COMPUTATIONAL ANALYSIS, SYNTHESIS AND CHARECTERIZATION OF NOVEL 1, 4-DISUBSTITUED PIPERAZINE DERIVATIVES

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Abstract: In this study, new entities of 1, 4-substitued piperazine derivatives were synthesized in good yields (60-89%). Scheme1 synthesis of Piperazine derivative compounds of 1-Piperazino-2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl)-1-ethanone was added to the P-Substituted Sulfonyl Chloride Derivatives forms the final products of 2-(2, 3, 4, 9-tetrahydro1H-9carbazolyl) Piperazine Substituted Sulfonyl Derivatives. Scheme2 synthesis of Piperazine and 1,3-Dioxoane derivative compounds of 6, 7, 8, 9-tetrahydro-9-((2-(piperazin-1-yl)-1, 3-dioxolan-2-yl) methyl)-5H-carbazole was added to the P-substituted Sulfonyl Chlorides forms the final products of 2-((5, 6, 7, 8-tetrahydrocarbazol-9-yl) 1, 3-dioxolan-2-yl) substituted Sulfonyl Derivatives. All the Synthesized compound derivatives were purified by appropriate solvents and characterized by Melting Point, TLC, IR and NMR (^{1}H&^{13}C) spectroscopy. Synthesized derivative compounds against 1EVE, 2BU8 molecular docking simulation was carried out with AUTODOCK VINA and Insilco ADMET Prediction Procedures. The results of docking study revealed that the binding profile for synthesized derivative compounds M_4, M_5, M_6, M_7, and K_6 was found significant interactions with Donepezil due to hydrogen bond, hydrophobic interactions like π–π Stacking interaction and π-alkyl stacking interactions with catalytic active site (CAS) of 1EVE. M_6, K_1, K_4, K_5, K_6 and K_7 was found significant interactions with Piperazine Analogue due to hydrogen bond, hydrophobic interactions like π–sigma Stacking interaction, π-alkyl stacking interactions and alkyl stacking interactions with CAS of 1BU8. The predicted ADMET properties revealed that all compounds fulfil drug-like criteria and could be considered as good candidate for drug development. All the synthesized compound derivatives have Standard Drug (Donepezil) like ADMET properties.

Index Terms -1, 4-substitued piperazine derivatives, 1,3-Dioxoane derivative compounds, 1-Piperazino-2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl)-1-ethanone, P-Substituted Sulfonyl Chloride Derivatives, 2-(2, 3, 4, 9-tetrahydro1H-9carbazolyl) Piperazine Substituted Sulfonyl Derivatives, 6, 7, 8, 9-tetrahydro-9-((2-(piperazin-1-yl)-1, 3-dioxolan-2-yl) methyl)-5H-carbazole, 2-((5, 6, 7, 8-tetrahydrocarbazol-9-yl) 1, 3-dioxolan-2-yl) substituted Sulfonyl Derivatives, 1EVE, 2BU8, Insilco ADMET Prediction, hydrogen bond, hydrophobic interactions, π–π Stacking interaction, π-alkyl stacking interactions, catalytic active site (CAS), π–sigma Stacking interaction, π-alkyl stacking interactions and alkyl stacking interactions.

I. INTRODUCTION

Piperazine (Figure 1) was used early in the 20th century for the treatment of gout. Giround discovered the anthelmintic activity of piperazine, synthesized by Cloez in 1853, fortuitously in 1942. The same effect was observed by Biosmare in 1948 and conformed by Bayared in 1949.[1] Piperazine is a six member cyclic secondary diamine with the nitrogen in the 1, 4 - position. It can also be called diethelenediamine for convenience its molecular formula can be written $C_4H_{10}N_2$. Piperazines and substituted piperazines are important pharmacophores that can be found in many marketed drugs, such as the Merck HIV protease inhibitor Crixivan[3], and drugs under development. Piperazinyl-linked ciprofloxacin dimers reported as potent antibacterial agents against resistant strains, a novel class of mixed D2/D4 receptor antagonists, dual calcium antagonist, and potential antipsychoticagents.[4]

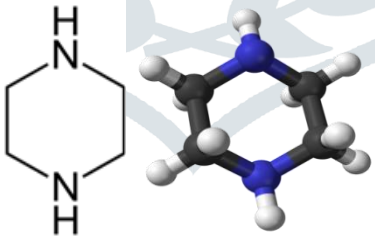


Figure 1

Dioxolane (Figure 2) is a heterocyclic acetal with the chemical formula $(CH_2)_2O_2CH_2$. It is related to tetrahydrofuran by interchange of one oxygen for a CH_2 group. The corresponding saturated 6-membered C_4O_2 rings are called dioxanes. The isomeric 1,2-dioxolane (where in the two oxygen centers are adjacent) is peroxide. 1,3-Dioxolane is used as a solvent and as a comonomer in polyacetals.[5] The perfluorinated bis(dioxolane) has been used as a I9F NMR imaging agent for tumors. The long-chain dioxolane inhibits leukotriene-B4 production in tumor cells and is an inhibitor of 5-lipoxygenase.[6] Benzodioxole-2,2-dicarboxylates and the corresponding dimethyl esters are effective anti-obesity agents. Spiro dioxolanes and oxathiolanes have been patented for the treatment of Alzheimer's disease.[7] The benzodioxole-2-thione has been used for the treatment of liver disease. A variety of simple benzodioxole-2-carboxylic acid derivatives have been examined in detail as diuretic and uricosuric agents. Dioxolane sulfamates are useful as anticonvulsants.[8] The different possible enantiomers and diastereomers of the dioxolane, oxathiolane, and its S-oxides have been separated and evaluated for their cholinergic agonist activity.[9] The bicyclic dioxolane has central nervous system (CNS) activity.[10] A series of 2-benzofuryl-4-aminomethyl-l,3-dioxolanes affect the cardiovascular system and offer protection against fibrillation, arrhythmia, and angina. The methylenedioxyphenyl pyrrolidinone inhibits blood platelet aggregation.[11]

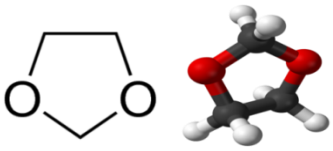


Figure 2

Docking (Figure 3) is an attempt to find the best matching between two molecules. Docking is a method which predicts the preferred orientation of one ligand when bound in an active site to form a stable complex. Lock and key finding the correct relative orientation of the "key" which will open up the "lock". On the surface of the lock is the key hole in the direction to turn the key after it is inserted. The protein can be thought of the "lock" and the ligand can be thought of as a "key". To achieve an optimized conformation for both receptor and ligand and the relative orientation between protein and ligand such that the free energy of the overall system is minimized. Successful docking methods search high dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings.[12]

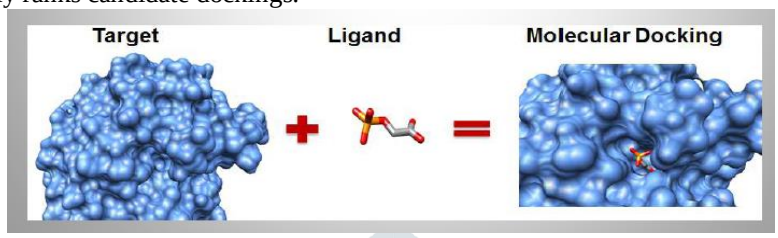


Figure 3

E2020 (Figure 4) is a member of a large family of N-benzylpiperidine-based AChE inhibitors that were developed, synthesized and evaluated by the Eisai Company in Japan, on the basis of QSAR studies, prior to elucidation of the three-dimensional (3D) structure of Torpedo californica AChE (TcAChE).[13] It was shown to significantly enhance performance in animal models of cholinergic hypofunction and to have high affinity for AChE **(Figure 5)**, binding to both electric eel and mouse AChE in the nanomolar range. Classification: SERINE HYDROLASE, Organism: Tetronarce californica, Resolution: 2.50 Å, Chains: A, Sequence Length: 543, PDB entry: 1EVE.

