DESIGN, SYNTHESIS OF NOVEL BENZOTHIAZEPINE DERIVATIVES AND ITS BIOLOGICAL EVALUATION AS POTENT Atu2422-GABA RECEPTOR INHIBITORS

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ABSTRACT

Starting with substituted aromatic aledehydes and aromatic ketones, a number of novel substituted derivatives benzothiazepine analogues were produced. By reacting with various aromatic aledehydes and aromatic ketones was transformed into substituted Chalcones, which were then reacted with 2-aminobenzenethiol under acidic conditions to produce the title compounds in good yields. The newly synthesized compounds were evaluated using elemental analyses, 1 H NMR, 13C NMR, and mass spectral studies. The ability of each new substance to inhibit Atu2422-GABA receptor in vitro was assessed. According to preliminary research, some of the designed series' molecules exhibited promising Atu2422-GABA receptor inhibitor properties. Rigid body docking studies were also carried out to comprehend the mode of binding and potential docking sites for the molecules on the target proteins.

Key-words: Chalcones, Anti-epileptic, GABA, inhibitors, Molecular docking

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INTRODUCTION

Numerous bioactive molecules contain the heterocyclic nucleus of thiazepine, which is recognised as a crucial building block for the synthesis of small molecules with potential pharmaceutical activities. [1]

Benzothiazepine derivatives are significant molecules in the field of medicinal chemistry due to their wide range of clinical applications and commercial success.[2-4] In the literature, numerous synthesis protocols for benzothiazepines have been developed. I'll give you two prime examples: 2,3-dihydro-l,4-benzothiazepine-3 is produced by the reaction of acid amides with phosphoryl chloride, and 1,4-benzothiazepines are created by the one-pot reaction of 2-aminobenzo[d]isothiazol-3-one and alkyl propiolates in the presence of triphenylphosphine.[5-7]

Additionally, molecules with a benzothiazepine skeleton have shown strong biological profiles. The anti-arrhythmic, angiogenic, central nervous system, antimicrobial, antioxidant, anti-inflammatory, analgesic, antitumor, and anticonvulsant properties of these classes of compounds have been well documented.[8-10] In addition to their demonstrated blockade of the mitochondrial sodium/calcium exchanger, 1,4-benzothiazepine derivatives also exhibit intriguing neuroprotective activity. A challenge for medicinal chemists is always the chemical modification of heterocyclic systems by developing a new protocol for the design of new compounds with high pharmacological profiles. Here, we present the synthesis of functionalized 1,4-benzothiazepine derivatives and the results of in vitro tests to determine how well they can acts as Atu2422-GABA receptor inhibitor.[11-13]

EXPERIMENTAL

Studies on molecular docking Software called VLifeMDS version 4.3 was used to analyse the enzyme-inhibitor complex's structural makeup. Three hundred expected 1,5-benzothiazepine derivatives were tested for our study. Using a semi-empirical free energy force field, VLifeMDS version 4.3 anticipated the binding free energies of enzyme-inhibitor complexes as well as the binding energies of both the bound and unbound states. Following PDBs' 3D structures were obtained from the RCSB Protein Data Bank. PDB identifier: 3IP9 (Structure of Atu2422-GABA receptor in complex with GABA). Selected 1,5-benzothiazepine derivatives' 3D structures were created in ACD-Chemsketch and then converted to 3D mol. format. Utilizing the Vlife MDS Tool, the automated docking model was created. In order to create the grid box for catalytic inhibition mode, the co-crystallized ligand was used.[14-15] The chosen grid box dimensions were 606060. To count the number of H-bonds and van der Waals interactions, PyMOL 1.7.4 and LigPlot+ were used. The amino acids with the binding pockets were predicted at the Q-site finder

server in order to ascertain the binding affinities between the ligand and receptor.

Figure 1 illustrates the method used to synthesize the target compounds **A1-A10**. The Claisen-Schmidt condensation reaction of aromatic ketones and aromatic aledehydes in the presence of potassium hydroxide in methyl alcohol produced the intermediate Chalcones, **a1-a10**. The target molecules A1-A10 were then created by the reaction of the Chalcones **a1-a10** with 2-aminobenzenethiol, concentrated hydrochloric acid, and methyl alcohol under ultrasonication for different time interval.

