. Step 1- Synthesis of Compound 2 (2-chloro-1-(10H-phenothiazin-10-yl)ethan-1-one)

Procedure- In a 250 ml RBF, 0.01 mole of phenothiazine and 0.01 mole of chloro acetyl chloride were taken, and to them, 100 ml anhydrous acetonitrile was added, and the flask was shaken to dissolve the solid. K_2CO_3 (0.02 mole) was added, and then this mixture was allowed to reflux for 6 hours. After 6 hours, the content was cooled and filtered. The solvent was evaporated by a vacuum pump to obtain the crude product. The obtained product was recrystallized by ethanol.

2.1.1.2. Step 2- Synthesis of Compound 3 (2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one)

Procedure- A 250 ml round bottom flask was taken, and to it, 2-chloro-1-(10H-phenothiazin-10-yl)ethan-1-one (0.01 mole) and p-amino acetophenone (0.01 mole) dissolved in 100 ml of anhydrous acetonitrile and anhydrous K_2CO_3 (0.02 mole) were added. The mixture of the reaction was then refluxed for six hours. After cooling and filtering, the substance was subjected to vacuum evaporation to remove the solvent. Ethanol was used to recrystallize the end product [6-8].

2.1.1.3. Step 3- General Procedure for the Synthesis of Compound 4a-4j (1-(10H-phenothiazin-10-yl)-2-((4-(1-(phenylimino)ethyl)phenyl)amino)ethan-1-one)

Procedure - In a 250 ml round bottom flask, 2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one (0.01 mole) and substituted anilines (0.01 mole) were dissolved in 100 ml anhydrous acetonitrile, and to this flask, anhydrous potassium carbonate (0.02 mole) was added. For 7-8 hours, this mixture was refluxed. After cooling and filtering, the substance was subjected to vacuum evaporation to remove the solvent. Ethanol was used to recrystallize the end product [9].

2.1.1.4. Step 4- General Procedure for the Synthesis of Compound P1-P5 (1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)-3-phenylprop-2-en-1-one)

Procedure- In a beaker, 2-((4-acetylphenyl)amino))-1-(10H-phenothiazin-10-yl)ethan-1-one (2 gm), substituted aldehyde (2 gm), and 50 ml of ethanol were taken. On a magnetic stirrer, this mixture was stirred, and 20 ml of 10% NaOH solution was gradually added. For 3–4 hours, stirring was done. After the formation of the solid, ice-cold water was poured into the beaker and it was then filtered. The separated solid was washed with cold water. The solid was then left overnight in a cool place to produce the dry product [10, 11].

2.2. Molecular Docking Studies

Molecular docking analysis was performed using Autodock Vina v.1.2.0 (The Scripps Research Institute, La Jolla, CA, USA) docking software [12, 13] on the Samson [14] platform by OneAngstrom 2022 for the visualisation and calculation of protein-ligand interactions. The receptor site was predicted using the MOE Site Finder program [15], which uses a geometric approach to calculate putative binding sites in a protein, starting from its tri-dimensional structure. This method is not based on energy models but on alpha spheres, which are a generalisation of convex hulls [16]. The protein structure was prepared with MOE Quick Prep using default program settings. Before the experiment, all ligands were converted to *.mol2 structure format using the Chem3D software.

Further, all ligands were set to minimise the preset of 1000 steps (N = 1000, M= 25, and Et = 0.05 kcal/mol, where N is the maximum number of minimisation steps, M is consecutive minimisation steps, and Et is the energy difference between steps being less than the threshold) before docking experiment was performed using Autodock Vina v.1.2.0. The crystal structure of human synaptic GABA-A receptor (PDB: 6D6U) [17] was retrieved from the Protein Data Bank, as done in the previous docking study [18], and utilised to perform docking simulations. The search domain box centre and size coordinates were 147.0 x 141.0 x 138.7 and 73.5 x 34.7 x 66.9 around the active binding site, as predicted by MOE. All coordinates used Angstrom units. The search parameters were used where the number of binding modes was 10, exhaustiveness was 32, and the maximum energy difference was 3 kcal/mol. After the docking experiment was run on Autodock Vina v.1.2.0 on the Samson platform by OneAngstrom 2022, the results were saved with further computational analysis. Various physicochemical parameters of test derivatives and standard drug diazepam were calculated by using Chem Draw ultra 12.0 and Chem 3D 12.0. Log P, MW, molar refractivity, MTI, ovality, nRB, and TPSA were the major parameters of computational studies. Fig. (**1**) shows the structure of the human synaptic GABAA receptor.

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2.3. Pharmacological Evaluation