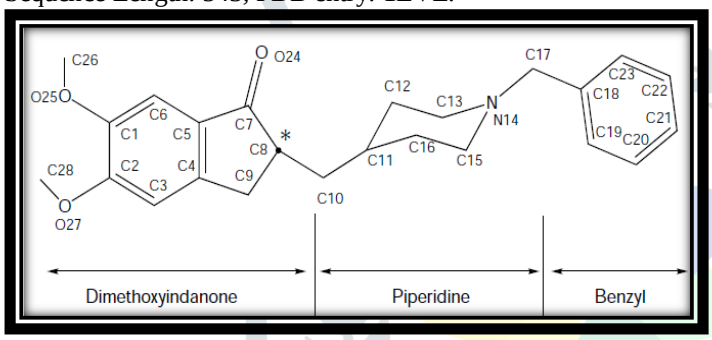


Figure 4

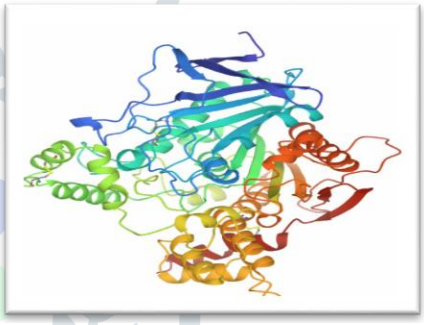


Figure 5

Pyruvate dehydrogenase kinase isoform 2 (PDK2) (Figure 6) also known as pyruvate dehydrogenase lipoamide kinase isozyme 2, mitochondrial was an enzyme that in humans was encoded by the PDK2 gene. PDK2 was an isozyme of pyruvate dehydrogenase kinase. To regulate glycolysis/carbohydrate oxidation and producing metabolites for oxidative phosphorylation and the electron transport chain. PDK2 activity is modulated by low levels of hydrogen peroxide; this happens because the compound temporarily oxidizes the cysteine residues 45 and 392 on the enzyme, resulting in an inactive PDK2 and greater PDH activity. These conditions also inactivate the TCA cycle, the next step in aerobic respiration.[14] Classification: TRANSFERASE, Organism: Homo sapiens, Resolution: 2.50 Å, Chains: A, Sequence Length: 394, PDB entry: 2BU8.[15]

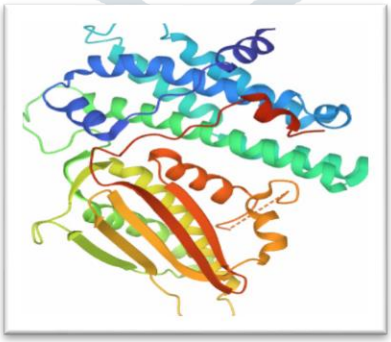


Figure 6

II. METHODOLOGY

2.1. SCHEME 1

Step 1: Synthesis of 6, 7, 8, 9-tetrahydro-5H-Carbazole[16]

A mixture of 0.1mol (1.737gms/1.83ml) Cyclohexanone, 0.1mol (2.964gms) Phenyl hydrazine hydrochloride, and 0.3mol (3gms) sodium acetate was refluxed with 50 ml ethanol for 2 h. After cooling, an excess of water was added; the precipitate of the crude 1-cyclohexylidene-2-phenylhydrazine was collected by suction, washed with water, and dissolved in 20 ml of the cyclization reagent (acetic acid and hydrogen chloride). After 5 min of heating on a water bath, the mixture was poured into water and the precipitate was washed thoroughly with water, dried it. Percentage yield of 6, 7, 8, 9-tetrahydro-5H-Carbazole 65-75%w/w was obtained.

Step 2: Synthesis of 2-(5, 6, 7, 8-tetrahydrocarbazol-9-yl) acetyl chloride[17]

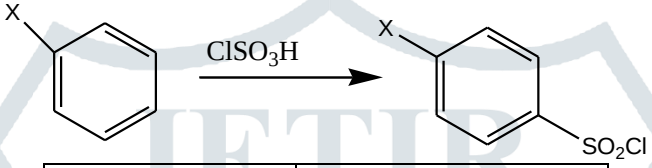
The synthesized product 6, 7, 8, 9-tetrahydro-5H-carbazole 0.0164mol (2.81gms) was taken into 250ml dissolved in 0.16mol (15ml) glacial acetic acid, 0.0251-0.0334mol (2.37-3.16gms/15-20ml). Heat for 30 min on a water both with continuous stirring. 6.92mol (15ml) Ether, 0.138mol (10ml) thionyl chloride mixed together and this mixture was kept it into a ice both. This reaction mixture was added slowly to the Carbazole mixture with continuous stirring for 1 hour. Than the reaction mixture was poured into crushed ice. Precipitates were filtered and wash with water and dry it.

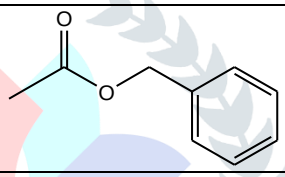
Step 3: Synthesis of 1-piperazino-2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl)-1-ethanone

The synthesized product 0.404mol (1gm), 1gm potassium carbonate were dissolved in 5ml of glacial acetic acid. Than 0.404mols (0.348) Piperazine was added in portions by continuous stirring for I hour. Kept a side for I hour than pour in ice water. The precipitates were filtered and dry the product.

Step 4: Synthesis of P-Substituted Phenyl Sulfonyl Chloride Derivatives[18]

Equip a 500 ml two necked flask with a dropping funnel and a reflux condenser. Attach the top of the latter to a device for the absorption of a hydrogen chloride. Place 0.148 mol of P-Substituted Phenyl compounds in the flask and 0.77 mol of chlorosulphonic acid in the dropping funnel and insert a calcium chloride guard tube. A small portion of chlorosulphonic acid was added and shakes the flask from time to time to ensure thorough mixing. After completion heat the reaction mixture on water bath for 1 hour in order to complete the reaction. Allow to cool and pour the oily mixture in a thin stream with stirring into 300g of crushed ice contained in q 1 liter beaker. Carry out this operation carefully under fuming cupboard since the excess of chlorosulphonic acid reacts vigorously with water, rinsed the flask with a little ice water and combusted the contents into the beaker. Break up any lumps of solid material and stirred the mixture for several minutes in order to obtain an even suspension of the granular solid. Filter off at the pump and washed it with a little cold water, press and drain it well.



Name	X
Acetanilide	$NHCOCH_3$
Anisole	OCH3
Benzyl Benzoate	
Methyl Salicilate	$COOCH_3$

Step 5: Synthesis of 2-(2, 3, 4, 9-tetrahydro1H-9carbazolyl) Piperazine Substituted Sulfonyl Derivatives[19]

1-Piperazino-2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl)-1-ethanone 0.00256mol (0.76gms) was added to the 20ml dichloromethane layer, containing P-Substituted Sulfonyl Chloride Derivatives 0.00256mol at 20-25^{0}C. The reaction was stirred for 4 h and then washed with 5% w/w aqueous sodium bicarbonate (100ml) followed by DM water (100ml). The dichloromethane layer was concentrated at <40^{0}C under reduced pressure to obtain a foamy material. The products were recrystallized with methanol and it was dried.

