Accepted Manuscript

Discovery of potent anti-convulsant carbonic anhydrase inhibitors: Design, synthesis, in vitro and in vivo appraisal

Chandra Bhushan Mishra, Shikha Kumari, Andrea Angeli, Silvia Bua, Martina Buonanno, Simona Maria Monti, Manisha Tiwari, Claudiu T. Supuran

PII: S0223-5234(18)30575-0

DOI: 10.1016/j.ejmech.2018.07.019

Reference: EJMECH 10554

To appear in: *European Journal of Medicinal Chemistry*

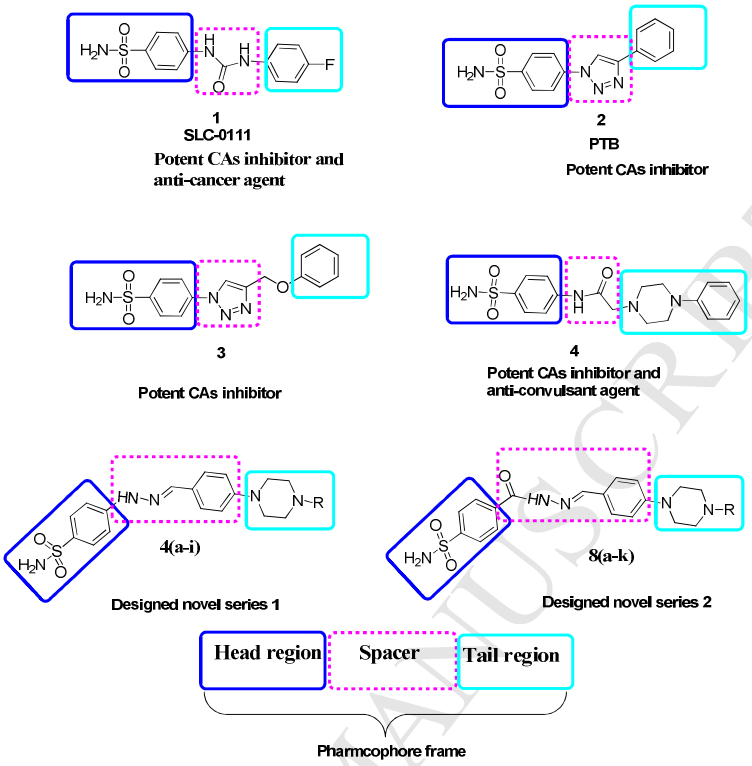
Received Date: 18 May 2018

Revised Date: 5 July 2018

Accepted Date: 7 July 2018

Please cite this article as: C.B. Mishra, S. Kumari, A. Angeli, S. Bua, M. Buonanno, S.M. Monti, M. Tiwari, C.T. Supuran, Discovery of potent anti-convulsant carbonic anhydrase inhibitors: Design, synthesis, in vitro and in vivo appraisal, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.07.019.

Graphical Abstract



Discovery of Potent Anti-convulsant Carbonic Anhydrase Inhibitors: Design, Synthesis, In vitro and In vivo Appraisal

Chandra Bhushan Mishra[1], Shikha Kumari[1], Andrea Angeli[2], Silvia Bua[2], Martina Buonanno,[3] Simona Maria Monti, [3] Manisha Tiwari[1]*, and Claudiu T. Supuran[2]*

[1]Bio-Organic Chemistry Laboratory, Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, 110007, Delhi, India

[2]Dipartimento Neurofarba, Universita` degli Studi di Firenze, Sezione di Scienze Farmaceutiche e Nutraceutiche, 50019 Sesto Fiorentino, Florence, Italy.

[3] Institute of Biostructures and Bioimaging, CNR, via Mezzocannone, 16, 80134, Naples, Italy.

Corresponding Authors. MT, Phone: +91-01127666272; E-mail:: mtiwari07@gmail.com; CTS, Phone: +39-055-4573729; fax: +39-055-4573385; E-mail: claudiu.supuran@unifi.it

Abstract

We report the design, synthesis and pharmacological assessment of novel benzensulfonamide derivatives acting as effective carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. All the synthesized compounds were screened for their CA inhibitory action against four isoforms of human origin (h), i.e. hCA I, hCA II, hCA VII and hCA IX. In-vitro carbonic anhydrase inhibition studies have shown that first series, 4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamides (**4a- 4i**) bestowed low nanomolar range to medium nanomolar range inhibitors against hCA II and hCA VII, effectively involved in epileptogenesis. Furthermore, compounds belonging to the second series, 4-(2-(4-(4-substitutedpiperazin-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamides (**8a-8k**) showed effective inhibition against hCA VII, being less effective against other hCA isoforms. Inspiring with obtained CA inhibition results, we have chosen some of the potent hCA II and hCA VII inhibitors (**4g, 4i** and **8d**) to test their anti-convulsant efficacy in MES and sc-PTZ seizure tests in Swiss Albino male mice. In result, these compounds significantly attenuated both electrical (MES) as well as chemical (sc-PTZ) induced seizures. Next, in advance anticonvulsant tests, compound **8d** displayed long duration of action in time course study and successfully attenuated MES induced seizure in mice up to 6 h after drug administration without showing neurotoxicity in rotarod test. Moreover, this compound was also found to be orally active and effectively abolished generalized tonic-clonic seizures in male Wistar rats upon oral administration, being non-toxic in sub acute toxicity studies.

1. Introduction

Epilepsy is a life-shortening neurological disorder, characterized by repeated, uncontrolled seizures and approximately affecting 1% population worldwide [1]. Epilepsy is considered as a complex disorder, consisting over 15 different types of seizure and more than 30 epileptic syndromes, associated with substantial comorbidity including, cognition deficit, depression and anxiety [2]. During the past decades, several third generation anti-epileptic drugs (AEDs) have been developed to treat epileptic patients; however, these AEDs are failing to control seizure in 20-30% of patients [3]. Currently available AEDs are also unable to prevent the development of drug resistant epilepsy which is considered as a major challenge in epilepsy management [4]. These serious drawbacks of existing AEDs are still insisting for development of novel and more efficacious drugs for the treatment of epilepsy. Pathophysiological studies of epilepsy revealed that several receptor, neurotransmitters, enzyme and ion channels are actively involved in seizure generation of epileptic patients. Therefore, several targets such as GABA (γ-aminobutyric acid), Cl^-, Na^+, K^+, Ca^{2+} ion channel, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor etc. are well studied to control epileptic seizure [3]. Apart from these targets, brain carbonic anhydrases (CAs, EC 4.2.1.1) appeared as an emerging target to control epileptic seizure from several past decades and some of potent CAs inhibitors are used clinically to control various types of seizure [5,6].

There are 15 different human carbonic anhydrases (hCAs) isoforms have been identified till date, CA I-III, CA VII and CA XIII are found in cytosol; CA IV, CA IX, CA XII and CA XIV are membrane bound; CA VA and CA VB are present in the mitochondrion and CA VI is found in milk and saliva. These CAs actively catalyze the interconversion between CO_2 and bicarbonate and are involved in numerous physiological functions associated with CO_2/bicarbonate equilibrium [7,8]

It is well established that CAs modulate numerous neuronal signaling mechanism associated with various central nervous system (CNS) disorders, including epilepsy [5,6]. Several reports have disclosed that epileptic seizures are associated with changes in the ionic composition within the brain, including enrichment of extracellular potassium concentration and pH shifts [6]. Brain pH plays an important role in epilepsy and the alteration in pH directly affects

seizure generation as well as its severity. It is found that generally alkalosis promotes seizure spreading, while acidosis blocks/halts seizure generation [5]. The pH buffering between extracellular and intracellular spaces is conducted by CO_2 and HCO_3^-. The regulation of both components is under control of CAs which catalyzes the reversible conversion of CO_2 and H_2O into H^+ and HCO_3^- [9]. Thus, with these vital evidences, brain CA appears as an important target for management of epileptic seizure syndromes. Several isoforms of CAs are present in mammalian brain, physiologically dominant hCA II isoform is widely expressed in oligodendrocytes, myelin sheaths as well as choroid plexus, which is engaged in various neurological disorders including epileptogenesis [10,11]. Halmi et al. showed that expression of CA II is increased in the CA1 cells after 3-12 h kainic acid treatment in status epileptic model [12]. The study has also displayed that CA deficient mice are more resistant to epileptic seizure and these mice also showed less mortality in repeated seizures [13]. CA VII isoform also play vital role in the generation of seizure and widely expressed in the cortex as well as hippocampus region of brain [14]. It is studied that CA VII promotes seizure generation via GABAergic excitation and Ruusuvuori et al. experimentally proved that this isoform is also actively involved in febrile seizures onset by activating GABAergic excitation in brain [15,16]. During the seizure episode, extracellular potassium concentration enhances due to GABAergic depolarization and intense neuronal firing depolarizes GABAA-mediated responses which are linked with HCO_3^-, efflux induced by GABAA receptors [16,17]. Rusuvuori et al. have also revealed that these GABA associated electrophysiological activities are under the control of brain CA VII [16a]. Bicarbonate reflux through GABAA receptor produces external alkalosis, supplied by intracellular CA VII and alkalosis may influence pathological incidents such as epileptic seizures [18,19].