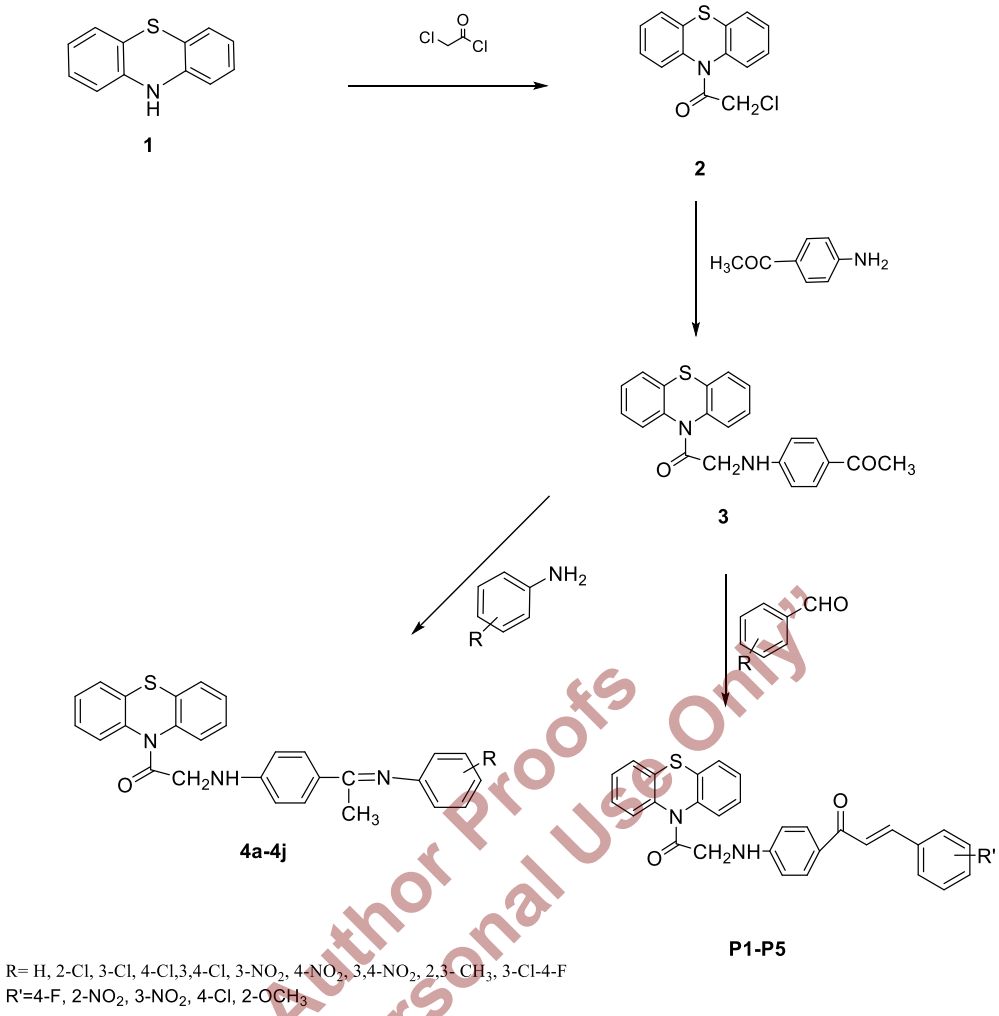
2.3.1. Experimental Animals

From the animal house of IFTM University, Moradabad, India, adult Wistar albino rats of either sex (150–200 g) were taken. They were kept in groups in cages made of polypropylene that measured 11 cm x 17 cm x 28 cm, with wood shavings used as bedding and under-regulated lighting and temperature regimes (25 ± 3°C). Food and water were freely available to the mice. The institutional animal ethical committee gave proper approval for the experimental animal protocols.

2.3.2. Evaluation of Anxiolytic Activity

2.3.2.1. Elevated Plus Maze Model

On the test day, all derivatives with a concentration of 5mg/kg were made in the suspension of 1% tween 80 and administered intraperitoneally at a dose of 0.2 ml of the mouse's body weight. Suspending agent, *i.e*., 1% tween 80 with normal saline, was given to the control group [19, 20]. Diazepam (2mg/kg, i.p.) was used as a standard anxiolytic agent. The device was made up of two open arms (50 x 10 cm)



Scheme **1**. Synthesis of phenothiazine derivatives.

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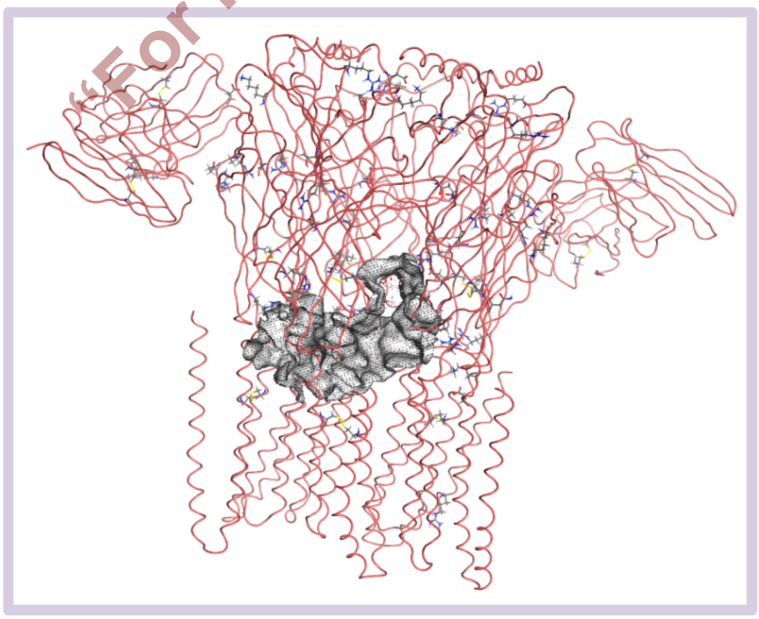

Fig. (1). The structure of human synaptic GABA-A receptor PDB ID: 6D6U; binding site in black carbon colour. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Docking score and physicochemical parameters of the derivatives.