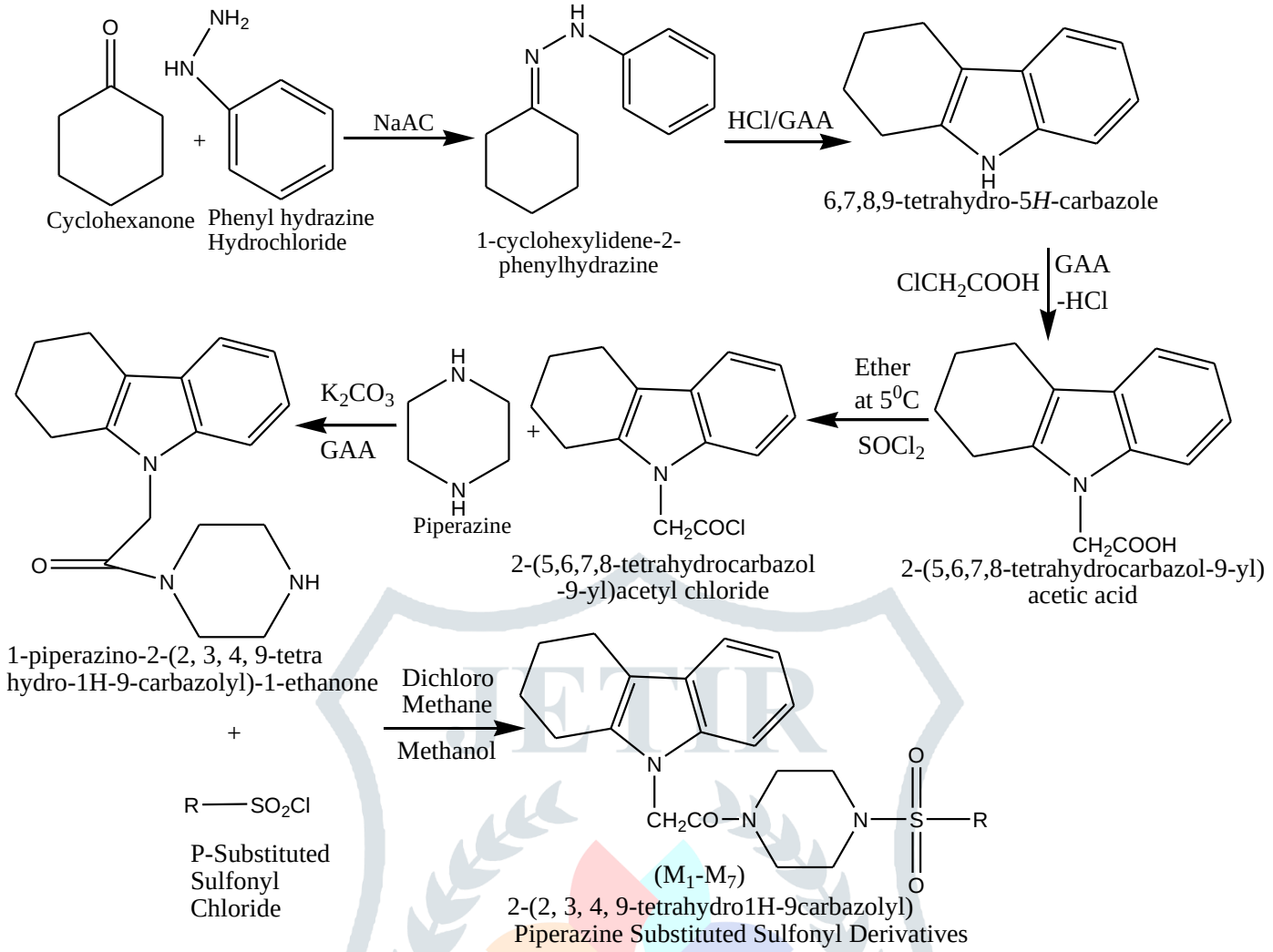
2.2. SCHEME 2

Step 1: Synthesis of 6, 7, 8, 9-tetrahydro-9-((2-(piperazin-1-yl)-1, 3-dioxolan-2-yl) methyl)-5H-carbazole[20]

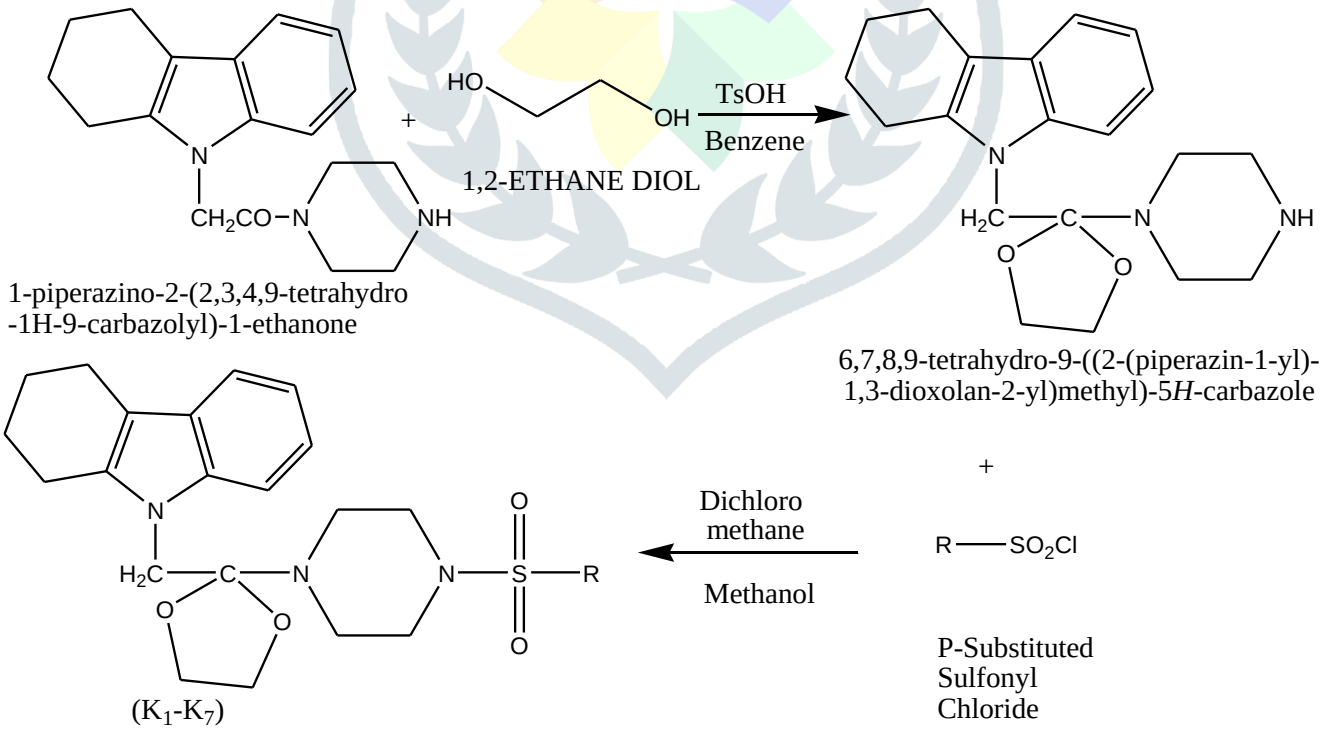
A mixture of 0.012moles (3.56gms) of N-substituted piperazine, 0.0132moles (0.819gms/0.738ml) of 1,3-ethanediol, 0.005gms of TsOH, 2.5ml of benzene was boiled for 20 h at constant stirring using a Soxhlet apparatus filled with 20 g of silica gel mesh. The catalyst was neutralized with 1 g of $Ca(OH)_2$, the precipitate was filtered off, acetone and benzene were distilled off from the filtrate, the residue was distilled in a vacuum. Percentage yield was 70-80% was obtained.

Step 2: Synthesis of 2-((5, 6, 7, 8-tetrahydrocarbazol-9-yl) 1, 3-dioxolan-2-yl) substituted Sulfonyl Derivatives

6, 7, 8, 9-tetrahydro-9-((2-(piperazin-1-yl)-1, 3-dioxolan-2-yl) methyl)-5H-carbazole 0.00256mol (0.87gms) was added to the 20ml dichloromethane layer, containing P-substituted Sulfonyl Chlorides 0.00256mol at 20-25^{0}C. The reaction was stirred for 4 hours and then washed with 5% w/w aqueous sodium bicarbonate (100ml) followed by DM water (100ml). The dichloromethane layer was concentrated at <40^{0}C under reduced pressure to obtain a foamy material. The products were recrystallized with methanol and it was dried.

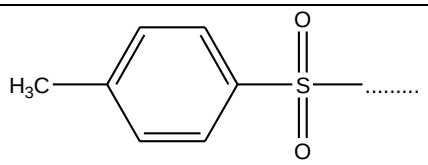
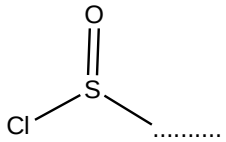
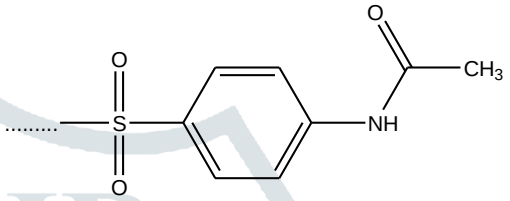
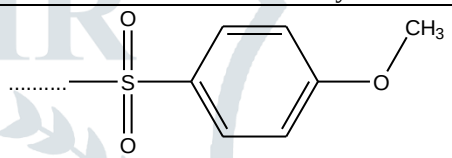
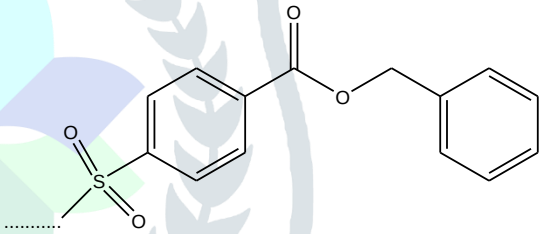
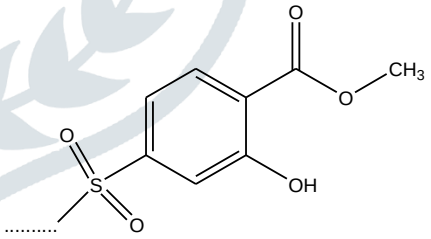

Cyclohexanone
Phenyl hydrazine Hydrochloride
NaAC
1-cyclohexylidene-2-phenylhydrazine
HCl/GAA
6,7,8,9-tetrahydro-5H-carbazole
ClCH2COOH
GAA
-HCl
2-(5,6,7,8-tetrahydrocarbazol-9-yl) acetic acid
Ether at 5⁰C
SOCl2
2-(5,6,7,8-tetrahydrocarbazol -9-yl)acetyl chloride
Piperazine
K2CO3
GAA
1-piperazino-2-(2, 3, 4, 9-tetra hydro-1H-9-carbazolyl)-1-ethanone
P-Substituted Sulfonyl Chloride
Dichloro Methane
Methanol
(M1-M7)
2-(2, 3, 4, 9-tetrahydro1H-9carbazolyl) Piperazine Substituted Sulfonyl Derivatives