Presently, a number of potent CA inhibitors such as acetazolamide (AAZ), zonisamide (ZNS), methazolamide (MZA) and topiramate (TPM) possess good anti-convulsant activity (**Chart-1**) and these potent CA inhibitors are still used by clinicians against numerous forms of seizures such as partial, myoclonic, generalized tonic-clonic and absence seizures [20,21,22].

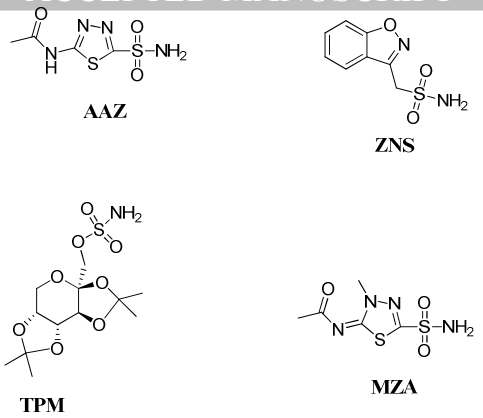


Chart 1: CA Inhibitors with potent anti-epileptic activity

However, it is clinically proven that some of these CA inhibitors do not show full effectiveness in long term therapy of epilepsy [23]. Therefore, development of potent CA inhibitors is required, which hold reasonable anti-convulsant activity along with long lasting efficacy. Aromatic sulfonamide moiety ($Ar\text{-}SO_2\text{-}NH_2$) has classical recognition to bind to the active site of CA, with deprotonated sulfonamide moiety coordinating to the Zn^{2+} in the active site, impairing the catalytic acivity of these highly efficient enzymes [24,25,26]. In the discovery of potent CA inhibitors, the installation of aryl/ heteroaryl sulfonamide moieties has provided various clinically useful therapeutic agents such as anti-glucoma, diuretics, anti-high altitude sickness drugs, as well as AEDs. Additionally, our research group has been constantly utilizing this functionality and numerous potent CA inhibitors are being reported, investigated for their inhibitory action against CA isoforms, with many inhibitors displaying a low nanomolar activity range against various such enzymes [27,28,29,30]. Several sulfonamide /sulfamate bearing CA inhibitors have been also developed which are endowed with excellent anti-convulsant activity in vivo model of epilepsy [31]. Continuing our interest in the design and development of potent CA inhibitors, herein we report synthesis, CA inhibition and in-vivo anticonvulsant activity of new 4-(2-(4-(4-substituted piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamides (series 1; 4a- 4i) and 4-(2-(4-(4-substituted piperazin-1-yl)benzylidene) hydrazinecarbonyl)benzenesulfonamides (series 2; 8a- 8k).

2. Results and Discussion

2.1. Compound design and synthesis

We aimed to design two series of novel CA inhibitors by utilizing the well studied tail approach for potent CA inhibitor development [32]. We chose benzenesulfonamide as a pharmacophoric head, which has attracted much attention in this field [33, 34]. In due course, several benzenesulfonamide based compounds have been prepared by installing a wide range of alkyl, aromatic, heterocyclic, or sugar scaffolds and these derivatives displayed excellent inhibitory action against various CA isoforms of medicinal importance, such as CA II, CA IV, CAVII, CA IX, CA XII and CAIV [33,34,35,36]. Such category of compounds, mostly containing three key molecular fragments; the benzenesulfonamide head, spacer/linker and alkyl/aryl/heteroaryl tail region [34,36] (**Chart-2**). It is also observed that the introduction of polar spacers/linkers offer numerous potent CA inhibitors including **SLC-0111** which contain urea as a linker between head and tail region, completed Phase I clinical trials for the therapy of metastatic hypoxic tumors [33,34,37,38]. 4-(4-Phenyl-[1,2,3]triazol-1-yl)-benzenesulfonamide (**PTB**, chart 2) and its analog **3** have also shown potent CA inhibition. Piperazine is also a well studied flexible heterocyclic compound in drug discovery research [39].

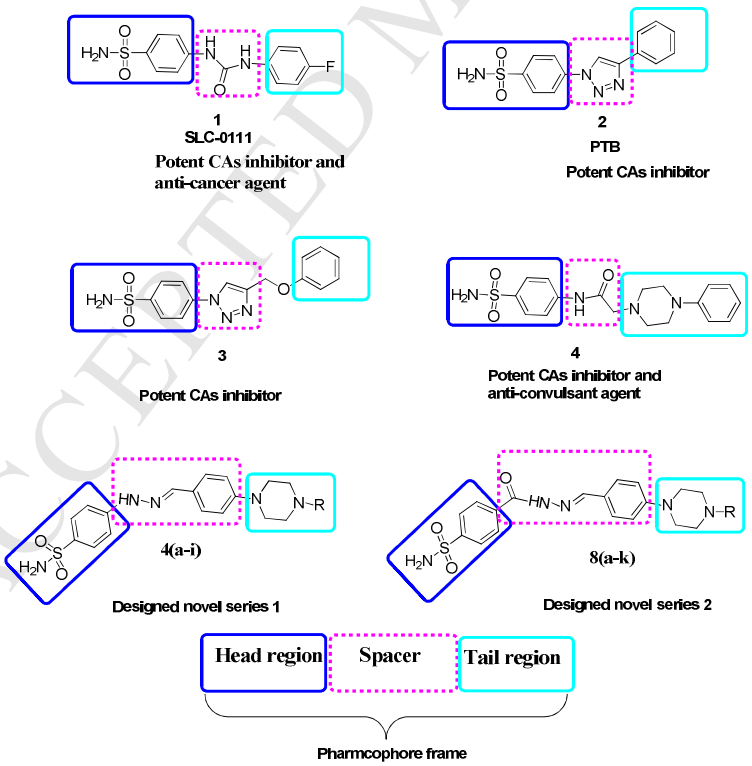
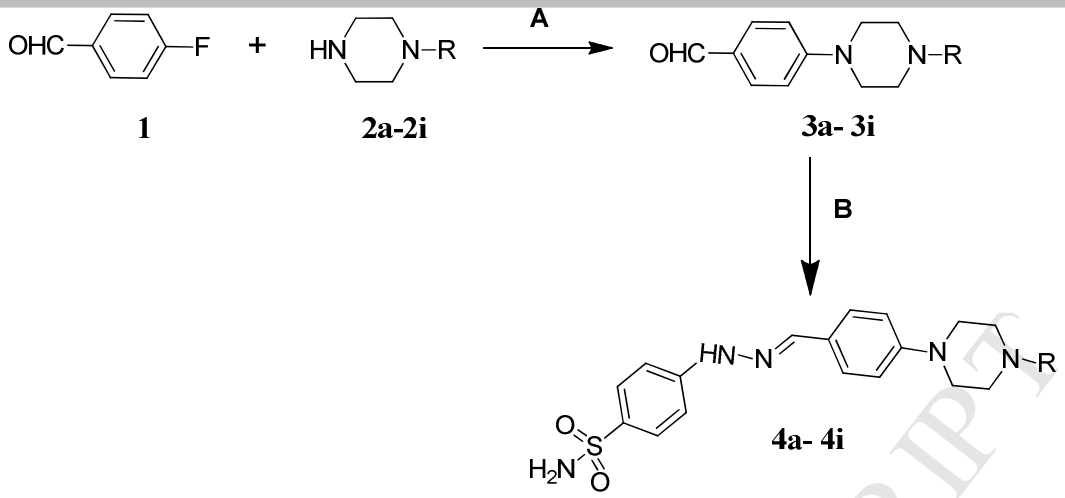
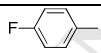
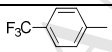
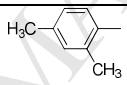
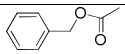