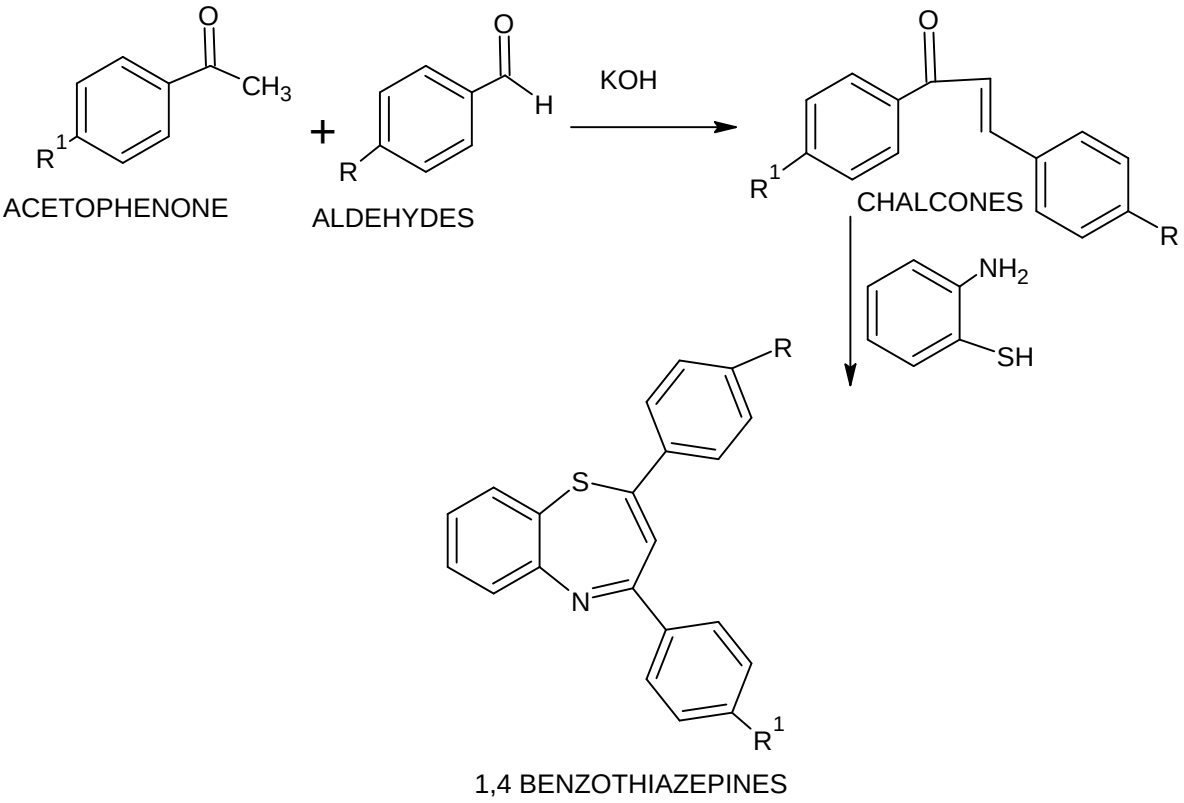

Fig. no. 01: Scheme for the synthesis of 1,4-Benzothiazepines

Comp. code	-R	-R1
A1	-H	-OH
A2	-H	$-OCH_3$
A3	-H	-Cl

A4	**-H**	**-F**
A5	**-H**	**-NO_2**
A6	**-OH**	**-Br**
A7	**-OH**	**-H**
A8	**-OH**	**-Cl**
A9	**-OH**	**-F**
A10	**-OH**	**-NO_2**

SYNTHESIS OF CHALCONE (a1-a10)

The well-known Claisen-Schmidt condensation of acetophenones and substituted aldehyde using alcoholic KOH (10%) at room temperature was the primary method used to create,-unsaturated carbonyl compounds or chalcones. The reaction took 30 minutes to complete, and TLC was used to monitor it. Residue was then poured onto ice water (100 mL). It was kept in the fridge all night. In order to obtain the corresponding substituted chalcones,(a1-a10) the resulting solid was filtered, collected, and washed with distilled water. Re-crystallization was used to filter and purify the precipitate from hot ethanol.[16]

SYNTHESIS OF SUBSTITUTED BENZOTHIAZEPINES (A1-A10)

Different substituted chalcone (a1-a10) and 0.01 mole of substituted mercapto aniline was dissolved in 10 ml of 2-methoxyethanol. To this 0.001 mmol of piperidine was added to reaction mixture and refluxed for 10-15 min (TLC). Then reaction mixture was cooled to room temperature. Solid separated was isolated by simple Buchner filtration; final purification was achieved by crystallization from ethanol to give A1-A10. The details are depicted in Fig. no.01.[16]

***IN-VIVO* ANTI-CONVULSANT ACTIVITY [17, 18]**

Experimental animals

Wistar albino rats, weighing 180–200 g, were used collectively as test subjects. The institute's central animal house is where the animals were obtained. Standard pelletized feed (Amrut mice feed, Pune, India) and unlimited access to water were given to the animals. Before starting the experiment, animals were acclimated to the lab environment. All experiments used animals that were available in the right size, age, weight, and sex. In CMC, the standard medications and test compounds were suspended and given intraperitoneally (IP). All experimental protocols were carried out

in accordance with ethical principles and guidelines and with approval from the institutional animal ethical committee (Protocol No. SCOP/IAEC/63/14-15), which was established by the Ministry of the Environment to control and oversee the use of experimental animals.