SCHEME 1


1,2-ETHANE DIOL
TsOH
Benzene
1-piperazino-2-(2,3,4,9-tetrahydro -1H-9-carbazolyl)-1-ethanone
6,7,8,9-tetrahydro-9-((2-(piperazin-1-yl)-1,3-dioxolan-2-yl)methyl)-5H-carbazole
Dichloro methane
Methanol
P-Substituted Sulfonyl Chloride
(K1-K7)
2-((5, 6, 7, 8-tetrahydrocarbazol-9-yl) 1, 3-dioxolan -2-yl) substituted Sulfonyl Derivatives

SCHEME 2

Table 1: Synthesized Derivative Compounds

SCHEME 1 CODE	SCHEME 2 CODE	R
M_1	K_1	 4-Toulene Sulfonyl chloride
M_2	K_2	CH_3SO_2..... Methane Sulfonyl chloride
M_3	K_3	 Thionyl chloride
M_4	K_4	 4-Acetamido benzene-1-sulfonyl chloride
M_5	K_5	 4-Methoxybenzene sulfonyl chloride
M_6	K_6	 Benzyl-4-chloro sulfonyl benzoate
M_7	K_7	 Methyl-4-chloro sulfonyl benzoate

2.3. DOCKING PROCEDURE[21]

To understand the binding interactions and selectivity of our compounds against two ChE and PDK, molecular docking simulation was carried out with AUTODOCK VINA. The crystal structure of AChE complexed with E2020 (code ID: 1EVE; resolution 2.5-Å) and Pyruvate dehydrogenase kinase 2 (code ID: 2BU8 resolution 2.5-Å) used in the docking study were obtained from the Protein Data Bank (http://www.rcsb.org/pdb). The original inhibitor, hetero atoms and water molecules in the PDB files were eliminated in the beginning of docking study. The enzyme was set up for docking with standard protocol. Add polar hydrogen atoms to amino acids residues and for assigning Gasteiger charges to all atoms of the enzyme. 3D structures of the AChE complexed with E2020, Pyruvate dehydrogenase kinase 2, synthesized compounds(14 compounds), standard drugs(Donepezil, Piperazine analogue) were generated and energy optimized by Discovery Studio 2.5 package (Accelrys Inc., San Diego, CA) employing CHARMm force field. Full hydrogens were added to the ligands and Gasteiger-Marsili partial atomic charges were caculated using the BABEL-2.2.338 program and saved in the PDBQT format for further analysis. All possible rotatable bonds of the resultant ligand molecules were defined by using standerd protocol. All side chains and the backbone of the protein were kept rigid as in the crystal structure. Docking was performed first by placing the ligand in a random position by centering the grid on the macromolecule and setting the grid with 2BU8-Co-ordinates are X-56.344, Y-44.67, Z-80.94, 1EVE-Co-ordinates are X-28.66, Y-64.62, Z-7.92 spacing on the entire proteins; after the identification of the best binding sites, further analysis was performed by starting with the ligand in the binding pockets and setting the grid.[72] The affinity expressed in kcal/mol was calculated as the difference in free energy of binding (ΔG) between the protein and the complex. The prepared PDBQT format of ligands was used as input files for AutoDock Vina subsequently. The docking simulation was performed using the Lamarckian Genetic Algorithm. Finally, the top-posed docking conformations were submitted to post

docking energy minimization on Discovery Studio 2.5. The resultant structure files were evaluated using PyMOL visualization program. All the Docking results of the synthesized compounds were shown in **Table 6.5, 6.6 and Figure 6.1-6.12.**

2.4. ADMET PREDICTION[22]

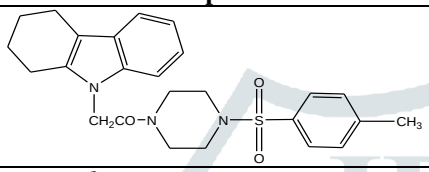
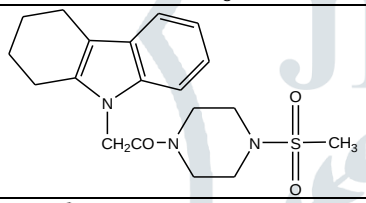
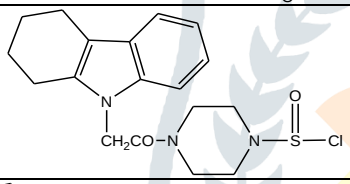
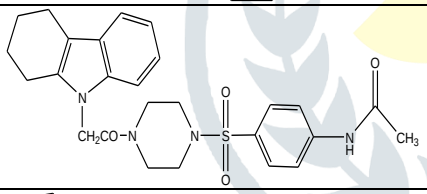
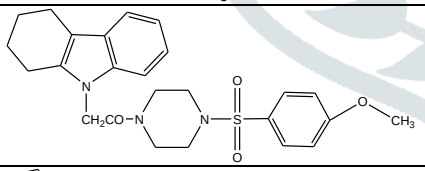
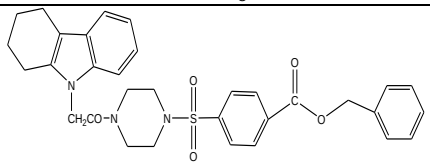
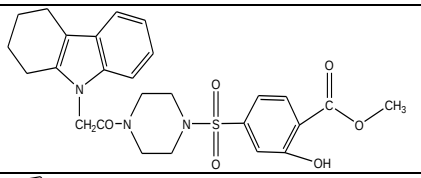
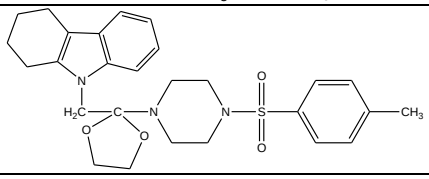
ADMET properties of a compound deal with its absorption, distribution, metabolism, excretion, and toxicity in and through the human body. ADMET, which constitutes the pharmacokinetic profile of a drug molecule, is very essential in evaluating its pharmacodynamic activities. Today a lot of online tools and offline software programs are available which helps us in predicting this behaviour of the drug candidate. In this study, I have used the SWISSadme, admetSAR prediction tools (http://lmmd.ecust.edu.cn:8000/). In the ADMET-SAR, web based query tools incorporating a molecular build-in interface enable the database to be queried by SMILES and structural similarity search. It provides the latest and most comprehensive manually curated data for diverse chemicals associated with known ADMET profiles (ADMETSAR@LMMD). All the ADMET results of the synthesized compounds were shown in **Table 6.7, 6.8 and 6.9.**

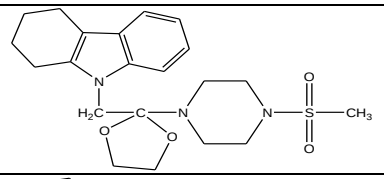
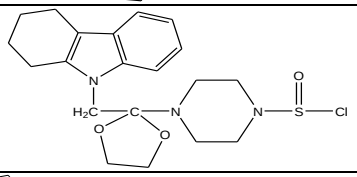
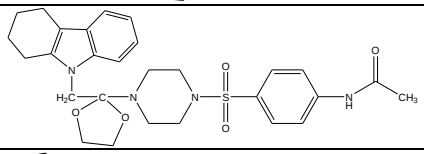
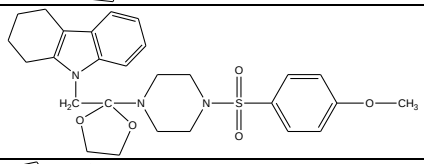
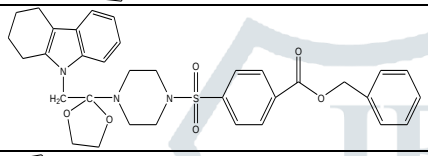
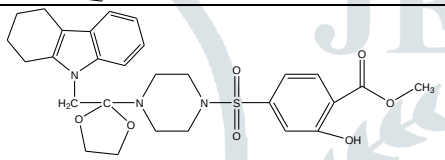
III. RESULTS AND DISCUSSION

3.1. Characterization of Synthesized Derivative Compounds

A total of 14 compounds were synthesized and recrystallized by appropriate solvents. They were identified and characterized by various spectral methods. All the compounds characterization was shown in the **Table 2.**

Table 2: Physical Characterization

Comp. Code	Compound	Molecular Formula	Mol. Wt.	M.P	% Yield	Rf value*
M_1		$C_{25}H_{29}N_3O_3S$	451.58	100-120	89.47	0.57
M_2		$C_{19}H_{25}N_3O_3S$	375.49	95-110	81.58	0.61
M3		$C_{18}H_{22}ClN_3O_2S$	379.9	90-105	78.11	0.59
M_4		$C_{26}H_{30}N_4O_4S$	494.61	110-125	76.34	0.61
M_5		$C_{25}H_{29}N_3O_4S$	467.58	115-125	71.93	0.52
M_6		$C_{32}H_{33}N_3O_5S$	571.69	130-142	68.63	0.56
M_7		$C_{26}H_{29}N_3O_6S$	511.59	120-135	61.72	0.47
K_1		$C_{27}H_{33}N_3O_4S$	495.22	104-108	61.2	0.62