Chart: 2. Designing strategy of novel CA inhibitors (series1; 4a-k and series 2; 8a-m)

Piperazine containing molecules have shown a wide range of biological activity, including CA inhibitory action [39,40]. Recently, we have also reported novel benzenesulfonamide derivative **4** as a potent CA inhibitor and anticonvulsant agent [37e]. Several research reports disclosed that piperazine bearing compounds have shown very effective CA inhibition with nanomolar range affinity [41]. Another aspect of the piperazine ring is its flexible nature and this scaffold is widely used to enhance molecular flexibility to influence the adjustment of molecule within active sites of enzymes [41]. Considering these facts, the present study was designed to explore new 4-(2-(4-(4-substituted piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamides (4a-4i) and 4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene) hydrazinecarbonyl)benzenesulfonamides (8a-8k) as potent CA inhibitors. In the first series, the benzenesulfonamide head was tethered with substituted piperazine tails by using the benzylidenehydrazine spacer. Introduction of piperazine with aromatic tail is thought to bestow flexibility to the tail region, which may enhance activity and selectivity against different CA isofoms. Furthermore, we replaced the benzylidenehydrazine spacer with the more polar spacer benzylidenehydrazine carbonyl, in order to obtain 4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene) hydrazinecarbonyl)benzenesulfonamides (8a-8k) and studied whether this replacement will produce a better inhibitory activity and selectivity for CA isoforms. Various substituted piperazines were installed as tail region in both series to achieve conclusive SAR . Some of them such as furoyl, benzoyl, benzyl carbamate, pyridine and pyrimidine substituents are well used to produce biological active molecules [39b]. To achieve the target compounds of both series, the following synthetic strategy has been adopted. For the synthesis of 4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene)hydrazinyl) benzenesulfonamides (**4a-4i, Scheme 1**), commercially available *p*-fluorobenzaldehyde **1** was coupled with substituted piperazines **2a-2i** using K_2CO_3/Na_2CO_3 as a base, leading to the key intermediates 4-(4-substituted piperazin-1-yl) benzaldehydes **3a-3i**. Compounds **3a-3i** were then reacted with 4-hydrazinobenzenesulfonamide in acidic medium (acetic acid) to yield the target compounds **4a-4i**.

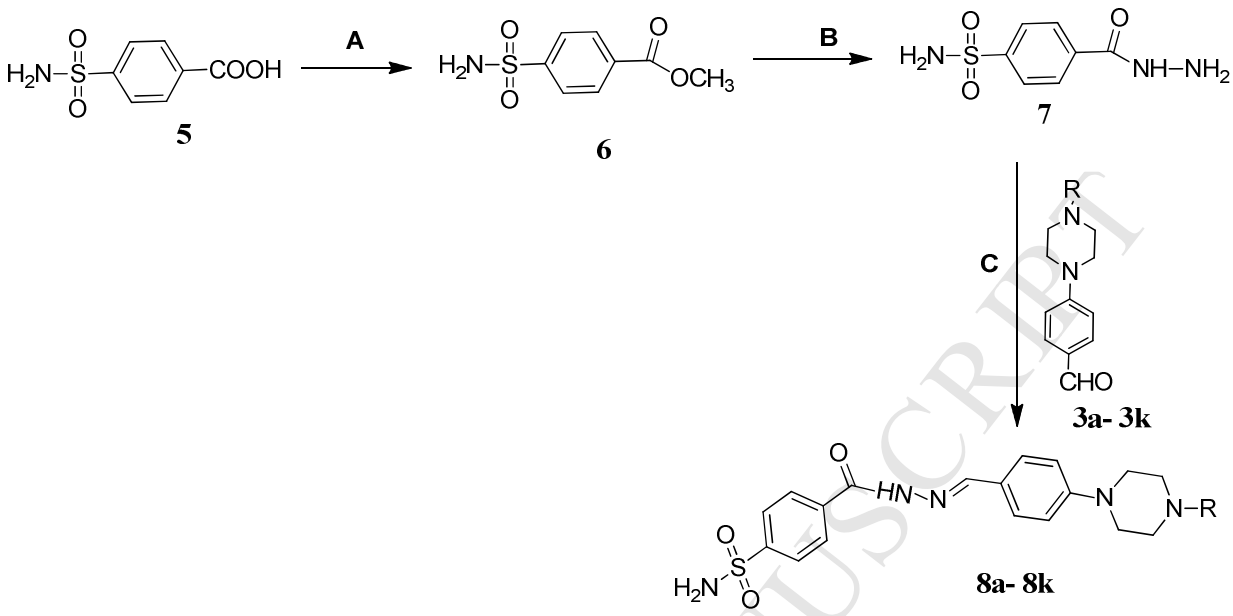


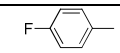
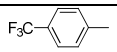
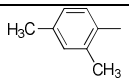
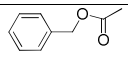
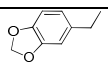
Compound No	R-Group
4a	
4b	
4c	
4d	
4e	
4f	
4g	
4h	
4i	

Scheme 1: Reagent & conditions: A; K_2CO_3/Na_2CO_3, DMF, reflux (90-100°C), B; Glacial acetic acid, ethanol, reflux (90°C).

Next, 4-sulfamoylbenzoic acid was used as starting material for the synthesis of 4-(2-(4-(4-substituted piperazin-1-yl) benzylidene)hydrazinecarbonyl)benzenesulfonamides (**Scheme 2**). The acid group of 4-sulfamoylbenzoic acid (**5**) was activated by converting it into methyl 4-sulfamoylbenzoate (**6**). Methyl 4-sulfamoylbenzoate (**6**) was treated with hydrazine hydrate in order to obtain 4-(hydrazinecarbonyl)benzenesulfonamide (**7**). Condensation of **7** with the corresponding formyl derivatives **3a-3k** in ethanol provided the target compounds 4-(2-(4-(4-substituted piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamides **8a-8k** in high yields. The synthesized target compounds were purified by column chromatography, followed by re-crystallization and have been fully characterized with ^1H

NMR, ^{13}C NMR and mass spectroscopy. Purity of all target compounds has been assessed by reverse phase HPLC and was > 95%.



Compound No	R-Group
8a	
8b	
8c	
8d	
8e	
8f	
8g	
8h	
8i	
8j	
8k	

Scheme 2: Reagent &conditions: A, Dried methanol, sulfuric acid reflux, 8h; B, Hydrazine hydrate, 1, 4 dioxane, reflux (100°C), 8h; C, Ethanol, glacial acetic acid, reflux (90°C), 8h

Additionally, synthesized target compounds **(4a-4i** and **8a-8k)** were screened to detect as a pan assay interference molecules (PAINS) which might be perturb biological activity [42]. According to SwissADME [43] and FAFDrugs4 [44] tools, none of these target compounds restrain substructural element documented as PAINS (supplementary table ST1).