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	449.15	5.80	13.67	730.55	44.7	27968	3636	1.62	3	1	6	-9.0
2	4b	484.01	6.42	6.82	669.36	44.7	29440	3945	1.59	3	1	6	-9.4
3	4c	483.11	6.42	14.16	697.47	44.7	29568	3972	1.60	3	1	6	-9.8
4	4d	483.11	6.42	14.16	698.45	44.7	29696	3999	1.63	3	1	6	-9.3
5	4e	518.45	7.04	14.65	717.58	44.7	31302	4338	1.60	3	1	6	-9.8
6	4f	494.14	5.69	14.28	707.78	96.51	34482	4714	1.61	4	1	7	-9.9
7	4g	495.57	5.70	14.28	713.67	96.51	34974	4795	1.63	4	1	7	-9.8
8	4h	539.12	5.59	14.89	734.94	148.32	41660	5912	1.62	5	1	8	-10.1
9	4i	477.18	6.46	14.59	702.78	44.7	33005	4284	1.59	3	1	6	-9.3
10	4j	502.00	6.58	14.17	702.37	44.7	31302	4338	1.60	4	1	6	-9.8
11	P1	480.13	6.34	14.27	683.39	49.41	32814	4448	1.62	4	1	7	-9.8
12	P2	507.56	6.08	14.86	702.26	101.22	37499	5142	1.60	4	1	8	-9.8
13	P3	504.56	6.08	14.86	721.82	101.22	38007	5226	1.63	4	1	8	-10.2
14	P4	496.10	6.80	14.74	714.04	49.41	32814	4448	1.61	3	1	7	-9.8
15	P5	492.15	6.08	14.87	695.70	58.64	35785	4766	1.59	4	1	8	-9.2
16	Diazepam	284.74	2.84	80.88	475.24	32.67	5393	726	1.421	2	0	1	-7.8

Note: [a]Molecular weight, [b]Log P, [c]Molecular refractivity, [d]Accessible surface area, [e]Topological surface area, [f]Molecular topological index, [g]Wiener index, [h]Ovality, [i]Hydrogen bond donor, [j]Hydrogen bond acceptor, [k]No. of rotatable bonds

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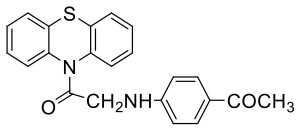
and two enclosed arms (50 x 10 cm) with a 40 cm high wall. The arms of the same kind were positioned opposite to one another, and a central square of 10 cm was used to create a plus sign. The wooden device was supported by a single central support, which raised it 50 cm off the ground. The animal was positioned on the maze's middle platform, facing an open arm. The maze was meticulously cleaned in between subjects and a standard 5-min test period was applied. The frequency and duration of arm visits, separately for open and closed arms, were recorded. Formula (open arm entries/total time spent) x 100 was used to compute the percentage of each mouse's entries that were made with open arms.

2.4. Chemistry

2.4.1. Spectral Data of the Compounds

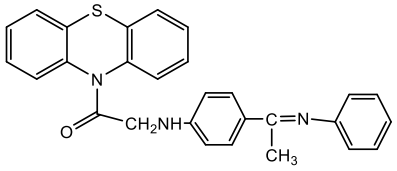
Compound 3



2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl) ethan-1-one

IR (KBr, cm^{-1}): 3226 (str, C-H Ar), 2553 (str, C-S Ar), 1739 (str, C=O Ali), 1636 (str, C=C Ali), 1638 (str, N-H Ali). 1470 (bend, C-H Ali), 1440 (str, C=C Ar), 1279 (str, CN Ali), 1214 (str, C-O Ali), 1033 (str, C-N Ar), 680 (bend, C-H Ar), ^{1}H NMR (500 MHz; DMSO) δ: 8.6 (s, 1H, C=CH), 7.7 (d, 2H, Ar-OH), 6.9 (m,4H, Ar-H), 6.7 (m, 6H, Ar-H), 2.4 (d, 3H, C=CH).

Compound 4a



1-(10H-phenothiazin-10-yl)-2-((4-(1-(phenylimino)ethyl) phenyl)amino)ethan-1-one

IR (KBr, cm^{-1}): 3421 (str, C-N Ar), 3226 (str, N-H Ali), 2165 (str, C=N Ali), 2105 (str, C-C Ali), 1739 (str, C=O Ali), 1738 (str, C=C Ali), 1636 (str, C-C Ar), 1440

Table 2. Evaluation of anxiolytic activity.

Compound Code	Consumed Time (Open Arm)	Number of Entries (Open Arm)	% Number of Entries (Open Arm)
4a	70.73±0.03	7.61± 0.17	65.89
4b	60.53±1.56	4.19±0.38	59.49
4c	61.39±2.01	2.60±0.37	59.89
4d	59.26±0.56	8.61±1.31	60.15
4e	40.58±1.35	3.71±0.86	45.76
4f	54.17±1.75	6.11±0.98	49.46
4g	41.51±1.67	7.13±0.67	41.49
4h	59.23±2.23	7.11±0.87	50.44
4i	54.12±0.85	9.77±1.55	43.28
4j	54.32±2.00	4.13±0.73	51.16
P1	70.56±2.89	7.33±0.85	63.18
P2	63.92±3.25	6.89±1.85	52.58
P3	72.52±1.20	6.62±1.53	66.16
P4	53.43±3.11	7.46±2.76	57.87
P5	69.93±2.62	5.56±0.69	59.21
Diazepam	91.87±3.54	10.97±0.67	65.46
Vehicle	41.15±4.22	3.24±0.81	21.24

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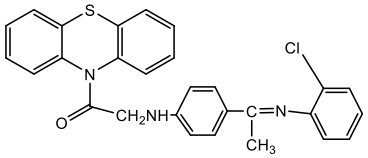
(str, C=C Ar), 958 (bend, C-H Ar), 521 (bend, C-H Ali),. ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.6 (d, 2H, C=CNH), 7.0 (m, 2H, C=CH), 6.8 (d, 2H, Ar-H), 6.7 (m, 4H, Ar-H), 6.5 (d, 2H, Ar-H), 6.0 (s, 2H, O=CNH), 2.4 (s, 1H, C-NH).