K_2		$C_{21}H_{29}N_3O_4S$	419.59	100-110	56.78	0.57
K_3		$C_{20}H_{26}ClN_3O_3S$	423.96	95-105	49.11	0.52
K_4		$C_{28}H_{34}N_4O_5S$	538.66	100-120	59.47	0.47
K_5		$C_{27}H_{33}N_3O_5S$	511.63	108-116	42.28	0.47
K_6		$C_{34}H_{37}N_3O_6S$	615.74	120-128	48.32	0.54
K_7		$C_{28}H_{33}N_3O_7S$	555.64	116-130	42.11	0.42

*Mobile Phase=Ethyl Acetate: n-Hexane (2:8)

Spectral data

M_1: 2-(5, 6, 7, 8-tetrahydrocarbazol-9-yl)-1-(4-tosylpiperazin-1-yl) ethanone, **FT-IR, $_{max}$, cm^{-1} (ATR):** C-H(str):3052; C-H(str):2846; C=O(str):1683.37; C-N(str):1230.03; C-S(str):1140.69**;** S=O(str):1061.36. **^{1}H NMR (in DMSO-d6, δ in ppm:** ^{25,29}Ar-H(S)-7.526;^{1}Ar-H(D)-7.426; ^{26,28}Ar-H(S)7.330; ^{2}Ar-H(D)-7.243; ^{3}Ar-H(S)6.833; ^{6}Ar-H(S)-6.813; ^{14}CH-C=O(Carbonyl), ^{16,17,19,20}Ar-CH(S,D)-3.455; ^{10}C=C-H(Vinylic, D)2.603; ^{13}C=C-H(Vinylic, D)-2.507; ^{30}C=C-CH$_3$(Allylic, S)-2.295; ^{11}C=C-CH$_3$(Allylic, M)-1.642; ^{12}C=C-CH$_3$(Allylic, M)-1.627. **^{13}C NMR (in DMSO-d6, δ, in ppm):** ^{4,22,27}Ar-C-135.65; ^{8}Ar-C-134.34; ^{5,26,28}Ar-C-127.95; ^{25,29}Ar-C-127.29; ^{1}Ar-C-123.71; ^{2}Ar-C-119.92; ^{6}Ar-C-117.89; ^{3}Ar-C-111.94; ^{9}Ar-C-108.0; ^{17,19}Secondary-CH$_2$-40.20; ^{16,20}Secondary-CH$_2$-39.99; ^{14}Secondary-CH$_2$-37.10; ^{30}Primary-CH$_3$-24.94; ^{13}Secondary-CH$_2$-22.99; ^{12}Secondary-CH$_2$-22.85; ^{11}Secondary-CH$_2$-22.75; ^{10}Secondary-CH$_2$-20.61.

M_2: 1-[4-(methylsulfonyl) piperazino]-2-(2, 3, 4, 9-tetrahydro-1H-9 carbazolyl)-1-ethanone, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3024.91; C-H(str):2928.38; C=O(str):1615.29; C-N(str):1323.96; C-S(str):1203.47; S=O(str):1038.82. **^{1}H NMR (in DMSO-d6, δ in ppm:** ^{1}Ar-H(D)-7.323; ^{2}Ar-H(D)-7.236; ^{3}Ar-H(S)-6.901; ^{6}Ar-H(S)-6.883; ^{14}CH-C=O(Carbonyl); ^{16,20}Ar-CH(D)-3.718; ^{22}C=C-CH$_3$(Allylic, D)-2.706; ^{17,19}Ar-H(D)-2.692; ^{10}C=C-H(Vinylic, D)-2.595;^{13}C=C-H(Vinylic, D)-2.505; ^{11,12}C=C-CH$_3$(Allylic, M)-1.810. **^{13}C NMR (in DMSO-d6, δ, in ppm):** ^{4,8}Ar-C-135.63; ^{5}Ar-C-127.28; ^{1,6}Ar-C-119.93; ^{2}Ar-C-117.90; ^{3}Ar-C-110.45; ^{9}Ar-C-107.99; ^{16,20}Secondary-CH$_2$-40.3; ^{17,19}Secondary-CH$_2$-40.23; ^{22}Primary-CH$_3$-39.82; ^{14}Secondary-CH$_2$-38.99; ^{13}Secondary-CH$_2$-22.98; ^{12}Secondary-CH$_2$-22.85; ^{11}Secondary-CH$_2$-22.75; ^{10}Secondary-CH$_2$-20.61.

M_3: 4-[2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl) acetyl]-1-piperazinesulfinyl chloride, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):2923; C-H(str):2844.61; C-N(str):1229.78; C=O(str):1551.94; S=O(str):1053.70, **^{1}H NMR (in DMSO-d6, δ in ppm:** $^{1-3}$H (D, T-aromatic)-2.59, 1.56; $^{4-7}$H (D, T-aromatic)-6.33, 7.26; ^{8}H (methylene)-4.74; ^{9,10}H (D)-2.92.

M_4: N$_1$-[4-(4-[2-(2, 3, 4, 9-tetrahydro-1H-9- carbazolyl) acetyl] piperazinosulfonyl) phenyl] acetamide, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3043.81; C-H(str):2924.24; N-H(str):3395.25; C-N(bend):1232.82; C=O(str):1703.59; S=O(bend):1056.20; C-S(str):1141.74**, ^{1}H NMR (in DMSO-d6, δ in ppm:** $^{1-3}$H (D, T-aromatic)-2.59, 1.56; $^{4-7}$H (D, T-aromatic)-6.33, 7.26; ^{8}H (methylene)-4.74; ^{9,10}H (D)-3.31; ^{11,12}H(D)-7.92; ^{13}H(N-H)- 8.0; ^{14}H (S)-2.02.

M_5: 2-(5, 6, 7, 8-tetrahydrocarbazol-9-yl)-1-(4-methoxybenzenesulfonylpiperazin-1-yl) ethanone, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3015.21; C-H(str):2933.05; C-N(str):1003.33; C=O(str):1670.11; S=O(str):1060.15; C-S(str):1278.23; C-O:1120.39, **^{1}H NMR (in DMSO-d6, δ in ppm:** $^{1-3}$H (D, T-aromatic)-2.59, 1.56; $^{4-7}$H (D, T-aromatic)-6.33, 7.26; ^{8}H (methylene)-4.74; ^{9,10}H (D)-3.34; ^{11,12}H (D)-7.13; ^{13}H (S)-3.73.

M_6: benzyl 4-(4-[2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl) acetyl] piperazinosulfonyl) benzoate, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3035.49; C-H(str):2924.57; C-N(str):1232.09; C=O(str):1690.21; S=O(str):1053.30; C-S(str):1232.09; C-O:1142.45, **^{1}H NMR (in DMSO-d6, δ in ppm:** $^{1-3}$H (D, T-aromatic)-2.59, 1.56; $^{4-7}$H (D, T-aromatic)-6.33, 7.26; ^{8}H (methylene)-4.74; ^{9,10}H (D)-3.31; ^{11,12}H (D)-8.15; ^{13}H (S)- 8.0; ^{14}H (D, T-aromatic)-7.19.

M_7: Methyl 2-hydroxy-4-(4-[2-(2, 3, 4, 9-tetrahydro-1H-9-carbazolyl) acetyl] piperazinosulfonyl) benzoate**, FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3057.45; C-H(str):2921.32;C-N(ben):1223.57; C=O(str):1684.41; S=O(str):1044.60; C-S(bend):1186.52; C-O(bend):1132.83; O-H(str):3393.92**, ^{1}H NMR (in DMSO-d6, δ in ppm:** $^{1-3}$H (D, T-aromatic)-2.59, 1.56; $^{4-7}$H (D, T-aromatic)-6.33, 7.26; ^{8}H (methylene)-4.74; ^{9,10}H (D)-3.31; $^{11-13}$H (D, S)-8.08, 7.51; ^{14}H(O-H)-8.0; ^{15}H (S)-3.88.

