

Design, Synthesis and Pharmacological Evaluation of Sulfonamide Derivatives Screened Against Maximal Electroshock Seizure Test

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Abstract

A series of substituted 4-amino-benzene sulfonamides were designed, keeping in view the structural requirement of pharmacophore. Lipinski rule of five has also been calculated; failure to Lipinski rule was not observed. The synthesized compounds were evaluated for anticonvulsant. Docking was performed through AutoDock Vina. Molecules have been screened out through docking. Compounds were synthesized and characterized through IR, ¹H NMR, ¹³C NMR, Mass and elemental analysis. The anticonvulsant activity of the synthesized compounds was assessed using the MES model. *In-silico* biological activity spectrum, toxicological studies, predicted oral rat LD50 and possible mechanism of action were investigated. The compound, 4-[2-(4-Acetyl-phenylamino)-ethyl]-benzenesulfonamide (K23) is found most active among the synthesized compounds.

Keywords: Anticonvulsant; Sulfonamide; Docking; In-silico studies; Synthetic compounds

Introduction

Epilepsy is one of the serious disorders with no age, sex or racial boundaries. Epilepsy imposes a large economic burden on health care systems of countries. There is also a hidden burden associated with stigma and discrimination against the patient and even their family in the community, workplace, and home. In general, the patients with epilepsy suffer severe emotional distress, behavioral disorders and extreme social isolation [1].

Epilepsy is usually associated with recurrent seizures. This implies that epilepsy is an ancient disorder; indeed, in all civilizations it can be traced as far back as medical records exist. In fact, epilepsy is a disorder that can occur in all mammalian species, probably more frequently as brains have become more complex. Epilepsy is also remarkably uniformly distributed around the world [2].

Among the reported potential agents to treat the epilepsy, sulphonamides are important and their significance cannot be ignored. Sulphonamides are of high attention for various pharmacological importance, as it is reported for chiefly anticonvulsant [3,4], antitubercular [5,6] and antimicrobial activities [7,8]. For all these activities, the sulfonamide derivatives are reported with multiple scaffolds such as Sulfonamides incorporating Valproyl derivatives, Sulfamates, Sulfamides, Thiazolidin derivatives, Aminobenzamides, Beta-Ketosulfonamide etc [3-8].

Being inspired from the previous developments, there is always an enthusiasm to design of novel sulphonamide with many scaffolds and to evaluate their possible pharmacological properties as well as mechanism of action. These novel designs will support for future drug developments for multiple diseases in human health care. There is always a chance to get a potential compound on hit and trial basis for design and synthesis of novel compounds. Beyond this, at present multi-disciplinary protocols are being suggested to screen the designed-only compounds through computational methods to get the possibility of pharmacological importance of compounds prior to their synthesis and in-vitro experiments. Sequential computational procedures include: (i) Collection of information about scaffolds, linkers and side-groups for possible novel compound; (ii) Structural designing of location specific derivative combinations with one or more scaffold; (iii) Virtual-screening of activity of designed compounds through structure-

activity-relationship (SAR) models; (iv) Ligand-protein docking studies of designed compounds; (v) Toxicity estimation of designed compound and (vi) Evaluation of possible pharmacological properties designed compounds. The newly designed compounds, which follow the criteria considered in multiple computational studies, are preceded for synthesis, structural elucidation and further experimental evaluation of activities.

In this paper, we have designed and synthesized compounds as carbonic anhydrase inhibitors, keeping in view the structural requirement of pharmacophore. All the designed compounds were initially gone through *in-silico* screening like Lipinski rule, QSAR modeling & screening and docking studies. The screened compounds were then synthesized and gone through analytical studies like IR, NMR and Mass spectroscopy. After that all the synthesized compounds were treated for pharmacological screening. An *In-silico* biological activity spectrum, toxicological studies, predicted oral rat LD50, possible mechanism of action and SAR studies were also investigated.

Steps followed for completing the current research work is shown in following illustration Figure 1.

Materials and Methods

Designing of molecules

Identification of pharmacophoric groups was done from the structures of well-known anticonvulsants. The structures of well-known anticonvulsants and identified pharmacophore are presented in Table 1 [9,10]. The essential structural features which could be responsible for an interaction with the active site were a hydrophobic (HP) unit, an electron donor (D) group, and a hydrogen donor/acceptor (HD/HA) unit.