2.2. Carbonic anhydrase inhibitory activity: The synthesized novel compounds (**4a-4i; 8a-8k**) were evaluated for their CA inhibitory activity against four CA isforms of human origin; hCA I, hCA II, hCA VII, and hCA IX. Among them, hCA II and hCAVII have been well studied for their role in epileptogenesis and seizure associated activity [14, 15, 16]. Obtained results have been resided in Table 1 and following structure activity relationship (SAR) was observed on the basis of inhibitory pattern:

i) Majority of synthesized compounds in both series did not display potent inhibitory action against hCA I isoform, generally considered as off target for epilepsy. However, compounds bearing benzyl carbamate (**4g**) and furoyl group (**4i**) were shown a medium range inhibitory potential against hCA I with a K_i value of 919.6 nM and 321.8 nM, respectively.

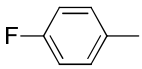
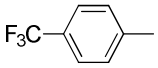
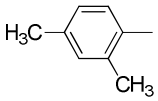
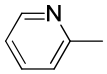
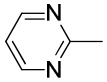
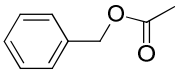
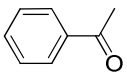
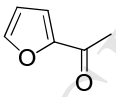
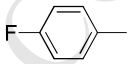
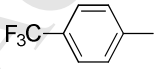
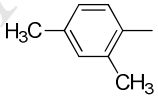
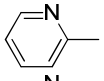
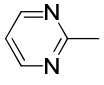
ii) Results indicate that synthesized derivatives exhibited medium to high nanomolar range inhibitory activity towards physiologically dominant hCA II isoform. Although, derivatives containing benzyl carbamate (**4g**) and furoyl group (**4i**) at the terminal end along with benzylidenehydrazine spacer bestowed satisfactory inhibitory action for hCA II isoform. Benzyl carbamate derivative and furoyl derivative have shown a K_i value of 93.9 nM and 30.8 nM, respectively. In this benzylidenehydrazine series, compounds with *p*-fluorophenyl (**4b**) and *o*, *p*-dimethylphenyl (**4d**) displayed medium nanomolar range inhibitory activity with a K_i value of 559.1 nM and 552.6 nM, respectively for hCA II. In benzylidenehydrazine carbonyl series, compounds with trifluoromethyl (**8c**), benzoyl (**8h**) and flexible side chain benzyl (**8j**) also have appeared as a medium nanomolar range inhibitor for hCA II, showed a K_is value range 307-555 nM. It was noticed that most of compounds which displayed medium nanomolar range inhibitory action against hCA II were also appeared to be selective over hCA I and hCA IX.

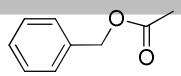
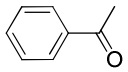
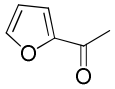
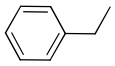
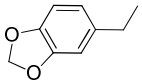
iii) It was observed that most of the synthesized compounds effectively inhibited hCA VII isoform with low nanomolar to medium nanomolar range K_is values. In the first series, compounds holding benzyl carbamate and furoyl tails demonstrated low nanomolar inhibitory activity for hCA VII. Benzyl carbamate piperazine derivative (**4g**)

showed a K_i value of 11.4 nM while furoyl piperazine derivative (**4i**) exhibited an impressive K_i value 6.2 nM. Interestingly, in benzylidenehydrazine carbonyl series, compound containing *o*, *p*-dimethyl piperazine (**8d**) tail was found to be a most selective hCA VII inhibitor among whole series. This compound displayed low nanomolar inhibitory action (17.8 nM) with satisfactory selectivity over other tested isoforms. Compound **8d** showed 561, 527 and 93 fold selective over hCA I, hCII, and hCA IX isoform. Compounds with phenyl (**4a**), *p*-fluorophenyl (**4b**) and *o*,*p*- dimethylphenyl (**4d**) side chain of benzylidenehydrazine series have also shown satisfactory inhibitory action towards hCA VII isoform. In addition, compound possess benzoyl (**8h**), furoyl (**8i**) and benzyl (**8j**) tails along with benzylidenehydrazine carbonyl spacer between the head and tail region also bestowed reasonable inhibitory potential and displayed K_is values below 100 nM. Other derivatives such as **4c**, **4e**, **4h**, **8a-8c**, **8e-8g** showed moderate hCA VII inhibitory action with K_is value ranges 118-180 nM. However, pyrimidine (**4f**) and piperonyl (**8k**) substituted derivatives did not appear to be potent inhibitor of hCA VII and these compounds showed high nanomolar range K_is value (606-750 nM).

iv) Cancer associated isoform hCA IX was poorly inhibited by most of synthesized derivatives. Most of derivatives showed high nanomolar range inhibitory activity and exhibited K_is value ranges 1046-2491nM. Although, compounds of benzylidenehydrazine carbonyl series, substituted with furoyl (**8i**) and piperonyl (**8k**) hetrocyclic group were appeared as effective hCA IX inhibitors and showed a Ki value of 30.4 and 95.2 nM, respectively. It was also noticed that four compounds, **4e**, **8a**, and **8e**, are medium potency inhibitors for hCA IX. Thus, it seems that benzylidenehydrazine carbonyl spacer bestowed some effective hCA IX inhibitor, while benzylidenehydrazine spacer failed to produce such action.

Table 1. Inhibition data of hCA I, II, VII and IX with compounds 4a-4k, 8a-8m & AAZ

Compound no	R-group	K_i (nM)			
		hCA I	hCA II	hCA VII	hCA IX
4a		7862.0	887.5	98.4	1243.6
4b		2377.3	559.1	49.6	1272.6
4c		9467.2	7524.5	118.2	1931.7
4d		4105.9	552.6	72.0	1738.1
4e		>10000	4590.8	135.3	235.3
4f		>10000	8396.7	606.7	2231.8
4g		919.6	93.9	11.4	1631.4
4h		>10000	2792.7	127.4	2491.8
4i		321.8	30.8	6.2	1788.6
8a		>10000	4131.1	180.8	275.7
8b		5795.9	822.3	147.1	1956.6
8c		8164.3	555.7	147.7	1853.6
8d		>10000	9385.9	17.8	1660.9
8e		>10000	4557.9	125.9	151.5
8f		2061.1	963.1	132.2	1841.0

8g		9638.6	1447.5	121.5	1903.1
8h		5503.9	307.9	94.7	1046.3
8i		9489.9	686.5	78.8	30.4
8j		7101.7	413.6	41.5	162.7
8k		8368.4	962.2	750.9	95.2
AAZ		250	12.1	2.5	25.8

*Mean from 3 different assays, by a stopped flow technique (errors were in the range of ± 5-10% of the reported values).

v) Overall, the SAR investigation revealed that introduction of benzylidenehydrazine spacer between benzenesulfonamide head and aryl/ heteroaryl tail, provided effective hCA II and hCA VII inhibitors, while more polar benzylidenehydrazine carbonyl spacer bestowed only effective and selective hCA VII inhibitors. Our results indicated that compounds **4g** as well as **4i** were very effective hCA II and hCA VII inhibitors and compound **8d** was a very selective hCA VII inhibitor which also displayed low nanomolar range inhibition against hCA VII. Therefore, these three compounds were selected to explore their anti-convulsant action in preclinical animal models of epilepsy.