K_1: 6, 7, 8, 9-tetrahydro-9-((2-(4-tosylpiperazin-1-yl)-1, 3-dioxolan-2-yl) methyl)-5H-carbazole, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3025.40; C-H(str):2928.36; C-N(str):1224.78; C-S(str):1147.60; S=O(str):1045.03; C-O(str):1002.90.
K_2: 9-(2-[4-(methylsulfonyl) piperazino]-1, 3-dioxolan-2-ylmethyl)-2, 3, 4, 9-tetrahydro-1H-carbazole, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):2983.09; C-H(str):2931.09; C-N(str):1309.24; C-S(str):1193.09; C-O(str):1111.66; S=O(str):1021.75.
K_3: 4-(2-((5, 6, 7, 8-tetrahydrocarbazol-9-yl) methyl)-1, 3-dioxolan-2-yl) piperazine-1-sulfinyl chloride, **FT-IR, υ_{max}, cm^{-1} (ATR):** C-H(str):3241.72; C-H(str):3135.79; C-N(str):1223.55; C-O(str):1154.97; S=O(str):1082.02.

3.2. Computational analysis of Synthesized Derivative Compounds

A total 14 synthesized Piperazine derivative compounds were carried out Molecular Docking studies with 1EVE Protein by using Autodock Vina software. The structure of 1EVE enzyme revealed that the active site was located at the bottom of a deep and narrow gorge containing Trp84, Phe329 and Phe330 residues in the catalytic active site (CAS) with Donepezil. It was observed that the most potent 1EVE inhibitor in our synthesized derivative compounds M_4, M_5, M_6, M_7, and K_6 displayed significant interactions in the CAS binding sites of the enzyme to provide maximal inhibitory profile as similar to Donepezil Standard drug. The positions of compounds M4, M5, M6, M7, and K6 with respect to the key residues in the binding sites were shown in **Figure 7-12.** The formation of strong Hydrogen bond between the synthesized Piperazine derivatives of M_4, M_5, M_6, M_7, and K_6 compounds were Hydrogen bond interaction with hydroxyl group of Tyr333, Hydrophobic bonds between aromatic rings interaction with π–π Stacking with the active site residues of Trp83, rings interaction with π-alkyl stacking with the alkyl groups of Phe329 and Phe330 respectively. The results of docking study revealed that the binding profile for synthesized derivative compounds M_4, M_5, M_6, M_7, and K_6 was found significant interactions with Donepezil due to hydrogen bond, hydrophobic interactions like π–π Stacking interaction and π-alkyl stacking interactions with CAS of 1EVE. The results of 14 synthesized derivative compounds with 1EVE were obtained as Molecular docking Studies were mentioned in **Table 3.**

Table 3: Docking Results of the Synthesized Compounds with 1EVE

S. No.	Comp. Code	Docking Score Kcal/Mole	Hydrogen bond Interactions (A°)	Hydrophobic bond Interactions (A°)		
				π–π Stacking	π-alkyl	
					PHE329	PHE330
1	M_1	11.8	-	4.30396	4.71159	3.97815
2	M_2	10.8	-	4.47491	4.27786	5.24422
3	M_3	10.8	2.81975	4.42876	4.35569	5.35569
4	M_4	11.7	2.91242	4.72482	5.58653	4.91909
5	M_5	11.7	2.85677	4.3723	4.50967	4.05876
6	M_6	12.9	3.48581	4.19792	5.39906	4.97908
7	M_7	11.7	3.07005	4.72952	5.65742	4.99557
8	K_1	10.6	2.75266	4.41858	4.28638	4.71038
9	K_2	9.9	2.34453	5.43824	4.54756	4.71038
10	K_3	9.9	3.22251	5.14755	4.54756	5.40406
11	K_4	10.7	2.71438	4.5213	-	-
12	K_5	10.8	2.82366	4.88208	5.18852	-
13	K_6	11.5	2.75911	4.19608	-	-
14	K_7	10.4	2.60506	5.54375	4.64783	-
Donepezil		10.9	2.55598	4.0411	4.04208	4.98362

A total 14 synthesized Piperazine derivative compounds were carried out Molecular Docking studies with 2BU8 Protein by using Autodock Vina software. To determine the binding sites and binding orientation of the synthesized compounds to 2BU8, the results were compared with Piperazine Analogue (4-(2-(5, 6, 7, 8-tetrahydrocarbazol-9-yl) acetyl) piperazine-1-sulfonyl chloride). The structure of 2BU8 revealed that the active site was located at the bottom of a deep and narrow gorge containing Asn242, Leu322, Leu332, Ala246, Ala290 residues in the catalytic active site (CAS) with Piperazine Analogue. It was observed that the most potent 2BU8 inhibitor in our synthesized derivative compounds M6, K_1, K_4, K_5, K_6 and K_7 displayed significant interactions in the CAS binding sites of the enzyme to provide maximal inhibitory profile as similar to Piperazine Analogue Standard drug. The position of compounds M_6, K_1, K_4, K_5, K_6 and K_7 with respect to the key residues in the binding sites were shown in **Figure 13-18.** The formation of strong Hydrogen bond between the synthesized Piperazine derivatives of M_6, K_1, K_4, K_5, K_6 and K_7 compounds were Hydrogen bond interaction with hydroxyl group of Asn242, Hydrophobic bonds between aromatic rings interaction with π–sigma Stacking with the active site residues of Leu322, rings interaction with π-alkyl stacking with the alkyl groups of Ala246, Ala290 and alkyl stacking interaction with the alkyl groups of Leu322, Leu332 respectively. The result of docking study revealed that the binding profile for synthesized derivative compounds M_6, K_1, K_4, K_5, K_6 and K_7 was found significant interactions with Piperazine Analogue due to hydrogen bond, hydrophobic interactions like π–sigma Stacking interaction, π-alkyl stacking interactions and alkyl stacking interactions with CAS of 1BU8. The results of 14 synthesized derivative compounds with 2BU8 were obtained as Molecular docking Studies were mentioned in **Table 4.**

Table 4: Docking Results of the Synthesized Compounds with 2BU8

S. No.	Comp. Code	Docking Score Kcal/Mole	Hydrogen bond Interactions (A°)	Hydrophobic bond Interactions (A°)		
				Alkyl	π-alkyl	π-sigma
1	M_1	8.1	3.6501	4.23619	5.15765	3.79737
2	M_2	8.2	2.91739	4.25368	4.26553	-
3	M_3	7.9	2.93315	4.23191	3.68357	-
4	M_4	8.6	2.86281	4.19027	4.2073	-
5	M_5	8.0	2.7589	4.54456	4.78292	3.70851
6	M_6	9.8	2.91254	4.94661	4.97111	-

7	M_7	8.8	2.62176	5.02248	4.9964	3.7974
8	K_1	9.0	2.90572	4.92185	4.68327	-
9	K_2	8.3	2.75759	3.37889	4.94896	3.88915
10	K_3	8.1	2.69357	3.34237	4.91591	3.8314
11	K_4	9.1	2.72466	5.49601	5.10581	-
12	K_5	9.0	2.88703	3.41353	4.9552	3.91597
13	K_6	9.3	3.00088	4.26732	4.46085	-
14	K_7	9.3	2.78095	4.33912	4.78712	-
Piperazine analogue		9.0	2.32713	4.47141	4.95202	3.78768