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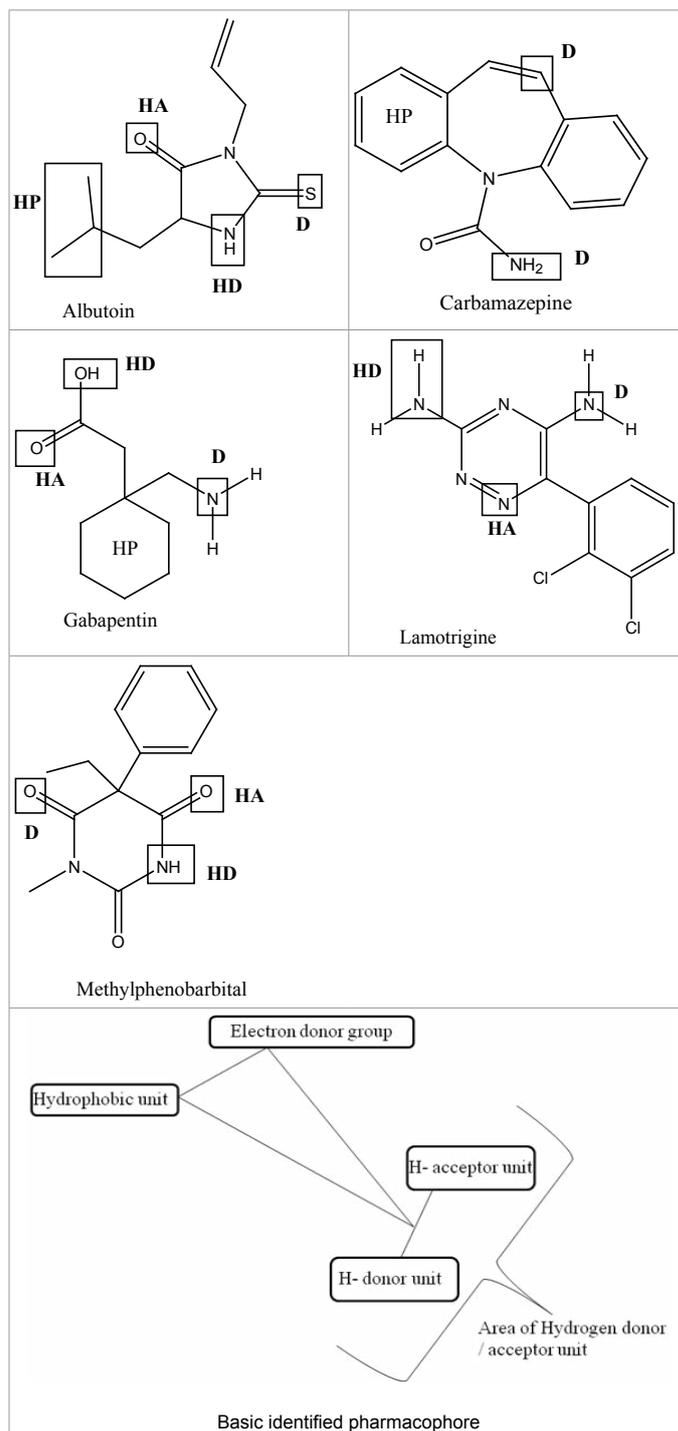
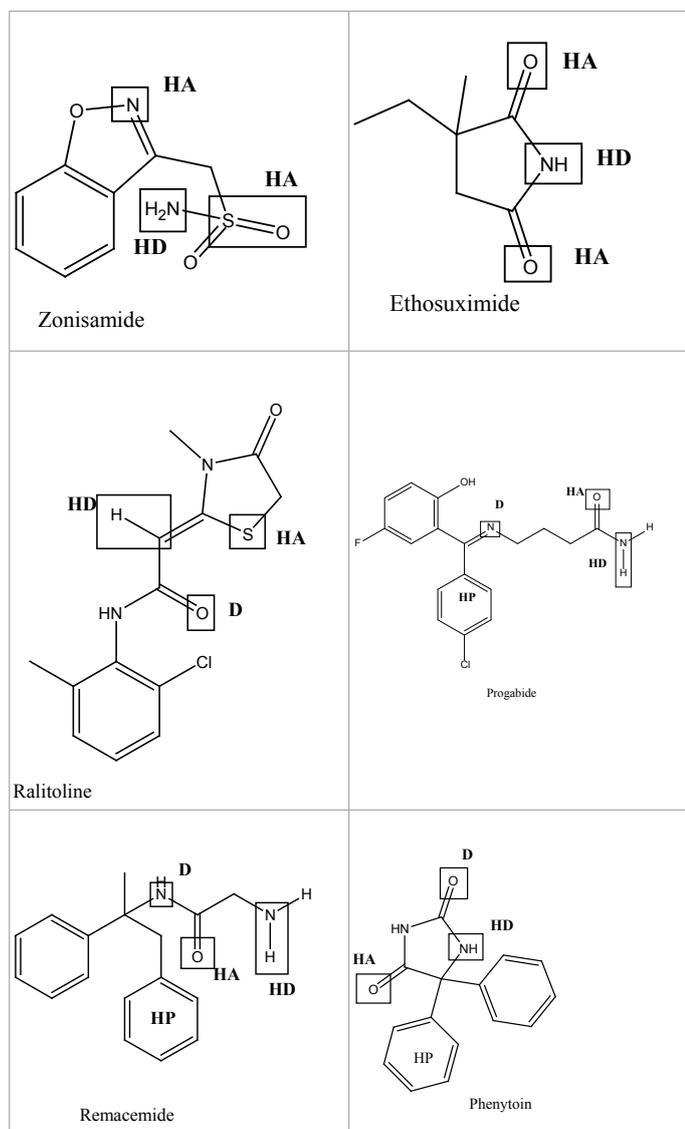
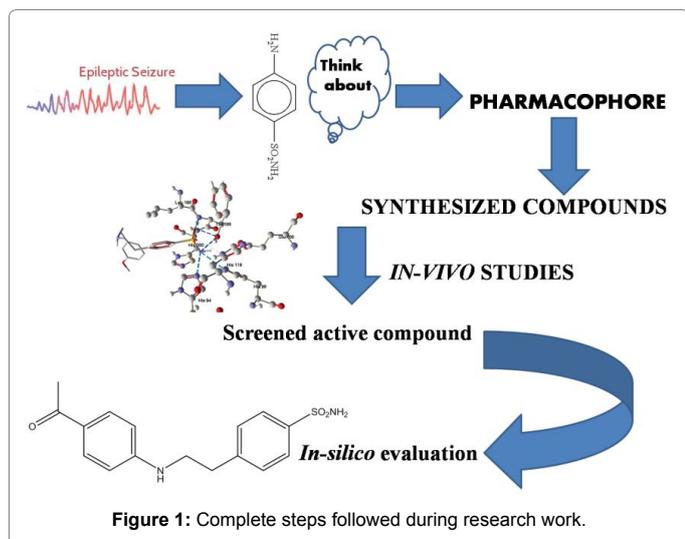


Table 1: Pharmacophoric pattern of well-known anticonvulsants.

Docking studies

Docking tool: Docking was performed with AutoDock Vina (PyRx-Python Prescription 0.8) docking software [11]. It is virtual screening software for computational drug discovery that can be used to screen libraries of compounds against potential drug targets. It enables medicinal chemists to run virtual screening from any platform and helps users in every steps of this process from data preparation to job submission and analysis of the results [12].

Receptor: Lyase (Oxo-acid)-Human Carbonic Anhydrase-I.

Experimental: All the chemicals and solvents, purchased from Merck (India), Spectrochem (India), Sigma-Aldrich (India), Hi-Media (India) and S.D. Fine (India) were used without further purification. Thin layer chromatographic (TLC) analysis of compounds was performed on silica gel G coated glass plates. The adsorbent silica gel G was coated to a thickness of about 0.3 mm on previously cleaned TLC plates of 20x5 cm² using conventional spreader. The plates were placed in hot air oven at 105°C for 30 min. The solutions of compounds were applied as a spot on the activated plate about 2 cm above from the lower edge. The mobile phases were selected according to the polarity of compounds.

Melting points were determined by using open capillary melting point apparatus and are reported uncorrected. The ¹H-NMR spectra were recorded on Avance-III, Bruker, 400 MHz High Resolution NMR spectrometer and ¹³C-NMR were recorded with Avance-III, Bruker, 100 MHz. Signals were described as singlet (s), doublet (d), triplet (t) and multiplet (m). FT-IR spectra (KBr) were recorded on a Perkin-Elmer Spectrometer BX-II spectrophotometer. The mass spectra were recorded on a Waters Micro-Mass ZQ 2000 mass spectrometer.