2.3. In vivo studies

2.3.1. Anti-convulsant action: CA isoforms, CAI-CAVIII, X, XI, XII and XIV are generally present in the brain and associated with numerous neurosignaling/neuromodulatory functions [14,15]. The role of CA II and CA VII in epileptogenesis is well studied and its inhibition either suppresses or blocks epileptic seizure [15,16]. It is also evidence that many potent hCA II and hCA VII inhibitors including AAZ and TPM showed excellent anti-epileptic effects. Compounds **4g**, **4i**, and **8d** reported here displayed potent hCA II and hCA VII inhibitory activity, therefore, anti-seizure effect of these compounds was evaluated in vivo models of epilepsy. In addition, two standard anti-epileptic drugs AAZ and TPM were taken as a reference to correlate the findings. The evaluation of new molecules for their anti-epileptic activity mainly relies on maximal electroshock test (MES) and pentylenetetrazole (PTZ) test. These two tests are considered as “gold standards” for evaluation of novel anti-epileptic drug as compared to other test method [45]. Generally, it is observed that most of the AEDs are found to be effective in either both tests or at

least one test. Thus, in this study, we also included these two gold standard tests to evaluate anti-convulsant effect of most active hCA II and hCAVII inhibitors i.e. **4g**, **4i**, and **8d.**

2.3.1.1. Maximal electroshock method: MES test is considered to be very authentic method for identifying new anti-convulsants which block seizure through neuronal tissue [46]. In this test, animals receive an electric stimulus of adequate intensity to induce tonic-clonic generalized seizures and they are very similar to be human generalized seizures. In this experiment, anti-convulsant activity of compounds **4g**, **4i**, and **8d** was evaluated upon 30 and 100 mg/kg dose administration at two time intervals of 0.5 h and 3 h. Observed anti-convulsant activity of these three compounds in this test is depicted in Table **2**. Our results indicate that compound **4g** at 30 mg/kg dose showed 50% seizure protection at 0.5 h, the activity was slightly reduced at 3 h and displayed 33% seizure protection. 100 mg/kg dose of this derivative, also showed 50% protection at 0.5 h time interval similar to 30mg/kg dose. However, at 3 h time interval compound (**4g**) exhibited almost 67% seizure protection, which indicate it's quick and long duration action against MES induced seizure. Compound **4i** (30 mg/kg) had shown stable seizure protection at both time intervals, displayed 50% protection at 0.5 and 3 h against MES induced seizures. Although, 100 mg/kg dose of this compound showed excellent protection at 0.5 h and forcefully defend 83% animals against MES provoked seizures. However, activity of this compound decreased at 3 h time interval and protected only 50% of the animals. This compound possesses fast onset of action and short duration of seizure protection capability. Interestingly, compound **8d** at lower dose (30 mg/kg) demonstrated excellent protection against MES induced seizure and its activity was found to be sustained up to 3 h after drug treatment. This compound displayed 67% protection at 0.5 h as well as 3 h time intervals. Higher dose (100 mg/kg) of this compound also showed 50 % seizure protection at 0.5 h and 3 h time intervals. These findings clearly indicate that all three derivatives have shown reasonable protection against MES provoked seizures; however, compound **8d** was appeared very effective as compared to other two derivatives **4g** and **4i**.

2.3.1.2. Subcutaneous Pentylenetetrazole Test: PTZ is a strong chemoconvulsant and work as a γ-amino butyric acid (GABA) receptor antagonist that blocks GABAA receptor subtype in the brain. Administration of PTZ induces strong myoclonic and absence seizure in experimental animals that mimics seizures induced in human [47]. It is well validated pre-clinical test, being widely used to discover potent anticonvulsant agent which blocks seizure directly or

indirectly through GABA receptor. Several studies have shown that hCAs influence GABAA activity through HCO_3^- in the brain, especially hCAII and hCA VII are the main player. It is also seen that potent CA inhibitor AAZ and TPM also inhibit sc-PTZ induced seizures in experimental animals. Therefore, anti-convulsant effect of **4g**, **4i**, and **8d** have been also evaluated against PTZ induced seizures test at two doses 30 mg/kg and 100 mg/kg. In sc-PTZ test, compound **4g** (30 mg/kg) was able to protect 50% animals against sc-PTZ induced seizure at 0.5 h. At the same dose **4g** displayed almost 67% seizure protection at 3 h time interval, which indicates stable activity of this compound at lower dose of 30 mg/kg. Whereas, higher dose (100 mg/kg) of this compound showed fast action, but activity was unfortunately not retained up to 3 h and exhibited 67% and 33% seizure protection at 0.5 and 3 h time interval, respectively. A stable activity trend was also observed with compound **4i** at 30mg/kg dose, although this compound was less effective against sc-PTZ induced seizure and demonstrated 33% protection at both time intervals (0.5 and 3 h). However, some improvement was observed at 100 mg/kg dose of this compound and it displayed 67% and 50% seizure protection at 0.5 and 3 h, respectively. Noticeably, compound **8d** (30 mg/kg) has shown 50% seizure protection at 0.5 h, activity was enhanced over the time and exhibited 67% protection at 3 h. 100 mg/kg dose of compound **8d** displayed 50% protection at 0.5 h and 83% protection at 3 h time duration. Thus, compound **8d** appeared as potent anti-convulsant agent which has shown higher protection against sc-PTZ induced seizures as compared to standard drugs AAZ and TPM. Overall, in vivo anticonvulsant studies revealed that compound **8d** has shown very significant protection against MES as well as sc-PTZ induced seizures at a lower dose of 30 mg/kg. Thus, this compound was chosen for advance anti-convulsant studies such as oral bioavailability, time course, and neurotoxicity evaluations.

Table 2: In vivo anticonvulsant activity of compounds 4g, 4i and 8d against MES and sc-PTZ seizure tests

Compounds ([a]Dose in mg/kg)	MES[b] screen		scPTZ[c] screen	
	0.5 h	3.0 h	0.5 h	3.0 h
4g (30)	3/6	2/6	3/6	4/6
4g (100)	3/6	4/6	4/6	2/6
4i (30)	3/6	3/6	2/6	2/6
4i (100)	5/6	3/6	4/6	3/6
8d (30)	4/6	4/6	3/6	4/6
8d (100)	3/6	3/6	3/6	5/6

TPM (30)[d]	8/8	8/8	3/6	3/6
TPM (100)[d]	NT	NT	2/6	3/6
AAZ (30)[d]	7/8	4/8	3/6	3/6
AAZ (100)[d]	8/8	4/8	5/6	3/6

[a]30 and 100 mg/kg doses were administered intraperitoneally (i.p.). The animals were examined at 0.5 and 3.0 h after injections. [b]Maximal electroshock test (MES) (n = 6 mice for each tested dose). scPTZ test (n = 6 mice for each tested dose). [d]Data taken from reference [37e]. NT: not tested.

2.3.1.3. MES seizure protecting capability of compound 8d upon oral administration: Efficacy of compound **8d** against MES induced seizure was also examined by providing orally to rats. Oral efficacy of novel chemical entity is considered as an important feature to develop as a successful therapeutic agent. In this experiment, anti-MES activity of compound **8d** has been evaluated at five time intervals (0.25- 4 h) after its oral administration (Table 3).

Table 3: Anti-MES potential of compounds 8d (30 mg/kg, bwt) after oral administration.

Time (h)	MES test[a]	% Protection[b]
0.25	1/6	17
0.5	5/6	84
1	5/6	84
2	4/6	67
4	3/6	50

[a]MES test (number of animals protected/number of animals tested; n=6). [b]Percentage seizure protection (number of animals protected/number of animals tested x 100).

These results indicate that compound **8d** has shown only 17% protection after 0.25 h oral treatment as expected, because oral administration takes more time to display action as compared to intraperitonial (i.p) or intravenous (i.v) administration. Compound **8d** enhanced protection ability after 0.5 h of oral administration and displayed 84% protection against tonic-clonic seizures. The activity was found to be sustained up to 1 h and similar percentage protection (84%) was observed at 0.5 h. Slightly less activity was noticed at 2 h time interval and this compound displayed 67% protection against MES induced seizures. Finally, this compound protected 50% of tested animals against MES induced seizure after 4 h of oral administration. Thus, it is noticed that compound **8d** has shown

excellent protection against tonic-clonic seizures up to 4h of oral administration. However, 0.5 to 1h after oral administration may be considered as a best peak time effect of compound **8d** where it displayed 84% protection. Hence, compound **8d** appeared as a potent orally active anti-convulsant agent that appreciably abolished MES induced tonic-clonic seizures upon oral administration to rats.

2.3.1.4. Time course anti-convulsant study: Long duration action of drug molecule is regarded as a useful property which may reduces the chance of drug intolerance and repeated dose associated side effects. Therefore, a time course study of anti-convulsant agent **8d** was carried out in Swiss Albino male mice at 30 mg/kg (i.p) against MES induced seizure and anti-convulsant effect was monitored up to 6 h of drug administration (Table 4). Results indicated that the compound **8d** significantly attenuated MES provoked seizure at 0.5 h time interval and displayed 70% seizure protection. At 1h time point this compound protected 60% animals and activity was sustained up to next 2 h. Further, protective ability of this compound was increased and demonstrated 70% protection against MES provoked seizure at 3 h. After 4h of drug administration this compound successfully abolished tonic-clonic generalized seizures in 50% of the animals. Lastly, at 6 h time point compound **8d** shielded 60% tested animals against MES induced generalized seizures. Thus, this time course study evidently supports long duration action of novel CA inhibitor **8d**, which forcefully protected animals against MES provoked seizures up to 6 h of drug administration.