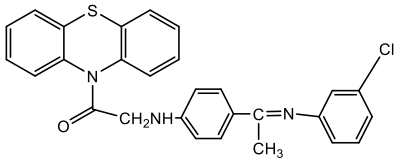
Compound 4b



2-((4-(1-((2-chlorophenyl)imino)ethyl)phenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

IR (KBr, cm^{-1}): 3217 (str, C-H Ar), 2162 (str, C=N Ali), 2126 (str, C-N Ar), 1738 (str, C-O Ar), 1639 (str, C-C Ali), 1635 (str, C=O Ali), 1637 (str, C-C Ar), 1499 (str, C-H Ali), 1480 (str, N-H Ali), 1439 (str, C=C Ar), 1213 (str, C-N Ali), 730 (str, C-Cl), 609 (bend, C-H Ar). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.9 (m, 2H, Ar-H), 6.8 (m, 4H, Ar-H), 6.5 (s,1H, Ar-H), 2.4 (s, 1H, Ar-CH).

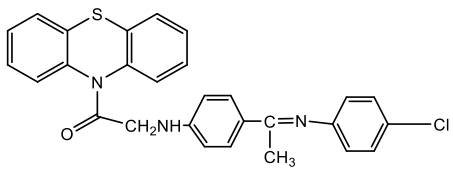
Compound 4c

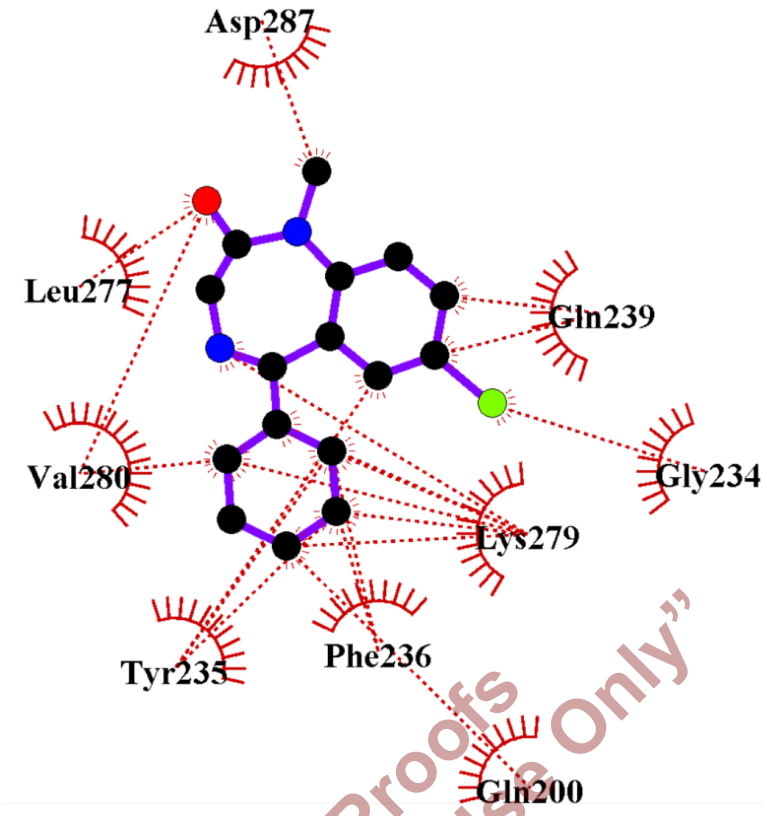


2-((4-(1-((3-chlorophenyl)imino)ethyl)phenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