Synthetic scheme

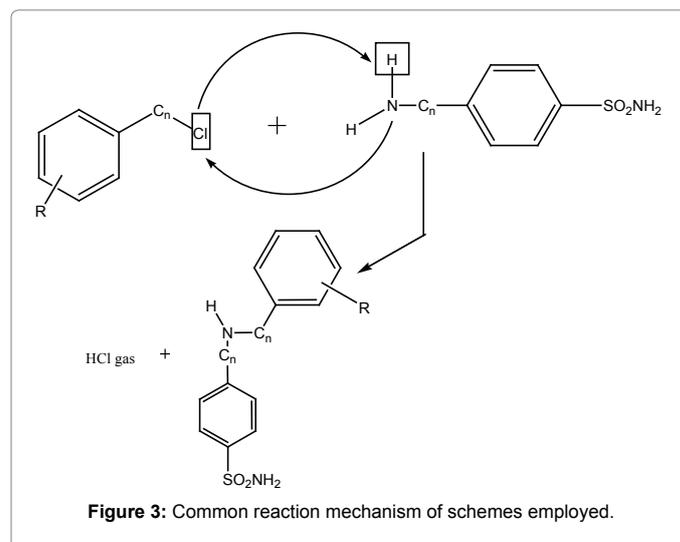
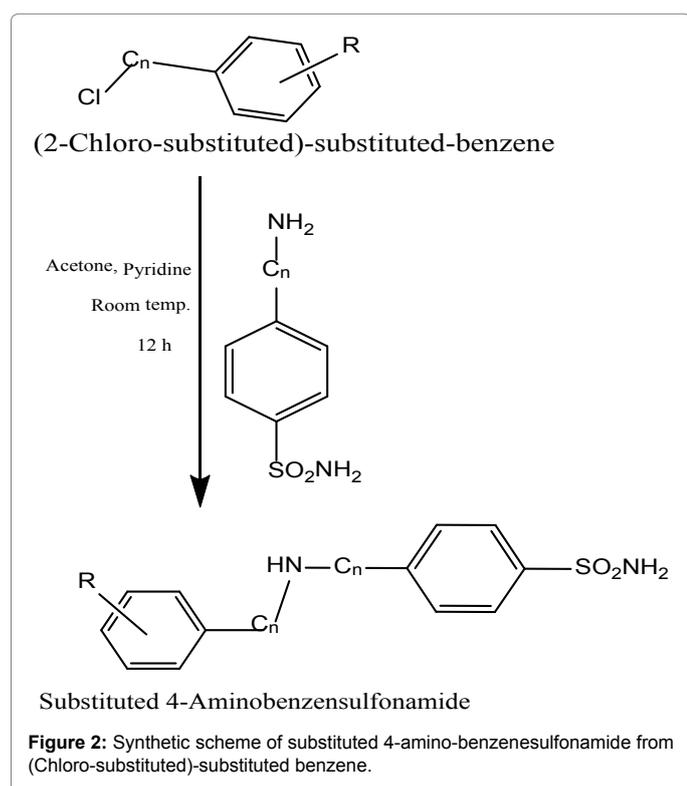
Shown in following illustration Figure 2.

Mechanism of reaction

A mechanism of reaction of given schemes has been depicted in Figure 3.

Synthesis of substituted 4-amino-benzenesulfonamides

For the synthesis of an appropriate amide, the substituted substituted chlorobenzene (0.009 mol) of an individual acid dissolved in 20 ml. of dry acetone was added drop wise to a stirred solution of suitable aminosulfonamide (0.0092 mol) and pyridine (0.0091 mol)



in 50 ml. of dry acetone. After addition, the reaction mixture was stirred for about 12 hour at room temperature and then the solvent was evaporated under reduced pressure. The residue was dissolved in 100 ml ethyl acetate and the organic phase washed three times with 20 ml of distilled water (3x20 ml). Then 10% HCl solution was added until pH 1 was reached, and the organic phase was separated from the aqueous phase and washed three times with brine. The aqueous solutions were combined and extracted with ethyl acetate (3x50 ml). The ethyl acetate extracts were combined and dried over MgSO₄ and product obtained was filtered and evaporated under reduced pressure [13].

Pharmacology

Anticonvulsant activity

Animals: Rats of weight (125-160 g) were used to study the effect of test drug on MES induced seizures via "Electro convulsometer". Female animals were excluded because of the fact that estrus cycle influences the seizure threshold. The protocol for animal based experiments was approved by Institutional Animal Ethical Committee, IFTM University, Moradabad (Resolution no. 2015/837ac/PhD/04) as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). Animals were housed in polypropylene cages with dust free rice husk as a bedding material under laboratory condition with controlled environment of temperature 25°C ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines. They were provided with balanced food and water, before subjecting them to experimentation, the animals were given a week's time to get acclimatized with the laboratory conditions. The animals were fasted overnight before the experiment.

The synthesized compounds were suspended in 0.5% methyl cellulose and the test compound was usually manipulated with a mortar pestle to help preparation of suspension. In the preliminary screening by MES, each compound was administered, at dose level of 100 mg/kg (i.g.,) and anticonvulsant and neurotoxicity was assessed at 30 min and 2 h intervals after administration with different groups at each time interval having same species of animals.

The Maximal Electroshock Seizure (MES) model or Maximal Seizure Pattern Test

In the MES test, an electrical stimulus of 0.2 s in duration (150 mA in rat at 60 Hz) is delivered via trans-auricular electrodes. Rats

are tested at 30 minutes and 2 hours following doses of 100 mg/kg of test compound. Rats are tested at time intervals between 30 min. and 2h following a standard dose of 100 mg/kg (i.g.). Abolition of the hind limb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread [14-16]. Control animals were also treated with the same MES procedure as of the test animals. Different behavioral phases under MES model including Flexion (legs in particular are bent), Extensor (arms and legs being flung outwards), Clonus (sustained rhythmical jerking) and Stupor (reduced level of consciousness) were also recorded for their duration of action (Sec) so that a reduction in duration of phases can be observed as an additional parameter for MES.

Minimal neurotoxicity

For minimal toxicity, 3 assessments i.e. rota rod test positional sense test, and gait and stance test were used.

Toxicity induced by a compound is detected in rats using the standard rota rod test. Untreated control rats, when placed on a 6 rpm. Rotation rod can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of a mouse to maintain equilibrium for one minute in each of three successive trials [17].