MES (Maximal Electroshock method)

Using predictable animal models, the synthesized compounds A1–A10 were first evaluated for their ability to treat convulsions. The animal seizure models most frequently used in the search for novel anticonvulsants are represented by the MES seizure models. Ten groups of albino rats, each weighing between 100 and 120 gm, were used for the anticonvulsant activity. Six animals each made up each of the three groups of animals (control, standard, and test). Rats were given the test compounds A1-A10 by i.p. route 60 minutes before the start of the experiments, at a concentration of 100 mg/kg in comparison to the reference drug phenytoin. After an hour, they received a 150 mA convulsiometer shock delivered through ear electrodes for 0.2 seconds. Extensor response-free animals were regarded as protected rats. Students used the "t" test and one-way ANOVA in Graph Pad Prism to analyze the data. Statistical significance was defined as a value of P 0.05. Standard error of the mean (SEM) is expressed as mean SEM for all values.

RESULTS AND DISCUSSION

To estimate the geometrics of the enzyme inhibitor interaction for the chosen compounds, a molecular docking approach was used. The docking results for 1,5-benzothiazepine derivatives (300 1,5-benzothiazepine moieties) with interacting 3IP9 (Structure of Atu2422-GABA receptor in complex with GABA) residues, including hydrogen bond, van der Waals, and hydrophobic interacting residues, The potential of A1-A10 and sodium phenytoin against 3IP9 (Structure of Atu2422-GABA receptor in complex with GABA) was observed to be related to the binding energy and the number of bonds formed at the catalytic site out of 300 1,5-benzothiazepine moieties that were chosen (Figure no. 2). The enzyme-inhibitor interaction was observed to be further stabilized by the hydrogen bond and hydrophobic interactions of A1-A10 and sodium phenytoin with proteins. It was found that the 3IP9 had interactions with Sodium Phenytoin, A1-A10 through hydrogen bonds. The LYS637A amino acid at distance 2.142 of the 3IP9 proteins further stabilized the enzyme-inhibitor interaction. Due to the LEU282B amino acid at 2.173, 2.164, and 2.173, respectively, the hydrogen bond interactions A1-A10 with

3IP9 proteins further stabilized the enzyme- .
inhibitor interaction

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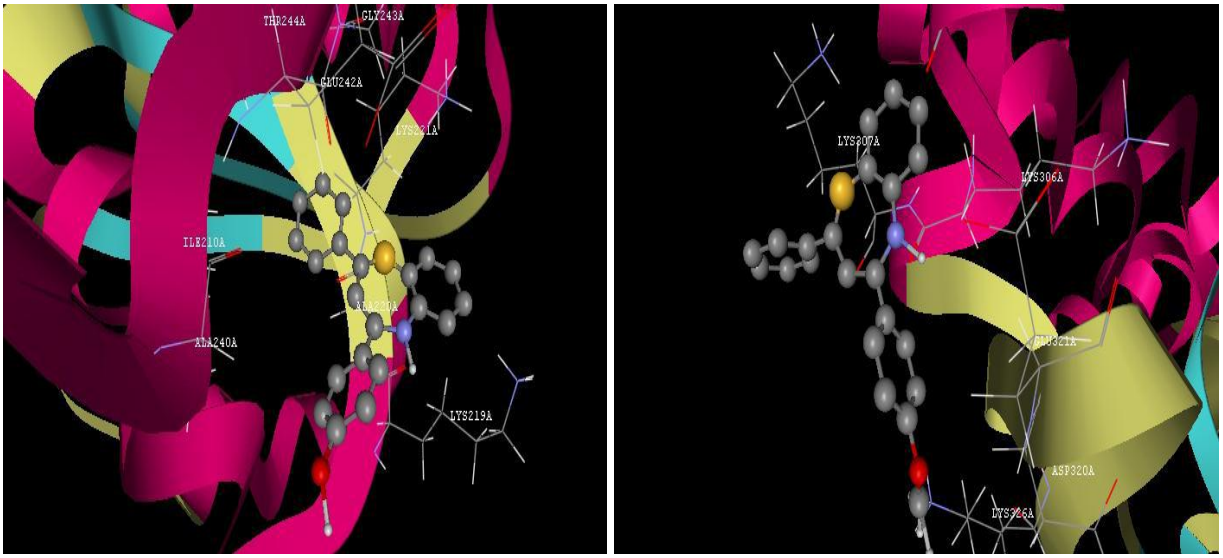

Fig. no. 02: Interaction of Benzothiazepine with GABA receptors

Table no. 01: Physicochemical properties of compounds (A1-A10)