Table 4: Time course study of compound 8d (30 mg/kg, bwt) in the MES test

Time (h)[a]	MES Test[b]	% Protection[c]
0.5	7/10	70
1	6/10	60
2	6/10	60
3	7/10	70
4	5/10	50
6	6/10	60

[a]Time after drug treatment.[b]MES test (n=10; number of protected animals/total number of tested animals). [c]Percentage protection (number of protected animals/ total number of tested animals x 100).

2.3.2. Neurotoxicity assessment: It is well studied that neurotoxicity induced by any drug molecule affects motor co-ordination system of the body. The rotarod test is considered as a simple and validated test to observe neurotoxicity associated motor co-ordination failure [40e]. Therefore, we planned to evaluate the neurotoxic effect of compound **8d** (30 mg/kg; i.p.) by using the rotarod test at different time intervals (30, 60, 90, 120 min) after compound administration (**figure 1**). To examine neurotoxicity, vehicle treated and compound **8d** pre-treated mice were placed on a rotating rod for 180s. Results indicated that compound **8d** and vehicle pre-treated mice retained almost equal time after 30, 60, 90 and 120 min of treatment. Thus, results signified that compound **8d** did not display any significant neurotoxicity at active dose 30 mg/kg.

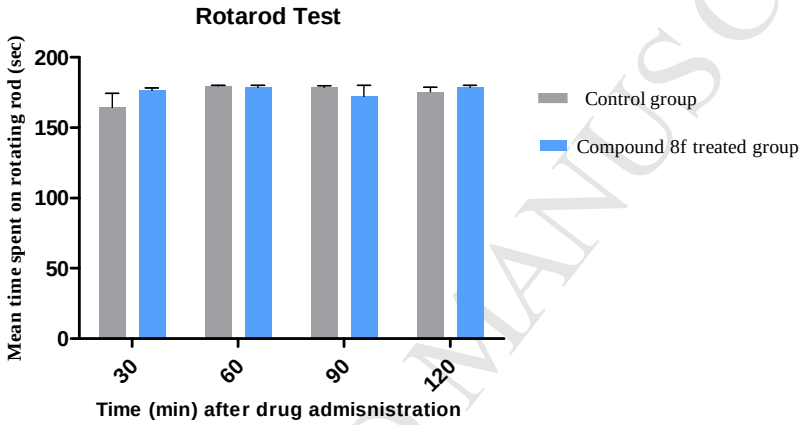


Figure 1: Time spent on the rotarod in second up to 120 min after the vehicle and compound **8d** (30mg/kg, i.p.) treatment. Each point indicates the mean ± S.E.M (n= 8, two-way repeated measures ANOVA followed by Bonferroni post-test).

2.3.3. Sub-acute toxicity study: Toxicity of biologically active agents is considered as a major barrier in the development of potent therapeutic agents. Numerous potent pre-clinically active pharmacological agents were not approved for clinical use due to their toxic nature [40d]. Therefore, a sub-acute toxicity study has been performed to evaluate safety nature of novel compound **8d** in normal, healthy Wistar rats at the dose of 100 mg/kg/bwt. During the whole experimental period, no signs of clinical toxicity or change in the animal's behavior and no death were observed. All animals took the normal amount of food and water during the whole experimental period. The results showed that compound **8d** treatment did not induce any noteworthy alteration in hematological parameters such as WBC, RBC and platelet count as compared to vehicle treated animals (Table 5).

Table 5: Hematological analysis upon oral administration of vehicle and compounds 8d for 14 days in male Wistar rats.

Parameters	Control(vehicle, *po*)	Compound **8d** (100mg/kg, *po*)± SD[a]
Hb (g/dl)	13.1 ± 0.9	12.2 ± 1.5
TLC (10^3/ml)	7.88 ± 2.5	9 ± 2.3
Neutrophils (%)	23.4 ± 2.9	23.1 ± 3.7
Lymphocytes (%)	72.3 ± 6.7	71.5 ± 3.7
Eosinophils (%)	2 ± 1.4	2.6 ± 1.7
Monocytes (%)	2.6 ± 1.5	2 ± 1.2
Basophils (%)	0.16 ± 0.4	0.33 ± 0.5
RBC (mill/mm^3)	7.9 ± 0.56	7.2 ± 0.44
Platelet count (thou/mm^3)	760.8 ± 179.9	622.3 ± 132.1

[a]The data are presented as the mean ± standard deviation; n = 6 in each group.

Thus, this compound did not display significant toxicity in the hematological system of animals. Further, toxic effect of compound **8d** on liver function was also analyzed and results showed that continuous treatment of compound **8d** restored optimum level of liver associated biomarkers such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, and total protein similar to control animals. This analysis evidently proved that compound **8d** did not induce liver toxicity in experimental animals (Table 6).

Table 6: Evaluation of Liver function associated biomarkers after compounds 8d treatment for for 14 days in rats.

Parameters	Control(vehicle, *po*)	Compound **8d** (100mg/kg, *po*)± SD[a]
SGOT (U/l)	69.9 ± 7.7	65 ± 13.9
SGPT (U/l)	58.8 ± 18.8	60.3 ± 15.2
Alkaline Phosphatase (U/l)	113.2 ± 15.1	96.5 ± 21.4
Total bilirubin(mg/dl)	0.37 ± 0.11	0.35 ± 0.10
Total protein (g/dl)	6.43 ± 0.58	6.62 ± 0.73

[a]The data are presented as the mean ± standard deviation; n = 6 in each group.

Furthermore, we have also examined several renal toxicity parameters after 14 days treatment of compound **8d**. The result clearly indicated that compound **8d** did not produce any significant renal toxicity and associated biomarkers such as creatinine, urea and uric acid level were found optimum, being similar to control animals. Hence, in this toxicological study compound **8d** appeared as a non-toxic chemical entity which had not shown any significant toxicity on liver function, renal function and hematological function (Table 7). Finally, compound **8d** has been proven as a safe CA inhibitor, which also endows excellent anti- convulsant action.

Table 7: Assessment of Kidney function associated biomarkers after oral administration of vehicle and compound 8d up to 14 days in rats.

Parameters	Control (vehicle, *po*)	Compound **8d** (100mg/kg, *po*)± SD[a]
Blood urea (mg/dl)	40.7 ± 3.3	39.6 ± 4.1
Creatinine (mg/dl)	0.62 ± 0.3	0.75 ± 0.1
Uric acid (mg/dl)	3.9 ± 2.8	4.33 ± 2.47
Calcium (mg/dl)	10.5 ± 1.5	10 ± 1.2
Phosphorus (mg/dl)	6.71 ± 1	6.48 ± 1.5
Na^+ (mEq/l)	143.8 ± 2.3	142.5 ± 2.1
K^+ (mEq/l)	4.79 ± 0.45	4.83 ± 0.38
Cl^- (mEq/l)	104.1 ± 6.2	105.6 ± 7.4

[a] The data are displayed as the mean ± standard deviation; n = 6 in each group.

3. Conclusion

Herein, two series of novel benzenesulphonamide derivatives (4a- 4i; 8a-8k) as potent CAs inhibitors have been synthesized using benzylidenehydrazine/ benzylidenehydrazine carbonyl spacer between benzesulphonamide head and substituted piperazine tails. SAR studies revealed that benzylidenehydrazine spacer containing derivatives showed moderate to good inhibitory action against hCA II and hCA VII, while benzylidenehydrazine carbonyl spacer bearing derivatives displayed effective and selective inhibition against hCA VII. Most of these derivatives poorly inhibited hCA I as well as hCA IX. The results exhibited that compounds **4g** and **4i** inhibited h CA II as well as hCA VII very effectively and compound **8d** was effectively inhibited only hCA VII. These three potent inhibitors noticeably inhibited MES as well as sc-PTZ induced seizures in mice. Among these three, compound **8d** appeared most potent anti-convulsant agent which bestowed almost similar potency as standard drug AAZ. Additionally,

Compound **8d** appeared as a long duration acting anti-convulsant without exhibiting significant neurotoxicity. This compound also appeared to be orally active anti-convulsant agent and powerfully abolished MES induced seizure in rats upon oral administration. In sub-acute toxicity study, this compound showed non-toxic behavior and did not provoke significant toxicity to liver, kidney and normal physiology.