Rats are examined for behavioral toxicity by the positional sense test and a gait and stance test. In the positional sense test, one hind leg is gently lowered over the edge of a table, whereupon the rat, experiencing neurological deficit, will fail to lift its leg quickly back to a normal position. In the gait and stance test, neurotoxicity is indicated by a circular or zigzag gait, ataxia, abnormal spread of the legs, abnormal posture, tremor, hyperactivity, lack of exploratory behavior and stupor. Neurotoxicity tests have been carried with a dose of 100 mg/kg body weight at 30 min and at 4 hours from dose administration.

In-silico Studies

In-silico biological activity spectrum

The biological activity spectrum of a chemical compound is the set of different types of biological activity that reflect the results of the compound's interaction with various biological entities. Biological activity is defined qualitatively ("yes"/"none") suggesting that the biological activity spectrum represents the "intrinsic" property of a substance depending only on its structure and physical-chemical characteristics.

PASS (Prediction of Activity Spectra for Substances) [18] is a software product designed as a tool for evaluating the general biological potential of an organic drug like molecule. PASS provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. Thus, we have used PASS to estimate the biological activity profiles for virtual molecules.

Probability "to be active" estimates the chance that the studied compound is belonging to the sub-class of active compounds (resembles the structures of molecules, which are the most typical in a sub-set of "actives" in PASS training set).

Predictive toxicology

Chemical safety is an important concern of 'The Organization for Economic Co-operation and Development' OECD (<http://www.oecd.org/chemicalsafety/>). OECD runs 'Quantitative Structure-Activity Relationships Project' under the flag of assessment of chemicals. United States Environment Protection Agency (EPA) leads U.S. engagement

with the OECD's Environment Policy Committee (EPOC) and related subsidiary bodies. EPA pays attention towards safer chemical research. Toxicity Estimation Software Tool (TEST) is an *in-silico* predictive toxicology application, which is used by EPA for screening of new coming compound during chemical research. TEST software includes a set of QSAR models for screening of small molecules against various toxicity parameters as defined at its website: (<https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test>). In the present study, we have used TEST software inbuilt QSAR models for estimation of mutagenicity as well as Oral rat 50% lethal dose (LD50) of newly synthesized sulphonamides. Predictive toxicology studies are performed for approval of safe chemical research.

Mutagenicity

It's a capacity to induce mutation, as it said in genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.

Irritants

Irritants are chemicals that cause reversible skin damage (unlike corrosion, which is irreversible). Clinical signs of irritation include the development of a rash, inflammation, swelling, scaling, and abnormal tissue growth in the affected area.

In-silico mutagenicity and skin irritancy studies

The Ames laboratory test was developed in the 1970's by Bruce Ames, Professor of Biochemistry at UC-Berkeley, as a fast and sensitive assay of the ability of a chemical compound or mixture to induce mutations in DNA.

Ames *In-silico* test was performed for finding probability of being mutagenic and skin irritant with the help of trial version of Discovery Studio 3.5 using TOPKAT® (Toxicity Prediction by Komputer Assisted Technology) [19] and freely available version of TEST 4.2.1 (Toxicity Estimation Software Tool) (U.S. EPA, 2016) using Consensus method.

Predicted oral rat LD50

Lethal dose (LD50) is the amount of an ingested chemical substance that kills 50 percent to test animal. It is expressed in mg/kg, or milligrams of substance per kilogram of body weight. Commonly it is known as lethal doses.

Predicted oral rat LD50 has been calculated with help of freely available version of TEST 4.2.1 (Toxicity Estimation Software Tool) (U.S. EPA, 2016) using Consensus method.

Results and Discussion

Designed molecules

A list of designed molecules has been shown in Table 2.

Lipinski rule of five

An initial descriptor calculation has also performed in order to observe designed compounds with its drug ability property (Lipinski Rule of Five) (Table 3).

Lipinski rule of five [20] helps in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules:

- Molecular mass less than 500 Dalton

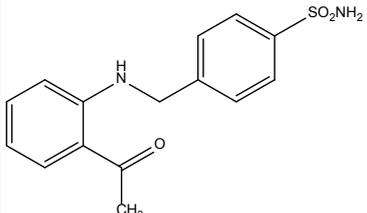
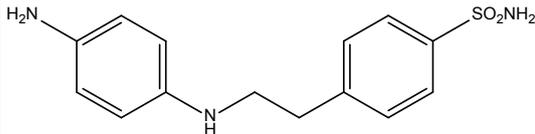
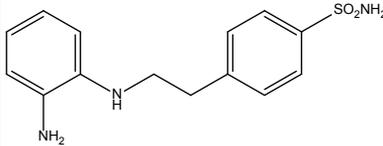
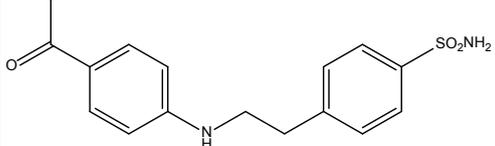
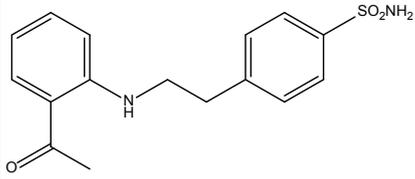
S. No.	Compound	Structure
K 16		4-[(2-Acetyl-phenylamino)-methyl]-benzenesulfonamide
K 20		4-[2-(4-Amino-phenylamino)-ethyl]-benzenesulfonamide
K 22		4-[2-(2-Amino-phenylamino)-ethyl]-benzenesulfonamide
K 23		4-[2-(4-Acetyl-phenylamino)-ethyl]-benzenesulfonamide
K 25		4-[2-(2-Acetyl-phenylamino)-ethyl]-benzenesulfonamide

Table 2: Designed molecules.

S. No.	Comp.	Molec. mass	AlogP	nHBD	nHBA	MR	Lipinski failure
1	K 16	304.0882	1.0802	2	5	88.051	0
2	K 20	291.1041	1.0688	2	5	86.5023	0
3	K 22	291.1041	1.0288	2	5	86.5023	0
4	K 23	318.1038	1.1084	2	5	92.5028	0
5	K 25	318.1038	0.1784	2	5	92.5028	0

Table 3: Descriptors of Lipinski rule of five.

- High lipophilicity (AlogP less than 5)
- Less than 5 hydrogen bond donors (nHBD)
- Less than 10 hydrogen bond acceptors (nHBA)
- Molar refractivity (MR) should be between 40-130
- This filter helps in early preclinical development and could help avoid costly late-stage preclinical and clinical failures.