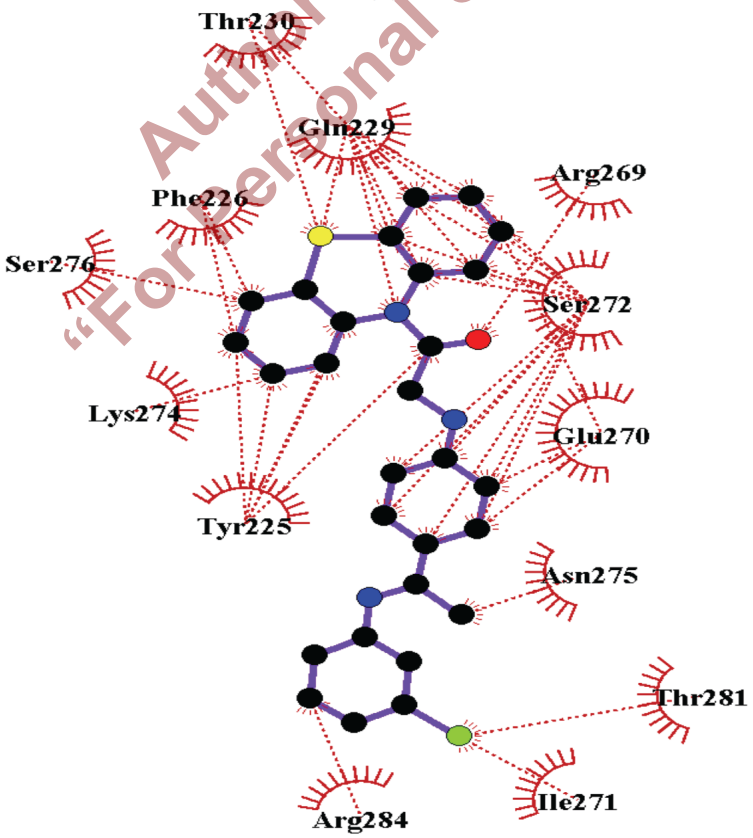
IR (KBr, cm^{-1}): 3217 (str, C-H Ar), 2162 (str, C=N Ali), 2126 (str, C-N Ar), 1738 (str, C-O Ar), 1635 (str, C-C Ar), 1639 (str, C-C Ali), 1635 (str, C=O Ali), 1499 (str, C-H Ali),1480 (str, N-H Ali), 1439 (str, C=C Ar), 1213 (str, C-N Ali), 730 (str, C-Cl), 609 (bend, C-H Ar). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.6 (d, 2H, Ar-H), 7.0 (m, 5H, C=CH), 6.9 (d, 5H, Ar-H), 6.8 (m, 5H Ar-H), 6.6 (d, 2H Ar-H), 6.0 (s, 2H, C=CH), 2.3 (s, 3H, O=CH).

Compound 4d





(A)



(B)

Fig. (2). contd….

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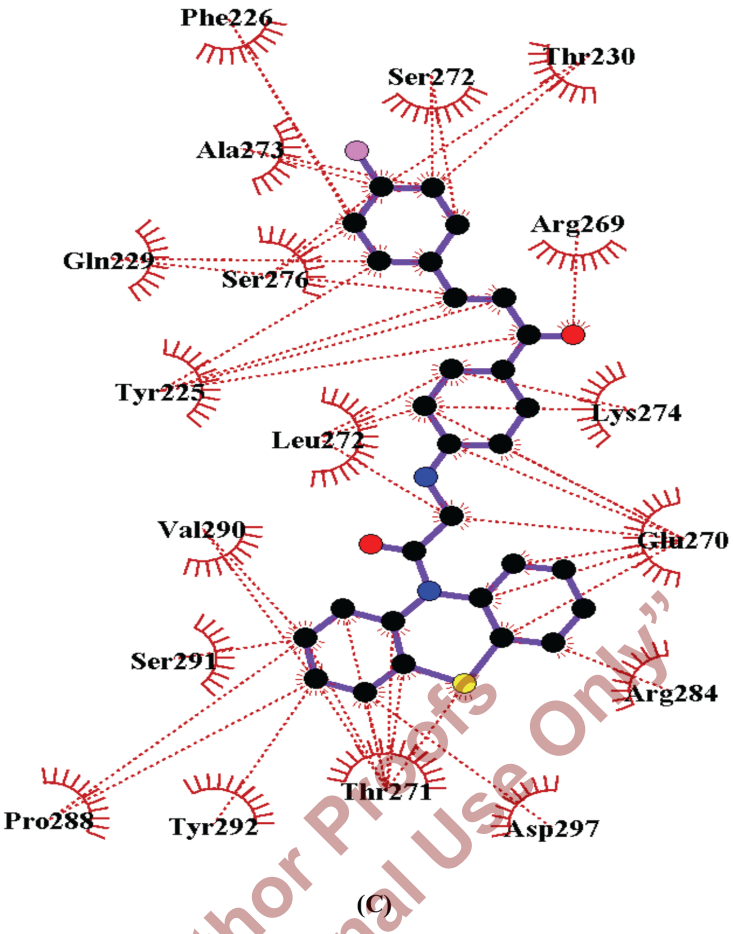

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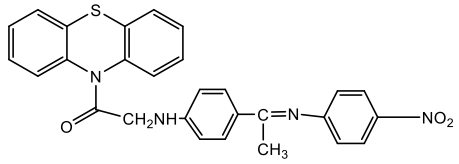
Fig. (2). Ligplot showing the interaction of diazepam (A) and phenothiazine derivatives (4f and P3). Purple lines - phenothiazine structure ligand bond; black circles - carbon atoms; blue circles - nitrogen atoms; green circles - chlorine atoms; pink circle - fluorine atom; red circles - oxygen atoms; yellow circles - sulphur atoms; red dotted lines - hydrophobic interactions; radial lines - non-ligand residues involved in hydrophobic contacts. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2-((4-(1-((4-chlorophenyl)imino)ethyl)phenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

IR (KBr, cm^{-1}): 3435 (str, N-H Ali), 1739 (str, C=O Ali), 1632 (str, C=N Ali), 1493 (str, C-C Ar), 1441(str, C=C Ar), 818 (bend, C-H Ar), 734 (str, C-Cl). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.6 (d, 2H, O=CNH), 7.0 (m, 3H, Ar-H), 6.9 (d, 2H, Ar-H), 6.7 (m, 5H, Ar-H), 6.5 (d, 3H, Ar-H), 6.0 (s, 2H, O=CNH), 5.2 (s, 1H, OCH).