4. Experimental Section

Chemistry: Reagents were acquired from Sigma-Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan) and solvents were obtained from Merck (Darmstadt, Germany). Thin layer chromatography (TLC) was performed on commercially accessible silica gel (Kieselgel 60, F254) coated on aluminium sheets (Merck) by using methanol/chloroform as mobile phase. Purification of synthesized compounds was carried out by column chromatography using 100−200 mesh (Merck) silica gel as stationary phase and chloroform: methanol as mobile phase. Hicon melting point apparatus (Hicon, India) was used to determine melting points of all the compounds and mass spectra of compounds were recorded with ESI-LC/MS (Agilent 6310 mass spectrometer). NMR spectra were taken by Jeol-400 MHz NMR spectrophotometer (USA), DMSO-d_6 used as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were described in parts per million relative to standard TMS, and the following abbreviations were employed to express obtained peak patterns: s, (singlet); d, (doublet); t, (triplet); m, (multiplet); brs, (broad singlet). The purity level of test compounds was investigated with reverse phase Shimadzu HPLC (Kyoto, Japan) coupled with a PDA detector and C-18 column. Samples were prepared in methanol and acetonitrile (50:50) and an injection volume of 20 μL was injected. Methanol + acetonitrile were employed as the mobile phase gradient, and the flow rate was fixed at 1 mL/min. All target compounds demonstrated more than 95 % purity. The target compounds were also screened for PAINS alerts by using SwissADME (http://www.swissadme.ch/) and FAFDrugs4 (http://fafdrugs4.mti.univ-paris-diderot.fr/) software tools.

4.1. General procedure for synthesis of 4-(4-substituted piperazin-1-yl)benzaldehyde (3a-k)

Intermediates (**3a-k**) were synthesized according to our previously reported method and their spectral data were matched with our earlier publication [40a] and supplementary material.

4.2. General procedure for the synthesis of (E)-4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4a-4i; scheme1)

An equimolar mixture of formyl derivatives (3a-3i) and 4-hydrazinobenzenesulfonamide were refluxed in ethanol with a catalytic amount of glacial acetic acid for 6-8 h. Reaction was monitored with TLC and appeared precipitate was filtered, washed with hot ethanol to accomplish target compounds 4a-4i. Target compounds 4a-4i were purified by column chromatography using $CHCl_3$: MeOH (95: 05) as eluent followed by re-crystallization by using ethanol

4.2.1. (E)-4-(2-(4-(4-phenylpiperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4a)

Yellowish-white solid; yield 88%; mp: 216-218°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.24-3.29 (m, 4H, piperazine, CH_2), 3.33-3.40 (m, 4H, piperazine, CH_2), 6.80 (t, 1H, Ar, J= 7.2Hz), 6.97-7.09 (m, 8H, Ar + NH_2), 7.23 (t, 2H, Ar, J= 7.6Hz), 7.55 (d, 2H, Ar, J= 8.4Hz), 7.63 (d, 2H, Ar, J= 8.4Hz), 7.85 (s, 1H, CH), 10.55 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.6, 48.1, 110.7, 115.1, 115.6, 119.1, 125.7, 127.1, 127.4, 128.9, 132.7, 139.5, 148.0, 150.8, 151.0; LC–MS: m/z; 436 (M^{+1}). HPLC purity: 97.9 %.

4.2.2. (E)-4-(2-(4-(4-(4-fluorophenyl)piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4b)

Creamish solid; yield 78%; mp: 225-227°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.19-3.23 (m, 4H, piperazine CH_2), 3.36 (s, 4H, piperazine, CH_2), 7.01-7.09 (m, 10H, Ar+ NH_2), 7.54 (d, 2H, Ar, J= 9.1Hz), 7.62 (d, 2H, Ar, J= 9.1Hz), 7.85 (s, 1H, CH), 10.55 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.6, 49.0, 110.7, 115.2 (d, J= 22Hz), 117.4, 125.7, 127.2, 132.7, 139.5, 147.7, 148.0, 151.0, 156.1 (d, J= 235 Hz); LC–MS: m/z; 454 (M^{+1}). HPLC purity: 98.5 %.

4.2.3. (E)-4-(2-(4-(4-(trifluoromethyl)phenyl)piperazinyl)benzylidene)hydrazinyl)benzenesulfonamide (4c)

Creamish solid; yield 68%; mp: 210-212°C ; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.34-3.47 (m, 8H, piperazine, CH_2), 7.12-7.00 (m, 8H, Ar + NH_2), 7.51-7.56 (m, 4H, Ar), 7.62 (d, 2H, Ar, J= 9.1Hz), 7.85 (s, 1H, CH), 10.55 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 46.7, 47.2, 110.8, 114.3, 115.0, 118.2, 123.6, 126.2 (q, J=21 Hz), 126.8 (q, J=166 Hz), 132.7, 139.5, 148.0, 150.8, 153.0; LC–MS: m/z; 504(M^{+1}). HPLC purity: 99.9 %.

4.2.4. (E)-4-(2-(4-(4-(2,4-dimethylphenyl)piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4d)

Yellow solid; yield 76%; mp:204-206°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 2.20 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.93 (t, 4H, piperazine, CH_2, J= 4.9Hz), 3.31-3.32 (m, 4H, piperazine, CH_2), 6.94-7.09 (m, 9H, Ar+ NH_2), 7.54 (d, 2H, Ar, J= 8.4Hz), 7.62 (d, 2H, Ar, J= 9.1Hz), 7.85 (s, 1H, CH), 10.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.4, 20.3, 48.1, 51.4, 110.7, 114.9, 118.6, 127.1, 131.5, 131.8, 132.7, 139.6, 148.0, 148.5, 151.2; LC–MS: m/z; 464(M^{+1}). HPLC purity: 96.7 %.

4.2.5. (E)-4-(2-(4-(4-(pyridin-2-yl)piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4e)

Creamish solid; yield 82%; mp: 227-229°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.43 (s, 4H, piperazine, CH_2), 3.88(s, 4H, piperazine, CH_2), 6.92-7.10 (m, 7H, Ar+ NH_2), 7.39(d, 1H, Ar, J= 9.1Hz), 7.55 (d, 2H, Ar, J= 8.4Hz), 7.62 (d, 2H, Ar, J= 9.1Hz), 7.89 (s, 1H, CH), 7.98-8.05 (m, 2H, Ar), 10.70 (brs, 1H, NH), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 45.4, 46.5, 110.7, 111.8, 112.2, 112.8, 115.0, 126.0, 127.3, 132.7, 137.8, 139.4, 143.1, 148.0, 150.2, 152.1; LC–MS: m/z; 437(M^{+1}). HPLC purity: 99.1 %.

4.2.6. (E)-4-(2-(4-(4-(pyrimidin-2-yl)piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4f)

Yellowish-white solid; yield 89%; mp: 193-195°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.28 (s, 4H, piperazine CH_2), 3.87 (s, 4H, piperazine, CH_2), 6.64 (t, 1H, Ar, J= 4.5Hz), 6.99-7.09 (m, 6H, Ar+ NH_2), 7.54 (d, 2H, Ar, J= 8.4Hz), 7.63 (d, 2H, Ar, J= 9.1Hz), 7.85 (s, 1H, CH), 8.38 (d, 2H, Ar, J= 4.5Hz), 10.54 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.0, 47.5, 110.3, 110.8, 115.2, 125.7, 127.1, 127.4, 132.7, 139.5, 148.0, 151.1, 158.0, 161.1; LC–MS: m/z; 438(M^{+1}). HPLC purity: 96 %.