None of the designed molecules are found to be Lipinski failure. Hence, it implies that all the 10 designed compounds have the drug ability properties/drug likeness properties.

So, it may be concluded that all these designed molecules may proceed for further screening.

Docking

For performing docking, all receptors have been downloaded from NCBI website with PDB ID 1AZM (Lyase-Human Carbonic Anhydrase-I), all the designed ligands have been docked with protein (receptor) with AutoDock Vina (PyRx-Python Prescription 0.8) [11] software having its default settings.

Docking study of different proteins were performed with the designed inhibitors is given in Table 4 and Figure 4; number of hydrogen

bonds & binding pattern such as element, type of bond, atom number and residue at binding site were evaluated.

On docking analysis, designed compound K16 has been found to be strongly docked with 1AZM with 6 hydrogen bonds and binding affinity of: 7.4 Kcal/mol. On residue study Thr199, His96, His119 and His94 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound K20 has been found to

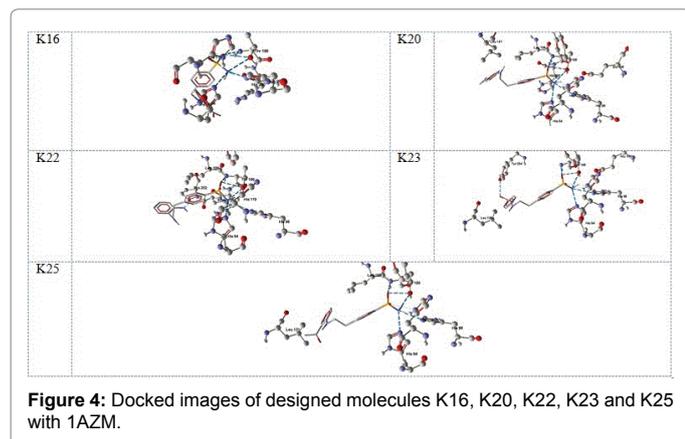


Figure 4: Docked images of designed molecules K16, K20, K22, K23 and K25 with 1AZM.

be strongly docked with 1AZM with 6 hydrogen bonds and binding affinity of: 7.3 Kcal/mol. On residue study Thr199, His96, His119 and His94 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound K22 has been found to be strongly docked with 1AZM with 6 hydrogen bonds and binding affinity of: 7.1 Kcal/mol. On residue study Thr199, His96, His119 and His94 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound K23 has been found to be strongly docked with 1AZM with 7 hydrogen bonds and binding affinity of: 7.5 Kcal/mol. On residue study Thr199, His96, His119, Tyr204 and His94 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

On docking analysis, designed compound K25 has been found to be strongly docked with 1AZM with 6 hydrogen bonds and binding affinity of: 7.4 Kcal/mol. On residue study Thr199, His96, His119 and His94 were found to be significant. On the account of ligand oxygen atom is significant in binding with donor bonds, whereas significant element in receptor is nitrogen.

Ligand	Affinity Kcal/mol	H-bond	H- Binding Ligand			H- Binding Receptor			
			Elem	At. ID	Type	Residue	Elem	At.ID	Type
K16	-7.4	6	O	21	Acceptor	Thr199	N	196	Donor
			O	21	Acceptor	Thr199	O	201	Both
			N	22	Donor	Thr199	O	201	Both
			N	22	Donor	His96	N	94	Acceptor
			N	22	Donor	His94	N	82	Acceptor
K20	-7.3	6	O	20	Acceptor	His119	N	113	Donor
			O	10	Acceptor	Thr199	N	196	Donor
			O	10	Acceptor	Thr199	O	201	Both
			N	11	Donor	Thr199	O	201	Both
			N	11	Donor	His96	N	94	Acceptor
K22	-7.1	6	N	11	Donor	His94	N	82	Acceptor
			O	9	Acceptor	His119	N	113	Donor
			O	10	Acceptor	Thr199	N	196	Donor
			O	10	Acceptor	Thr199	O	201	Both
			N	11	Donor	Thr199	O	201	Both
K23	-7.5	7	N	11	Donor	His96	N	94	Acceptor
			N	11	Donor	His94	N	82	Acceptor
			O	9	Acceptor	His119	N	113	Donor
			O	24	Acceptor	Tyr204	O	251	Both
			O	10	Acceptor	Thr199	N	196	Donor
K25	-7.4	6	O	10	Acceptor	Thr199	O	201	Both
			N	11	Donor	Thr199	O	201	Both
			N	11	Donor	His96	N	94	Acceptor
			N	11	Donor	His94	N	82	Acceptor
			O	9	Acceptor	His119	N	113	Donor

Table 4: Docking results of all the compounds with receptor 1AZM.

Experimental

Compound characterization

After synthesizing the designed compounds, percentage yield, retention factor (R_f), melting point and elemental analysis (CHNS analysis) were performed.

The findings of physical and elemental data of the compounds are reported in Table 5.

Spectral characterization of synthesized substituted 4-amino-benzenesulfonamides

Compound no. K16. 4-[(2-Acetyl-phenylamino)-methyl]-benzenesulfonamide (Figure 5).

IR (KBr, cm^{-1} , ν): 2851.6(> CH_2); 1685.7(>CO); 1585.7(C-C in ring); 1370.1(S=O); 1262.3(- CH_3); 1032.4(>NH); 721.3(- NH_2). ^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 2.54(t, 3H of C21); 4.49, 5.17(d, 2H of C11); 6.68(d, Ar-H of C14); 6.78(t, Ar-H of C16); 6.83(d, Ar-H of C3); 7.19(d, Ar-H of C4); 7.24(d, Ar-H of C6); 7.29(t, Ar-H of C15); 7.59(d, Ar-H of C1); 7.65(d 1H of C17); 7.66(d, 2H of $-\text{SO}_2\text{NH}_2$); 7.84(d, 1H of >NH). C13 NMR (CDCl_3 , 100 MHz, δ in ppm): 28.28(s, C21 of $-\text{COCH}_3$); 45.86(s, C11); 114.69(s, C14); 116.76(s, C16); 124.57(s, C18); 125.77(m, C1 and C3); 125.86(m, C4 and C6); 130.21(s, C17); 132.66(s, C15); 143.22(d, C5); 143.34(d, C2); 147.67(s, C13); 201.78(s, C19 of $-\text{COCH}_3$). MS (m/z, %): (304.36, M^+ , 67).

Compound no. K20. 4-[2-(4-Amino-phenylamino)-ethyl]-benzenesulfonamide (Figure 6).