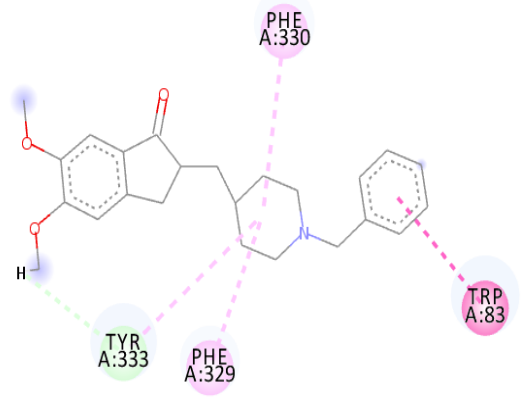


FIGURE 7: Binding interaction of Standard (Donepezil) with 1EVE

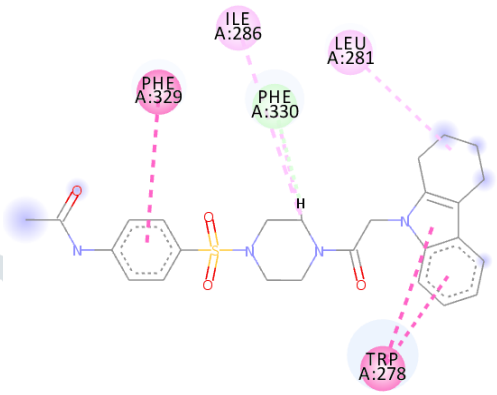


FIGURE 8: Binding interaction of M_4 with 1EVE

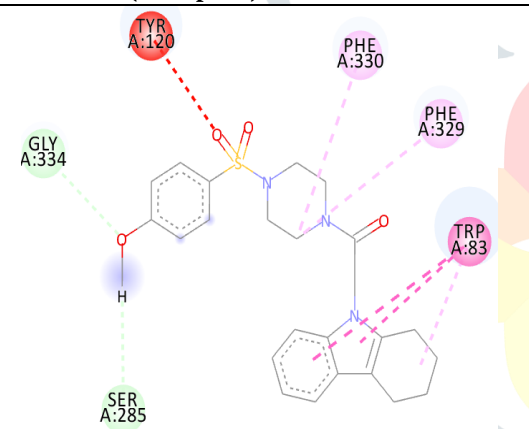


FIGURE 9: Binding interaction of M_5 with 1EVE

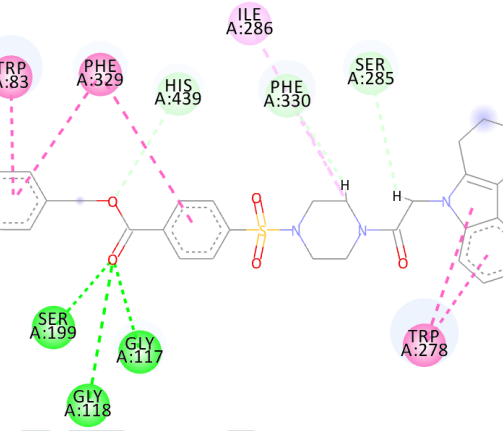


FIGURE 10: Binding interaction of M_6 with 1EVE

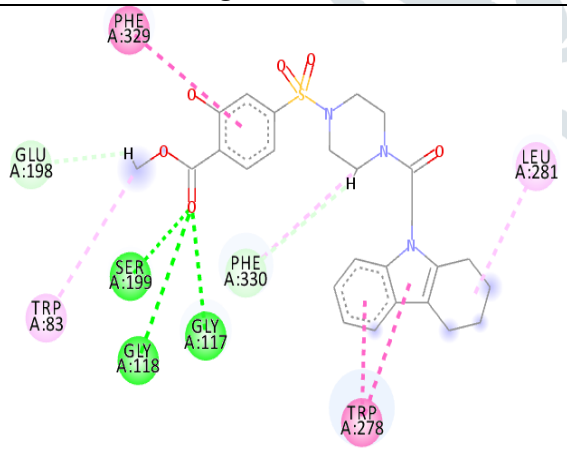


FIGURE 11: Binding interaction of M_7 with 1EVE

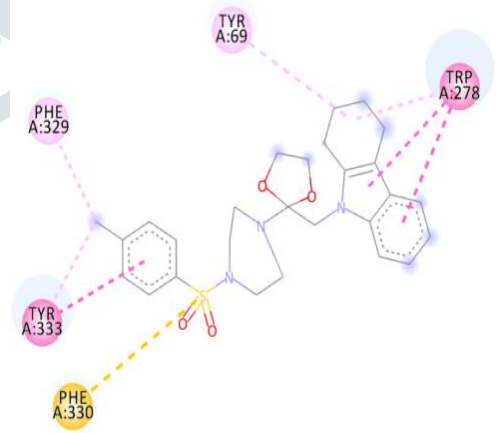


FIGURE 12: Binding interaction of K_1 with 1EVE

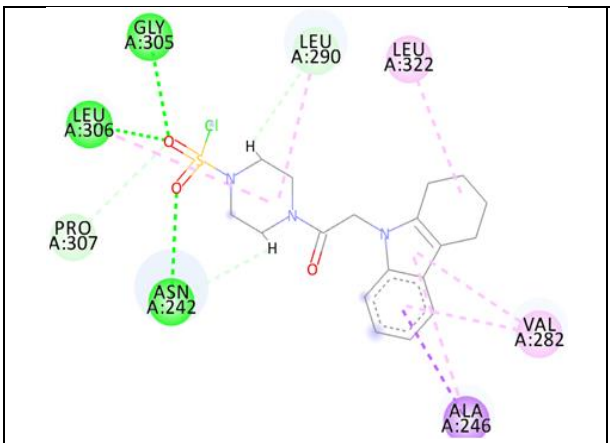


FIGURE 13: Binding interaction of Standard (Piperazine analogue) with 2BU8

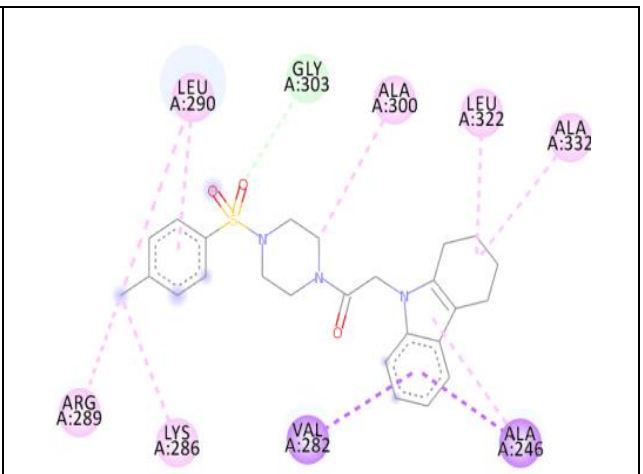


FIGURE 14: Binding interaction of M_1 with 2BU8

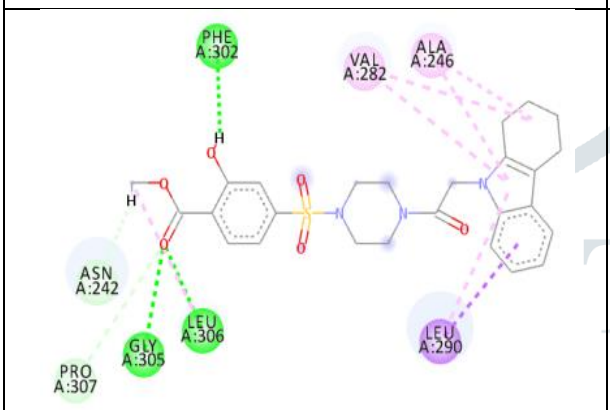


FIGURE 15: Binding interaction of M_7 with 2BU8

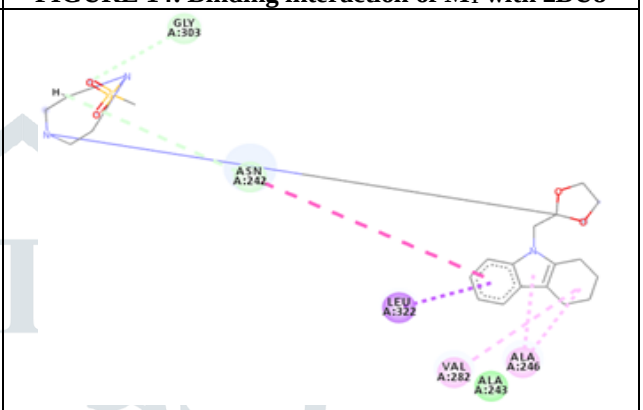


FIGURE 16: Binding interaction of K_2 with 2BU8

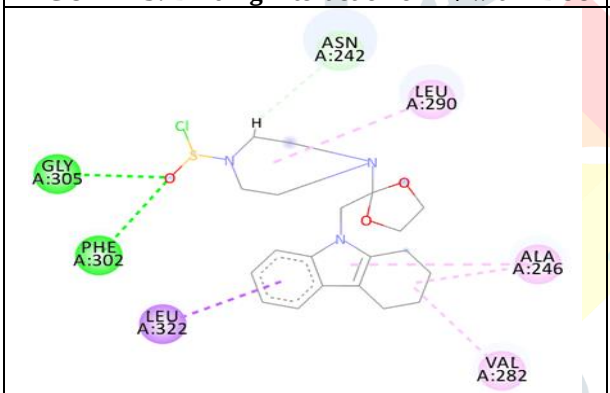


FIGURE 17: Binding interaction of K_3 with 2BU8

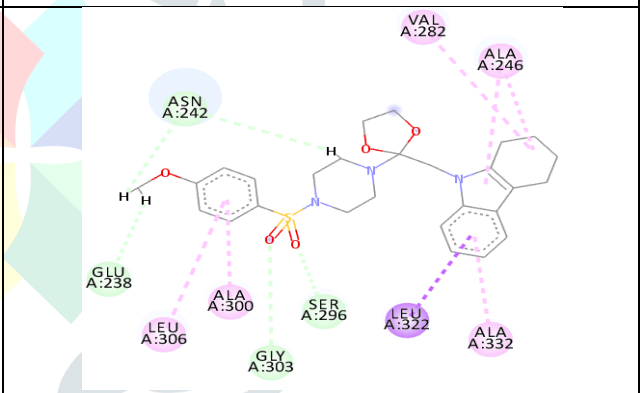


FIGURE 18: Binding interaction of K_5 with 2BU8

=Pi-Pi Stacking
=Conventional Hydrogen Bond
=Pi-Alkyl
=VanderWaals
=Pi-Sulfur
=Attractive Charges
=Carbon Hydrogen Bond
=Salt Bridges