COMP.CODE	MOLE FORMULA	MOL.WEIGHT	M.P. (^{0}C)	% YIELD	Rf VALUE
A1	C_{21}H19NOS	333.44	215-218	79.86	0.73
A2	C22H21NOS	347.47	221-225	80.65	0.71
A3	C21H18ClNS	351.89	231-234	86.75	0.62
A4	C21H18FNS	335.43	224-228	87.61	0.66
A5	C21H18BrNOS	412.34	219-221	88.43	0.61
A6	C21H18N2O2S	362.44	218-223	87.89	0.65
A7	C21H19NOS	333.44	210-214	78.87	0.54

A8	C21H18ClNOS	367.89	224-228	81.68	0.61
A9	C21H18FNOS	351.43	218-220	75.81	0.68
A10	C21H18N2O3S	378.44	222- 226	70.21	0.55

Table no. 02: Anticonvulsant activity of synthesized compounds (A1- A10)

Maximal electroshock seizure test			
Treatment (dose, mg/kg, i.p)	**Mean duration of tonic hind leg extension (THLE) ± SEM (s)**	**No. of animals recovered**	**Protection against mortality (%)**
Control	09.39 ± 1.09	3/6	50.00
Phenytoin (20)	Absence of extension	6/6	100.00
A1 (100)	01.18 ± 0.41**	4/6	50.00
A2 (100)	01.12 ± 0.022**	6/6	100.00
A3 (100)	08.53 ± 0.66*	6/6	100.00
A4 (100)	01.09 ± 0.07**	6/6	100.00
A5 (100)	12.63 ± 0.56*	2/6	33.33
A6 (100)	10.87 ± 0.41*	4/6	66.66
A7 (100)	09.15 ± 0.47*	3/6	50.00
A8 (100)	12.31 ± 0.56*	2/6	33.33
A9 (100)	11.00 ± 0.43*	1/6	16.66

A10 (100)	08.76 ± 0.65*	3/6	50.00
The results are shown as the mean values and SEM. * P<0.05, ** P < 0.01 & *** P<0.001 when compared to respective control values by Student‟s t-test			

In comparison to the other synthesized compounds, the series (A1 to A10) A2, A3 compounds had significantly lower mean durations of tonic hind leg extension (1.12, 8.53, 1.09, 1.12, 1.19, 1.08 respectively) and demonstrated significant activity against tonic seizures. MES was used to investigate the anti-seizure properties of all the synthesized compounds A1–A10 (Table no. 02). When assessing the anticonvulsant activity, it was found that compounds with phenyl rings introduced at the C2 and C4 positions of the substituted benzothiazepine ring, as well as electron withdrawing and electron donating groups had the strongest anticonvulsant effects.

SPECTRAL DATA

The TLC also displayed the development and culmination of the reaction. Initial IR analysis supported the final products' functional group and purity. The following functional groups, including C=C str, C-H str, C-H bend, N-H str, Ar C-N str, O-H stretch, C-H str, O-C, -C-Cl, -C-F, C-Br, -N-Osym, -N-Oasym, etc., have been observed in the IR of the compound A1-A10. A characteristic C-N band was assigned to compound A1 to A10 at 1290, 1299, 1309, 1330, 1300, 1316, 1320, 1330, 1310, and 1340 cm-1, respectively. The IR spectra of compound A1, A6, A7, A8, and A9 showed a broad band at 3210, 3150, 3060, 3101, 3110, and 3050 cm-1, respectively, attributed to the (OH) group. A2 compound, which has the -OCH3 group, displayed the -C-Ostr with a strong peak at 741 cm-1. There are sharp peaks of 741, 520, 580, 780, and 560 cm-1 for the halogenated compounds A3, A4, A6, A8, and A9. Two peaks of symmetry and asymmetry in the range of 1150 to 1350 with a strong and sharp edge were confirmed for the nitro-containing compounds (A5 and A10).

The hydrogen content of the compounds A1 to A10 was confirmed by 1H NMR. The hydrogen was identified in the 1H NMR as thiazepine (2H), H-Ar ring, -NH, -OH, and -CH3 (3H). A1 to A10 compounds were identified by their distinctive multiplet pattern and aromatic hydrogen in the range

of 6.2 to 7.6 ppm. All compounds showed thiazepine ring hydrogen at 7.8 and 5.9 ppm singlet peaks. The signals at 8.5, 8.5, 8.4, 8.6, 8.7, and 8.4 ppm in the 1H-NMR spectra of compounds A1, A6, A7, A8, A9, and A10, respectively, are attributed to the one protons of the (-OH) group attached to the benzyl group. The observed singlet 1H peak at 3.8 ppm was conformed to by the -CH3 group in the A2 compound.

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