IR (KBr, cm^{-1} , ν): 2855.4(> CH_2); 1461.1(C-C in ring), 1354.8(S=O); 1034.7(>NH); 721.8(- NH_2). ^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 2.90(d, 2H of C11); 3.81(d, 2H of C12); 4.45(t, 1H of >NH); 5.64(d, Ar-H of C19); 5.75(d, Ar-H of C18); 6.34(d, Ar-H of C15); 6.45(d, Ar-H

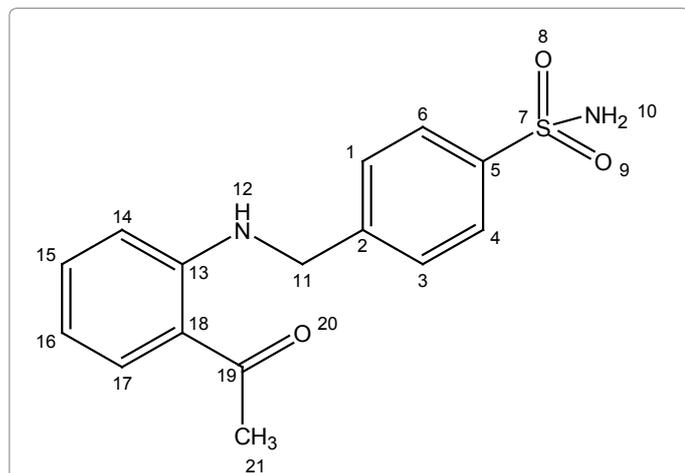


Figure 5: 4-[(2-Acetyl-phenylamino)-methyl]-benzenesulfonamide.

of C3); 6.47(d, Ar-H of C16); 6.78(d, Ar-H of C1); 6.84(d, Ar-H of C4); 6.98(d, Ar-H of C6); 7.43(d, 2H of $-\text{SO}_2\text{NH}_2$); 7.84(t, 1H of >NH). C13 NMR (CDCl_3 , 100 MHz, δ in ppm): 35.17(s, C11); 45.58(s, C12); 116(m, C15); 116.75(m, C16, and C18); 126.05(m, C1 and C3); 129.02(m, C4 and C6); 138.12(s, C17); 140.89(s, C14); 142.75(s, C2); 144.46(s, C5). MS (m/z, %): (291.37, M^+ , 08).

Compound no. K22. 4-[2-(2-Amino-phenylamino)-ethyl]-benzenesulfonamide (Figure 7).

IR (KBr, cm^{-1} , ν): 2859.9(> CH_2); 1461.2(C-C in ring); 1352.2(S=O); 1110.2(>NH); 722.7(- NH_2). ^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 2.92(t H of C11); 2.97(t, H of C11); 3.81(t, 2H of C12); 4.46(t, 2H of >NH); 5.83(d, Ar-H of C18); 5.84(t, Ar-H of C17); 6.36(d, Ar-H of C15); 6.45(d, Ar-H of C3); 6.53(t, Ar-H of C16); 6.78(d Ar-H of C1); 6.84(d, Ar-H of C4); 6.98(d, Ar-H of C6); 7.43(d, 2H of $-\text{SO}_2\text{NH}_2$); 7.83(t, 1H of >NH). C13 NMR (CDCl_3 , 100 MHz, δ in ppm): 35.17(s, C11); 45.45(s, C12); 115.60(s, C18); 116.64(s, C15); 119.01(s, C16); 120.10(s, C17); 126.05(m, C1 and C3); 129.02(m, C4 and C6); 134.84(s, C14); 138.14(s, C19); 142.75(s, C2); 145.46(s, C5). MS (m/z, %): (291.37, M^+ , 25).

Compound no. K23. 4-[2-(4-Acetyl-phenylamino)-ethyl]-benzenesulfonamide (Figure 8).

IR (KBr, cm^{-1} , ν): 2860.3(> CH_2), 1686.2(>CO); 1461.2(C-C in ring); 1353.4(S=O); 1110.9(>NH); 720.5(- NH_2). ^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 2.54(t, 3H of C22); 2.83, 2.93(t, 2H of C11); 3.47,

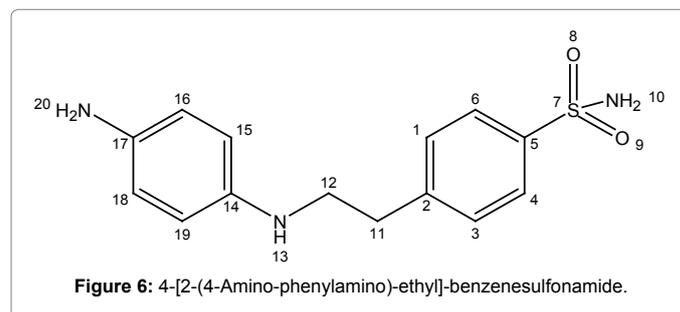


Figure 6: 4-[2-(4-Amino-phenylamino)-ethyl]-benzenesulfonamide.

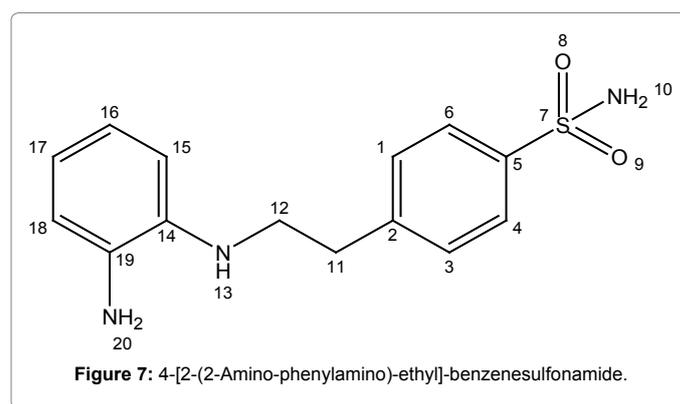
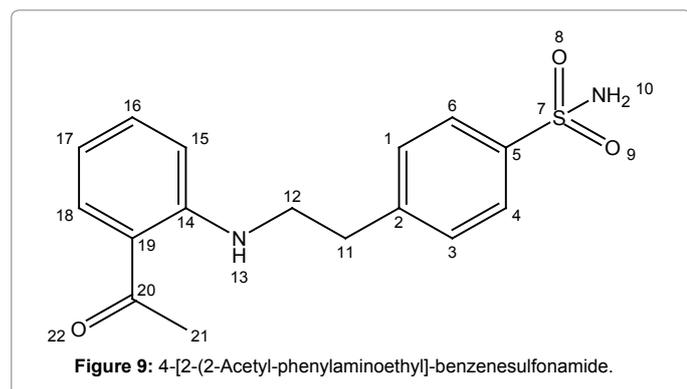
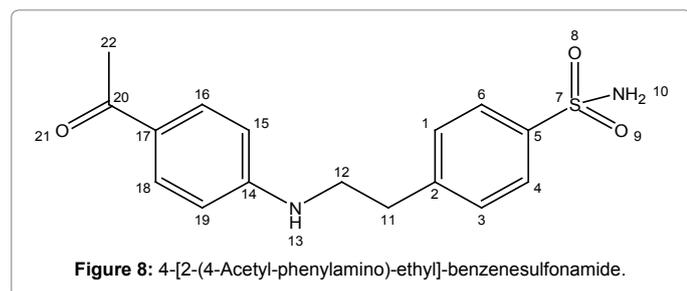


Figure 7: 4-[2-(2-Amino-phenylamino)-ethyl]-benzenesulfonamide.