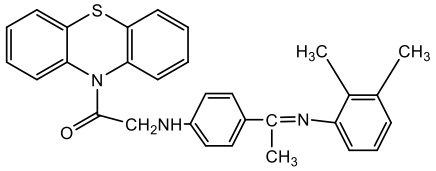
Compound 4g



2-((4-(1-((4-nitrophenyl)imino)ethyl)phenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

IR (KBr, cm^{-1}): 2164 (str, C=N Ali), 2109 (str, C-N Ali), 1738 (str, C=O Ali), 1634 (str, C-C Ar), 1505 (str, N=O Ali), 1467 (str, C-C Ali), 1442 (str, C=C Ar), 1116 (str, C-N Ar), 825 (bend, C-H Ar), 737 (str, C-H Ali), 530 (str, C-Cl).. ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.9 (s, 1H, Ar-OH), 7.6 (d, 2H, Ar-OH), 7.5 (s, 4H, Ar-H), 7.4 (m, 2H, C=CH), 7.0 (d, 2H, C=CH), 6.9 (t, 3H, C=CH), 6.8 (d, 2H, Ar-H), 6.7 (m, 5H, Ar-H), 6.5 (d, 2H, Ar-H), 6.0 (s, 2H, O=CNH), 2.3 (d, 3H, CNH).

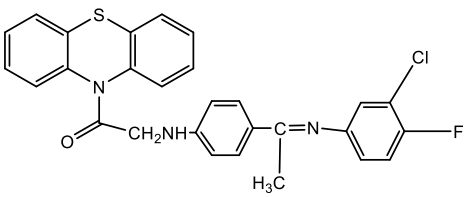
Compound 4i



2-((4-(1-((2,3-dimethylphenyl)imino)ethyl)phenyl) amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.8-7.3 (d, 5H, Ar-H), 7.0 (t, 2H, O=CNH), 6.8 (d, 2H, Ar-H), 6.7 (m,3H, Ar-H), 6.5 (d, 2H, Ar-H), 2.4 (s, 1H, C=CH), 2.1 (s, 1H, C=CH), 1.9 (s, 1H, C-CH).

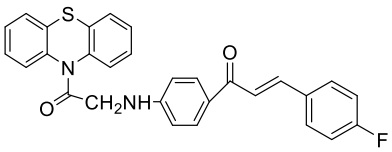
Compound 4j



2-((4-(1-((3-chloro-4-fluorophenyl)imino)ethyl)phenyl) amino)-1-(10H-phenothiazin-10-yl)ethan-1-one

IR (KBr, cm^{-1}): 2918 (str, C-H Ar), 1740 (str, C=O Ali), 1631 (str, C=N Ali), 1598 (str, C-N Ar), 1500 (str, C-C Ar), 1440 (str, C=C Ar), 1031 (str, C-F), 735 (str, C-Cl). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 7.7 (d, 2H, Ar-OH), 7.0 (m, 2H, Ar-H), 6.9 (d, 2H, Ar-H), 6.7 (m, 5H, Ar-H), 6.6 (d, 2H, Ar-H), 6.5 (m, 1H, Ar-H), 2.3 (s, 2H, C-NH)

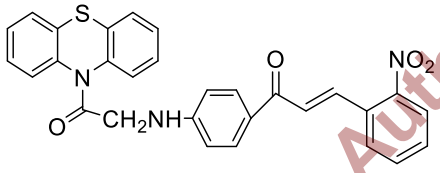
Compound P1



3-(4-fluorophenyl)-1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)prop-2-en-1-one

IR (KBr, cm^{-1}): 3436 (str, N-H Ali), 2555 (str, C-S Ar), 1734 (str, C=O Ali), 1635 (str, C=C Ali), 1468 (str, C=C Ar), 1411 (str, C-C Ali), 1225 (str, C-F), 1222 (str, C-N Ali), 1175 (str, C-N Ar), 821 (bend, C-H Ali), 783 (bend, C-H Ar).^{1}H NMR (500 MHz; DMSO) δ: 8.6 (s, 1H, C=CH), 7.6 (d, 1H, Ar-H), 7.0 (d, 3H, Ar-H), 6.9- 6.5 (m, 6H, Ar-H), 5.2 (s, 3H, C=CH).

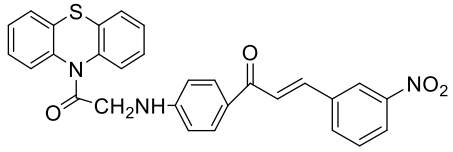
Compound P2



3-(2-nitrophenyl)-1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)prop-2-en-1-one

IR (KBr, cm^{-1}): 3433 (str, N-H Ali), 2468 (str, C-S Ar), 1633 (str, C=C Ali), 1444 (str, C=C Ar), 1388 (str, N=O Ali), 1308 (str, C-N Ar), 927 (str, C-H Ali), 755 (bend, C-H Ar). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 8.2 (d, 2H ArH), 7.6 (m, 2H, Ar-H), 7.1 (d, 2H, Ar-H), 6.8 (m, 3H, Ar-H), 6.7 (m, 2H, Ar-H).