To Predict the *In-silico* ADMET profile of synthesized molecules was done by using the admetSAR, SWISS ADME programmes physically significant descriptors and pharmacologically relevant properties of these compounds were predicted. Fundamental physiochemical features of CNS drugs are mainly related to their ability to penetrate the blood-brain barrier (BBB) and exhibit CNS activity. Our results indicated that compounds from all the two series **(M_1-M_7)** and **(K_1-K_7)** showed drug like characteristics based on Lipinski's rule of five (Mol MW<500, logP<5, donor HB<5, acceptor HB<10). All the synthesized derivative compounds were showed very low permeability for Caco-2, displayed good oral absorption, indicate their moderate binding with plasma protein and presented moderate in silico possible toxicity risks. The predicted ADMET properties revealed that all compounds fulfil drug-like criteria and could be considered as good candidate for drug development. All the synthesized compound derivatives have Standard Drug (Donepezil) like ADMET properties. The results of *in-silico* ADMET studies mentioned in **Table 5-7.**

Table 5.*In-Silico* Absorption and Distribution Studies of Synthesized Derivative Compounds

Comp. Code	ABSORPTION			DISTRIBUTION				
	Human Intestinal (HIA)	Aqueous Solubility (logS)	CaCO-2 Permeability (LogP, cm/s)	Plasma Protein Binding (PPB)	P-glycoprotein Substrate		P-glycoprotein Inhibitor	Blood Brain Barrier Penetration (BBB)
					Substrate	Inhibitor		
M_1	1.0000	-3.7114	0.7125	0.4745	0.6180*	0.6325*	0.5518	0.9123
M_2	0.9964	-3.5402	0.5988	0.4065	0.6937*	0.5000*	0.7489	0.9317
M_3	1.0000	-4.1693	0.9569	0.8480	0.5587*	0.8619*	0.7791*	0.9806
M_4	0.9936	-3.8094	0.6799	0.4208	0.6198*	0.5641*	0.5808*	0.8800
M_5	0.9973	-3.7580	0.6360	0.4980	0.6838*	0.6899*	0.6804*	0.8841
M_6	0.9843	-3.9030	0.3650	0.4786	0.5229*	0.7649*	0.6212*	0.8393
M_7	0.9791	-3.6529	0.4244	0.4865	0.6577*	0.5259	0.5753	0.5079
K_1	0.9952	-3.7333	0.5489	0.3533	0.5207	0.7055*	0.6878	0.8439
K_2	0.9853	-3.6298	0.4566	0.3780	0.5629*	0.6198*	0.7836	0.8531
K_3	1.0000	-3.9948	0.8472	0.4069	0.6398	0.6890*	0.7193	0.9261
K_4	0.9971	-3.7759	0.7288	0.3633	0.5335*	0.6769*	0.5889	0.8440
K_5	0.9917	-3.7460	0.6079	0.2966	0.5569*	0.7973*	0.6126*	0.8943
K_6	0.9924	-3.7935	0.4460	0.3817	0.5326	0.8080*	0.8001*	0.8826
K_7	0.9867	-3.6119	0.5830	0.3471	0.5919*	0.6450*	0.6619*	0.5989
Donepezil	0.9966	-2.4252	1.3424	0.8261	0.7721*	0.7641*	0.8202*	0.9953
Limit	1.0000	1-7.5	4.0000	1.0000				1.0000

*=Inhibitor/Substrate

Table 6.*In-Silico* Metabolism Studies Synthesized Derivative Compounds

Comp.Code	CYP450 Substrate			CYP450 Inhibitor				
	2C9	2D6	3A4*	1A2	2C9	2D6	2C19*	3A4
M_1	0.6387	0.7016	0.5410	0.9342	0.6075*	0.8406	0.7198	0.7641*
M_2	0.6667	0.6686	o.6152	0.9239	0.7662	0.8740	0.6594	0.6242
M_3	0.8500	0.6181	0.6015	0.7539	0.6844*	0.7400	0.9065	0.7092*
M_4	0.6446	0.7143	0.5634	0.9362	0.9194*	0.6863	0.7768	0.6524
M_5	0.6509	0.6873	0.6680	0.8941	0.7241*	0.9411	0.8280	0.8858*
M_6	0.7395	0.7516	0.5105	0.8755	0.7034*	0.8748	0.6615	0.6007*
M_7	0.6331	0.7412	0.6005	0.9121	0.5743*	0.9425	0.5815	0.5563*
K_1	0.6137	0.7538	0.5663	0.8927	0.7375*	0.8678	0.7581	0.8246
K_2	0.6435	0.7419	0.6315	0.8628	0.5102	0.9073	0.5327	0.9376
K_3	0.7566	0.7755	0.5989	0.5839	0.5184*	0.8160	0.7266	0.8422
K_4	0.6334	0.7482	0.5667	0. 8356	0.9111*	0.8040	0.8223	0.7739
K_5	0.6245	0.7291	0.6784	0. 8721	0.7010*	0.8873	0.8375	0.5348
K_6	0.6929	0.7578	0.5595	0. 8380	0.8093	0.8370	0.7981	0.7960
K_7	0.5883	0.7506	0.6429	0. 8779	0.6529*	0.8925	0.6518	0.6644
Donepezil	0.8465	0.8919*	0.7202	0.5072*	0.8189	0.8684*	0.8356	0.7411

*=Inhibitor/Substrate

Table 7.*In-Silico* Excretion and Toxicity Studies Synthesized Derivative Compounds

Comp. Code	Human Ether-a-go-go-Related Gene (hERG) Inhibitor	AMES Toxicity (non toxic)	Carcinogens (non-carcinogenic)	Tetrahymena Pyriformis Toxicity (Pigc50,ug/L)	Honey Bee Toxicity (HBT)	Biodegradation	Acute oral toxicity	Rat acute toxicity (LD50, mol/kg)	Fish Toxicity (pLC50, mg/L)
M_1	0.5162	0.5162	0.6959	0.4480	0.7343	0.9590	0.6328	2.4596	1.8023
M_2	0.5391	0.6407	0.8146	0.4614	0.7310	0.9376	0.6251	2.5952	1.7241
M_3	0.6505	0.7045	0.9178	0.7297	0.8882	0.9872	0.7319	2.5171	1.2941
M_4	0.5740	0.6808	0.7713	0.4896	0.8042	0.9854	0.6237	2.4386	1.6765
M_5	0.7534	0.6424	0.7656	0.4496	0.6341	0.9463	0.5963	2.5613	1.5706
M_6	0.5883	0.6558	0.8050	0.5123	0.6856	0.8889	0.5713	2.5009	1.6022
M_7	0.6103	0.6523	0.7507	0.4644	0.6770	0.9522	0.5735	2.5376	1.5297
K_1	0.6252	0.6129	0.7531	0.4956	0.7225	0.9854	0.5916	2.5144	1.5310
K_2	0.6698	0.5832	0.7895	0.5111	0.7181	0.9767	0.5839	2.6133	1.4879
K_3	0.7176	0.5699	0.7514	0.6400	0.7119	0.9699	0.5701	2.6330	1.3944
K_4	0.5901	0.6226	0.7445	0.4943	0.7864	0.9959	0.5965	2.5237	1.5049
K_5	0.7959	0.5903	0.7553	0.4828	0.6466	0.9796	0.5950	2.5819	1.4595
K_6	0.6068	0.6071	0.7670	0.5380	0.7114	0.9793	0.5759	2.5480	1.4876

K_7	0.7022	0.6025	0.7190	0.4974	0.7040	0.9840	0.5810	2.5632	1.4244
Donepezil	0.8095	0.6441	0.9528	0.2802	0.5853	0.9145	0.5250	3.0123	1.0750

IV. CONCLUSION

All the Synthesized compound derivatives of Novel 1, 4-disubstituted Piperazine Derivative Compounds were evaluated with Physical, spectral Characterization and its computational analysis by appropriate were compared with Donepezil, Piperazine Analog standard drugs respectively. The results of docking study revealed that the binding profile for synthesized derivative compounds M_4, M_5, M_6, M_7, and K_6 was found significant interactions with Donepezil due to hydrogen bond, hydrophobic interactions like π–π Stacking interaction and π-alkyl stacking interactions with CAS of 1EVE. The result of docking study revealed that the binding profile for synthesized derivative compounds M_6, K_1, K_4, K_5, K_6 and K_7 was found significant interactions with Piperazine Analogue due to hydrogen bond, hydrophobic interactions like π–sigma Stacking interaction, π-alkyl stacking interactions and alkyl stacking interactions with CAS of 1BU8. The predicted ADMET properties revealed that all compounds fulfil drug-like criteria and could be considered as good candidate for drug development. All the synthesized compound derivatives have Standard Drug (Donepezil) like ADMET properties.

The further scope of synthesized derivatives of Novel 1, 4-disubstituted Piperazine derivatives need to evaluation of various *in vivo* Pharmacological Studies to bring potentially active molecules.

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