Comp.	Molecular formula (MW)	Yield (%)	MP ($^{\circ}\text{C}$)	Elemental analysis (%) :				% purity	R_f
				Found (Calculated)					
				C	H	N	S		
K16	$\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ (304.088)	78.80	193-195	58.97 (59.19)	5.02 (5.30)	9.01 (9.20)	9.99 (10.54)	98.52	0.57
K20	$\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$ (291.104)	85.50	199-201	54.23 (57.71)	4.99 (5.88)	14.01 (14.42)	10.54 (11.00)	94.11	0.46
K22	$\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$ (291.104)	87.43	202-204	56.98 (57.71)	4.76 (5.88)	13.99 (14.42)	10.56 (11.00)	96.94	0.54
K23	$\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ (318.103)	81.23	206-207	60.22 (60.36)	5.01 (5.70)	8.12 (8.80)	9.87 (10.07)	97.98	0.56
K25	$\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ (318.103)	77.34	207-209	59.65 (60.36)	4.98 (5.70)	8.03 (8.80)	8.89 (10.07)	96.02	0.59

Table 5: Physical and elemental data of all the synthesized compounds.



3.52(t, 2H of C12); 6.68(d, 2H of C15, C19); 6.78(d, Ar-H of C3); 6.84(d, Ar-H of C6); 7.78(d, Ar-H of C4); 7.43(d, 2H of $-\text{SO}_2\text{NH}_2$); 7.52(d, Ar-H of C1); 7.66(d, Ar-H of C15, C16); 7.83(t, H of $>\text{NH}$). C13 NMR (CDCl_3 , 100 MHz, δ in ppm): 27.79(s, C22 of $-\text{COCH}_3$); 35.17(s, C11); 45.58(s, C12); 111.62(m, C15 and C19); 126.05(m, C1 and C3); 128.88(m, C17); 129.02(m, C4 and C6); 131.47(m, C16 and C18); 142.75(s, C2); 145.46(s, C5); 152.50(s, C14); 196.96(s, C20). MS (m/z, %): (318.39, M^+ , 50).

Compound no. K25. 4-[2-(2-Acetyl-phenylaminoethyl)-benzenesulfonamide (Figure 9).

IR (KBr, cm^{-1} , ν): 2846.9($>\text{CH}_2$); 1694.7($>\text{CO}$); 1488(C-C in ring); 1427(S=O); 1135.4($>\text{NH}$); 894.7($-\text{NH}_2$). ^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 2.54(t, 3H of C21); 2.83, 2.92(t, 2H of C11); 3.46, 3.96(m, 2H of C12); 6.70(d, Ar-H of C15); 6.78(d, Ar-H of C3); 6.79(t, Ar-H of C17); 6.85(d, Ar-H of C6); 7.17(d, 1H of C4); 7.32(t, 1H of C16); 7.45(d, 2H of $-\text{SO}_2\text{NH}_2$); 7.52(d, Ar-H of C1); 7.66(d, Ar-H of C18); 7.84(t, H of $>\text{NH}$). C13 NMR (CDCl_3 , 100 MHz, δ in ppm): 28.28(s, C21); 35.17(s, C11); 45.45(s, C12); 114.64(s, C15); 117.22(s, C17); 125.40(s, C19); 126.05(m, C1 and C3); 129.02(m, C4 and C6); 130.67(s, C18); 132.84(s, C16); 142.75(s, C2); 145.46(s, C5); 148.51(s, C14); 201.78(s, C20). MS (m/z, %): (318.39, M^+ , 20).

Pharmacology of Compounds

Anticonvulsant activity and neurotoxicity of substituted-4-amino-benzenesulfonamides

A maximal electroshock seizure test has been performed with an electrical stimulus of 0.2 s in duration (150 mA in rat at 60Hz) is delivered via trans-auricular electrodes. Animals were tested at 30 minutes and 2 h following doses of 100 mg/kg of test compound. Rats are tested at time intervals between 30 min. and 2h following a standard dose of 100 mg/kg. Abolition of the hind limb tonic extensor component indicates the test compound's ability to inhibit MES-induced seizure spread. It was observed that no animal has got neurotoxicity during a period of 30 min. to 4 hours.

MES activity and neurotoxicity studies are given in Table 6 and their various phase activities has been given in Table 7

As far as performance in MES model concerns, compounds K16 and K23 were observed comparable to standard drug Acetazolamide. Rest synthesized compounds have shown moderate performance.

While observing different phases of MES model induced convulsions, a reduction in duration of extensor was observed as compared to control. Extensor period of compounds K16 and K23 was in between 3.05 sec and 2.92 sec. The best and minimum duration of extensor among compounds K16 and K23 was 2.92 sec (For compound K23).

In-silico Studies

In-silico biological activity spectrum

Probability to be active of designed screened compounds against carbonic anhydrase have been calculated with the help of PASS and shown in Table 8.

In-silico mutagenicity and skin irritancy studies

Ames *In-silico* test results have been shown in Table 9

The model used in Ames *In-silico* test matches the predicted value with the experimental values used in modeling of software, depending upon them if probability comes 1 then the designed molecules may act as mutagenic.

Predicted oral rat LD50

The newly synthesized molecules were passed through TEST software inbuilt QSAR model for estimation of 'Oral rat 50 percent lethal dose' (LD50) (<https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test>). Predicted oral rat LD50 of screened compound is presented in Table 10, where it is converted to g/kg instead of mg/kg.