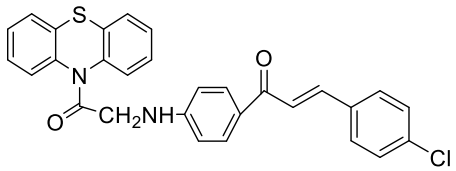
Compound P3



3-(3-nitrophenyl)-1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)prop-2-en-1-one

IR (KBr, cm^{-1}): 3446 (str, N-H Ali), 2470 (str, C-S Ar), 1643 (str, C=C Ali), 1526 (str, C=C Ar), 1390 (str, N=O Ali), 1318 (str, C-N Ar), 920 (str, C-H Ali), 788 (bend, C-H Ar). ^{1}H NMR (500 MHz; DMSO) δ: 8.7 (d-1HArH), 8.3 (d,1H C=CH), 8.1(d, 1H, ArOH), 7.9 (d, 1H, Ar-H), 7.7 (t,1H, Ar-H), 7.0 (t, 2H, Ar-H), 6.7 (m, 3H, Ar-H), 6.2 (s, 1H, O=CNH), 4.6 (s,1H, C=CH).

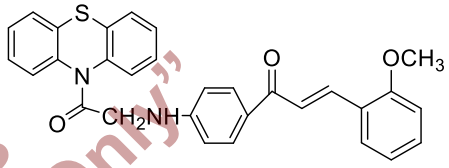
Compound P4



3-(4-chlorophenyl)-1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)prop-2-en-1-one

IR (KBr, cm^{-1}): 3450 (str, N-H Ali), 2550 (str, C-S Ar), 1750 (str, C=O Ali), 1468 (str, C=C Ar), 1446 (str, C-C Ar), 1250 (str, C-N Ar), 981 (bend, C-H Ali), 821 (bend, C-H Ar), 681 (str, C-Cl). ^{1}H NMR (500 MHz; DMSO) δ: 8.5 (s, 1H, C=CH), 8.0 (m,2H ArH), 7.6 (m, 1H, Ar-H), 7.3 (m, 3H, Ar-H), 6.9 (m, 4H, Ar-H), 6.7 (m, 4H, Ar-H).

Compound P5



3-(2-methoxyphenyl)-1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl)amino)phenyl)prop-2-en-1-one

IR (KBr, cm^{-1}): 3553 (str, N-H Ali), 2990 (str, C-H Ar), 2880 (str, C-H Ali), 2548 (str, C-S Ar), 1740 (str, C=O Ali), 1689 (str, C=C Ali), 1507 (str, C=C Ar), 1468 (str, C-C Ar), 1233 (str, C-N Ali), 1219 (str, C-O Ali),1175 (str, C-N Ar), 887 (bend, C-H Ar). ^{1}H NMR (500 MHz; DMSO) δ: 10.3 (s, 1H, O=CH). 8.6 (s, 1H, Ar-H), 7.7 (m, 3H, Ar-H), 7.5 (t, 2H, Ar-H), 7.2 (d, 2H, C=CH), 7.1 (m, 7H, Ar-H), 6.8 (m, 10H, Ar-H), 6.2 (s, 1H,C=CH), 5.1 (s, 1H, O-CH), 4.4 (s, 1H, C=CH), 3.9 (m, 4H, C-O-H), 3.7 (d, 4H, O-CH).

3. RESULTS AND DISCUSSION

15 derivatives in two series 1-(10H-phenothiazin-10-yl)-2-((4-(1-(phenylimino)ethyl) phenyl)amino)ethan-1-one (4a-4j) and 1-(4-((2-oxo-2-(10H-phenothiazin-10-yl)ethyl) amino)phenyl)-3-phenylprop-2-en-1-one (P1-P5) were prepared. 4a-4j derivatives were prepared as Schiff base by reacting 2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one with different substituted anilines. P1-P5 were prepared as chalcones by a reaction between 2-((4-acetylphenyl)amino)-1-(10H-phenothiazin-10-yl)ethan-1-one and substituted aldehydes. All of the derivatives exhibited a good practical yield. Molecular docking with a set of different parameters was done to characterise the derivatives. The log P values of the derivatives were found to be 5.69-6.80, which indicated them to have an active moiety. The docking score of the derivatives ranged between – 10.2 to -9. Among them, compound P3 had the lowest docking score of -10.2, showing to be the most potent; compound 4b showed a moderate docking score of -9.4, and compound 4a showed the highest docking score of -9.0. Autodock Vina v.1.2.0 was used to perform the docking study, and receptor structure (6D6U) was taken from the Protein Data Bank. The molecular docking score and physicochemical parameters of the prepared derivatives are presented in Table **1**. The

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result of the anxiolytic activity [21] of the derivatives is shown in Table **2**. According to the data obtained by the elevated plus maze method, compounds 4a, P1, and P3 showed maximum anti-anxiety activity. The potency of the compounds was compared with that of the standard drug diazepam. In the Ligplot (Fig. **2**), hydrogen bond interactions of compounds 4f and P3 with Gln229, Phe226, Ser276, Lys274, Tyr225, Asn275, Thr281, Arg284, Ile271, Ala273, Ser272, Thr230, Arg269, Leu272, Thr230, Val290, Pro288, and Asp297 amino acid residues have been shown.

CONCLUSION

This work has presented the design and execution of the synthetic scheme to prepare the derivatives of phenothiazine. In this study, the anxiolytic activity of the derivatives has been evaluated by using the elevated plus maze method. A set of molecular parameters has also been computed for the docking study of the prepared molecules. The results have shown three compounds to be active against anxiety in comparison to diazepam. For the evaluation of anxiolytic activity, diazepam was employed as a standard drug. The anti-anxiety activity of the compounds was assessed by using the elevated plus maze method, and out of them, compounds 4f, 4h, and P3 showed maximum activity as anti-anxiety agents.