Oral administration to rat ^a						
S. No.	Comp.	Dose (mg/kg)	30 min ^c	2 h ^c	Neurotox. ^b	
					30 min	4 hr
1.	K16	100	5/6	6/6	0/4	0/4
2.	K20	100	4/6	5/6	0/4	0/4
3.	K22	100	2/6	2/6	0/4	0/4
4.	K23	100	5/6	6/6	0/4	0/4
5.	K25	100	4/6	4/6	0/4	0/4
6.	Acetazolamide	100	5/6	6/6	0/4	0/4
7.	Control: HPMC 5% w/v	100	0/6	0/6	0/4	0/4

a-Indicates the number of rat out of six which are protected, b-Neurotoxicity evaluation, number of animals affected/number of animals tested. c-Time after drug administration

Table 6: Anticonvulsant (MES) activity and neurotoxicity of synthesized compounds.

S. No.	Compound	Time taken (sec)			
		Flexion	Extensor	Clonus	Stupor
1	K16	1.86±0.15	3.05±0.16	1.93±0.12	6.5±0.17
2	K20	4.17±0.09	4.86±0.20	10.7±0.17	11.41±0.23
3	K22	7.79±0.23	4.94±0.23	13.92±0.21	97.01±0.30
4	K23	2.06±0.10	2.92±0.15	2.16±0.21	7.08±0.19
5	K25	4.15±0.14	4.59±0.22	10.72±0.23	11.13±0.25
11	Acetazolamide	2.01±0.13	0±0.26	2.8±0.31	2.6±0.21
12	Control: HPMC 5% w/v	4.5±0.30	18.7±0.97	15.06±0.97	13.1±0.89

Data is expressed as Mean ± SEM

Table 7: Anticonvulsant effect on various phases of MES at 30 min from dose administration.

S. No.	Comp.	CA I inhibitor	CA II inhibitor	CA IV inhibitor	CA IX inhibitor	CA V inhibitor	CA VII inhibitor	CA XII inhibitor	CA XIV inhibitor
1	K16	0.223	0.340	0.213	0.359	0.285	0.139	0.263	0.256
2	K20	0.157	0.171	0.192	0.154	0.404	0.199	0.128	0.060
3	K22	0.265	0.276	0.236	0.114	0.214	0.176	0.197	0.256
4	K23	0.233	0.259	0.315	0.250	0.106	0.148	0.270	0.153
5	K25	0.022	0.036	0.012	0.039	0.082	0.139	0.064	0.050

Table 8: Biological activity Spectrum against Carbonic anhydrase (Probability to be active).

S. No.	Comp.	Mutagenicity		Skin irritancy		Method employed
		Probability	Status	Probability	Status	
1	K16	0.206	Non-Mutagen	0.568	Non-irritant	TOPKAT®
2	K20	0.616	Non-Mutagen	0.015	Non-irritant	TOPKAT®
3	K22	0.634	Non-Mutagen	0.321	Non-irritant	TOPKAT®
4	K23	0.432	Non-Mutagen	0.336	Non-irritant	TOPKAT®
5	K25	0.374	Non-Mutagen	0.298	Non-irritant	TOPKAT®

Table 9: Probability and current status to be mutagenic and skin irritant of designed screened compound.

S. No.	Compound	Oral rat LD50 (g/kg)
1	K16	4570
2	K20	3900
3	K22	4280
4	K23	3170
5	K25	3110

Table 10: Predicted oral rat LD50.

Possible mechanism of action of synthesized sulfonamide derivatives

Literature shows that variety of isoforms of Carbonic anhydrase is responsible as anticonvulsant target [21]. Sulfonamides are the most important class of Carbonic Anhydrase Inhibitors (CAIs) [4,22,23]

The inhibition and activation of CAs are well-understood processes: most types of classical inhibitors bind to the metal center [21].

CO₂, bicarbonate, and protons are essential molecules/ions in many important physiologic processes in all life kingdoms (Bacteria, Archaea, and Eukarya), throughout the tree of life, and for this reason, relatively high amounts of CAs are present in different tissues/cell compartments of most investigated organisms [22,23].

In many organisms, CAs enzymes are involved in crucial physiological processes connected with respiration and transport of CO₂/bicarbonate, pH and CO₂ homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (e.g. gluconeogenesis, lipogenesis, and ureagenesis), bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes (thoroughly studied in vertebrates). In algae, plants, and some bacteria they play an important role in photosynthesis and other biosynthetic reactions [24]. Many such enzymes from vertebrates, nematodes, fungi, protozoa and bacteria are well-known drug targets [22].

Carbonic anhydrase inhibition as anticonvulsant effect

One potential factor that may contribute to seizure is the modification of the environmental pH (alkalosis increases the neuronal excitability) [25]. The pH buffering of the extracellular and intracellular spaces is mainly carried out by the CO₂/HCO₃ buffer. The ratio of these two species is regulated by the activity of the carbonic anhydrase which catalyses the reversible conversion of CO₂ and H₂O into H⁺ and HCO₃⁻ [26]. Acetazolamide, Topiramate and Zonisamide (Figure 10) are a well-known Carbonic anhydrase inhibitors as anticonvulsants.

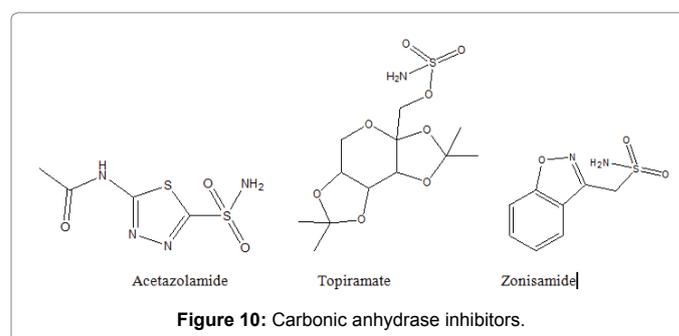


Figure 10: Carbonic anhydrase inhibitors.

These drugs act by blocking sodium and calcium channels, which leads to the suppression of neuronal hypersynchronization.

Conclusion

We have described the designing, synthesis, computational studies and pharmacological evaluation of synthesized compounds as anticonvulsant agents. The designed compounds were successfully synthesized and well characterized by analytical and spectral data. Docking studies showed good interaction with Lyase (Oxo-acid)-Human Carbonic Anhydrase-I (1AZM). The synthesized compounds showed moderate to good anticonvulsant activity in MES model. The *in-silico* studies proved them to be with good drug-likeness properties. Few of them showed promising results, especially 4-[2-(4-Acetylphenylamino)-ethyl]-benzenesulfonamide (K23), however, further clinical studies need to be carried.

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