LIST OF ABBREVIATIONS

°C	=	Degree Celsius
Ali	=	Aliphatic
Ar	=	Aromatic
bend	=	Bending
cm	=	Centimetre
d	=	Doublet
g	=	Gram
m	=	Multiplet
RBF	=	Round Bottom Flask
s	=	Singlet
str	=	Stretching

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Animal Ethical Committee, Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India, New Delhi (Reg. No. 837/PO/RE/S/04/CPCSEA).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All the reported works were in accordance with The US National Research Council's "Guide for the Care and Use of Laboratory Animals".

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Bernthsen B. Ultrasound-mediated synthesis of phenothiazine derivatives and their *in vitro* antibacterial and antioxidant studies. Dent Chem Ges 1883; 16: 2996.

[2] Barbara W, Joseph D, Terry S, Cecily D. Pharmacotherapy Handbook. 8th Edi. McGraw Hill Professional, pp. 1164.

[3] Kurihara T, Motohashi N, Pang GL, Higano M, Kiguchi K, Molnár J. Correlations between topological resonance energy of methyl-substituted benz[c]acridines, benzo[a]phenothiazines and chrysenes, and their carcinogenic or antitumor activities. Anticancer Res 1996; 16(5A): 2757-65. PMID: 8917383

[4] Kumar VD, Gupta AK, Yadav YC. Synthesis, characterization and antimicrobial activity of benzimidazolyl-phenothiazine derivatives. Int J PharmRes 2010; 2(2): 45-50.

[5] Pluta K, Morak-Młodawska B, Jeleń M. Recent progress in biological activities of synthesized phenothiazines. Eur J Med Chem 2011; 46(8): 3179-89. http://dx.doi.org/10.1016/j.ejmech.2011.05.013 PMID: 21620536

[6] Meyer M, Lang PT, Gerber S, *et al.* Synthesis and testing of a focused phenothiazine library for binding to HIV-1 TAR RNA. Chem Biol 2006; 13(1): 993. http://dx.doi.org/10.1016/j.chembiol.2006.07.009 PMID: 16984889

[7] Kalkanidis M, Klonis N, Tilley L, Deady LW. Novel phenothiazine antimalarials: Synthesis, antimalarial activity, and inhibition of the formation of β-haematin. Biochem Pharmacol 2002; 63(5): 833-42. http://dx.doi.org/10.1016/S0006-2952(01)00840-1 PMID: 11911834

[8] Bishnoi A, Pandey VK, Saxena R. Indian J Chem, Sect B: Org Chem Incl Med Chem 2002; 41(1): 1978.

[9] Chopde HN, Pagadala R, Jetti V. An efficient synthesis of novel bioactive azetidinones and thiazolidinones of 1, 5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one. Int J Pharm Biosci 2011; 2(1): 19-22.

[10] Saranya AV, Ravi S. Int J Clin Exp Pathol 2013; 3(2): 9-21.

[11] Saranya AV, Ravi S. Synthesis of 5-phenyl-3-(10H-phenothiazinyl)-Δ2-cyclohexen-1-ones by conventional and microwave-assisted methods and their antifungal activity. Res Chem Intermed 2014; 40(8): 3085-93. http://dx.doi.org/10.1007/s11164-013-1153-9

[12] Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. J Chem Inf Model 2021; 61(8): 3891-8. http://dx.doi.org/10.1021/acs.jcim.1c00203 PMID: 34278794

[13] Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010; 31(2): 455-61. PMID: 19499576

[14] SAMSON. Software for Adaptive Modeling and Simulation of Nanosystems. Available from: https://www.samson-connect.net (accessed on 11 November 2022)

[15] Zhu S, Noviello CM, Teng J, Walsh RM Jr, Kim JJ, Hibbs RE. Structure of a human synaptic $GABA_A$ receptor. Nature 2018; 559(7712): 67-72. http://dx.doi.org/10.1038/s41586-018-0255-3 PMID: 29950725

[16] Molecular Operating Environment (MOE). (202202)Chemical computing group ULC. Montreal, QC, Canada 2022.

[17] Edelsbrunner H, Liang J, Fu P, Facello M. Measuring proteins and voids in proteins. Proceedings of the Twenty-Eighth Annual Hawaii International Conference on System Sciences,. 256-64. 03-06 January 1995 , Wailea, HI, USA http://dx.doi.org/10.1109/HICSS.1995.375331

[18] Gabriel de Oliveira M, Kelle da Silva Moreira L, Turones LC, *et al.* Mechanism of action involved in the anxiolytic-like effects of Hibalactone isolated from Hydrocotyle umbellata L. J Tradit Complement Med 2021; 12(4): 318-29.

[19] Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 1996; 54(1): 21-30. http://dx.doi.org/10.1016/0091-3057(95)02126-4 PMID: 8728535

[20] Kumar D, Bhat ZA, Kumar V, Shah M. Nature: Anxiolytics in the lap of nature. Webmed Cent Pharmac Sci 2011; 2(9): WMC002140.

[21] Rodgers RJ, Cao BJ, Dalvi A, Holmes A. Animal models of anxiety: An ethological perspective. Braz J Med Biol Res 1997; 30(3): 289-304. http://dx.doi.org/10.1590/S0100-879X1997000300002 PMID: 9246227

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