4.2.7. (E)-benzyl 4-(4-((2-(4-sulfamoylphenyl)hydrazono)methyl)phenyl)piperazine-1-carboxylate (4g)

Cream solid; yield 84%; mp:254-256°C; ^{1}HNMR (DMSO-d_6,400 MHz):δ 3.27 (s, 4H, piperazine, CH_2), 3.54 (s, 4H, piperazine), 5.10 (s, 2H, CH_2), 7.00 (d, 2H, Ar, J= 9.1Hz), 7.31-7.37 (m, 5H, Ar), 7.51 (s, 2H, NH_2), 7.58 (d, 2H, Ar, J= 9.1Hz), 7.93 (d, 2H, Ar, J= 8.4Hz), 8.04 (d, 2H, Ar, J= 8.32Hz), 8.32 (s, 1H, CH), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.0, 47.0, 66.3, 110.7, 115.3, 125.9, 127.1, 127.4, 127.5, 127.8, 128.4, 132.7, 136.8, 139.4, 147.9, 150.9, 154.4; LC–MS: m/z; 494(M^{+1}). HPLC purity: 99.7 %.

4.2.8. (E)-4-(2-(4-(4-benzoylpiperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4h)

Creamish solid; yield 71%; mp: 225-227°C ; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.17-3.27(m, 4H, piperazine, CH_2), 3.41-3.82 (m, 4H, piperazine, CH_2), 6.97 (d, 2H, Ar, J= 8.4Hz), 7.03 (s, 2H, NH_2), 7.07 (d, 2H, Ar, J= 9.1Hz), 7.42-7.47 (m, 5H, Ar), 7.54 (d, 2H, Ar, J=8.4Hz), 7.62 (d, 2H, Ar, J= 8.4Hz), 7.84 (s, 1H, CH), 10.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): 49.3, 110.9, 117.0, 127.0, 127.1, 127.4, 128.5, 129.8, 133.1, 135.4, 138.8, 147.9, 169.1; LC–MS: m/z; 464(M^{+1}). HPLC purity: 99.8 %.

4.2.9. (E)-4-(2-(4-(4-(furan-2-carbonyl)piperazin-1-yl)benzylidene)hydrazinyl)benzenesulfonamide (4i)

Creamish solid; yield 80%; mp: 211-213°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.29 (s, 4H, piperazine, CH_2), 3.81 (s, 4H, piperazine, CH_2), 6.64 (s, 1H, Ar), 6.97-7.09 (m, 7H, Ar+ NH_2), 7.55 (d, 2H, Ar, J= 8.4Hz), 7.62 (d, 2H, Ar, J= 8.4Hz), 7.84 (d, 2H, Ar, J= 4.5Hz), 10.54 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.7, 110.8, 111.4, 115.2, 115.7, 125.9, 127.1, 127.4, 132.7, 139.5, 144.8, 146.9, 147.9, 150.7, 158.2; LC–MS: m/z; 454(M^{+1}). HPLC purity: 99.9 %.

4.3. Synthesis of (E)-4-(2-(4-(4-substituted piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8a-k; scheme 2)

4.3.1. Synthetic procedure of methyl 4-sulfamoylbenzoate (6)

Commercially available, 4-sulfamoylbenzoic acid **5 (**10 mmol) was refluxed in dried methanol with a catalytic amount of conc.H_2SO_4 acid for 8h. After completion of the reaction, the mixture was poured into ice cold water and obtained precipitate was filtered and dried to give methyl 4-sulfamoylbenzoate **6**.

4.3.1. Methyl 4-sulfamoylbenzoate (6)

White crystalline solid; yield 95%; mp: 148-150°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 3.87 (s, 3H, OCH_3), 7.55 (s, 2H, NH_2), 7.94 (d, 2H, Ar, J= 8.4 Hz), 8.12 (d, 2H, Ar, J= 8.4 Hz); LC–MS: m/z; 216 (M^{+1}).

4.3.2. Synthetic procedure of 4-(hydrazinecarbonyl)benzenesulfonamide (7)

A mixture of methyl 4-sulfamoylbenzoate **6** (10 mmole) and hydrazine hydrate (20 mmole) refluxed (100°C) in 1,4-dioxane for 8h. Reaction mixture was poured into ice cold water and obtained precipitate was filtered and dried to achieve 4-(hydrazinecarbonyl)benzenesulfonamide in high yield.

4.3.2. 4-(hydrazinecarbonyl)benzenesulfonamide (7)

White solid; yield 90%; mp: 214-216°C; ^{1}H NMR (DMSO-d_6,400 MHz):δ 4.56 (s, 2H, NH_2), 7.47 (s, 2H, NH_2), 7.86 (d, 2H, Ar, J= 8.4Hz), 7.94 (d, 2H, Ar, J= 8.4Hz), 9.95 (s, 1H, NH); LC–MS: m/z; 216(M^{+1}).

4.3.3. General process for the synthesis of (E)-4-(2-(4-(4-substitutedpiperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8a-8k)

An equal mole of 4-(hydrazinecarbonyl)benzenesulfonamide (**7**) and formyl derivatives (3a-3m) were refluxed (100°C) in ethanol with a catalytic amount of glacial acetic acid for 8h. The precipitate appeared at the end of the reaction was filtered and washed with hot ethanol to bestow crude compounds and then after, target compounds (E)-4-(2-(4-(4-substituted piperazin-1-yl) benzylidene)hydrazinecarbonyl)benzenesulfonamide (8a-8k) were purified by column chromatography using $CHCl_3$/MeOH (90: 10) as eluent and recrystallization was performed using ethanol as a solvent.

4.3.3.1. (E)-4-(2-(4-(4-phenylpiperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8a)

Light yellow solid; yield 88%; mp: 278-280°C; ^{1}H NMR (DMSO-d_6, 400MHz): δ 3.23 (t, 4H, piperazine, CH_2, J= 4.9Hz), 3.37 (t, 4H, piperazine, J= 4.2Hz), 6.76 (t, 1H, Ar, J= 6.8Hz), 6.95 (d, 2H, Ar, J= 8.4Hz), 7.02 (d, 2H, Ar, J= 8.4Hz), 7.19 (t, 2H, Ar, J= 7.6Hz), 7.47 (s, 2H, NH_2), 7.56 (d, 2H, Ar, J= 8.4Hz), 7.89 (d, 2H, Ar, J= 8.4Hz), 8.01 (d, 2H, Ar, J= 8.4Hz), 8.29 (s, 1H, CH), 11.74 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.2, 48.1, 114.7, 115.7, 119.1, 124.0, 125.6, 128.1, 128.4, 128.9, 136.5, 148.7, 150.7, 152.0, 161.7; LC–MS: m/z; 464(M^{+1}). HPLC purity: 97.8 %.

4.3.3.2. (E)-4-(2-(4-(4-(4-fluorophenyl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8b)

Cream solid; yield 78%; mp: 265-267°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.21 (t, 4H, piperazine, CH_2, J= 4.9Hz), 3.40 (t, 4H, piperazine, J= 4.9Hz), 6.99-7.09 (m, 6H, Ar), 7.50 (s, 2H, NH_2), 7.60 (d, 2H, Ar, J= 9.1Hz), 7.94 (d, 2H, Ar, J= 8.4Hz), 8.05 (d, 2H, Ar, J= 8.4Hz), 8.34 (s, 1H, CH), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.2,

48.9, 114.7, 115.3 (d, J=22 Hz), 117.3, 124.1, 125.7, 128.3, 136.5, 146.4, 147.6, 148.7, 152.1, 156.1 (d, J=230 Hz), 161.7; LC–MS: m/z; 482(M^{+1}). HPLC purity: 97.2 %.

4.3.3.3. (E)-4-(2-(4-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8c)

Creamish-white solid; yield 75%; mp: 270-272°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.43 (s, 8H, piperazine, CH_2), 7.05 (d, 2H, Ar, J= 8.7Hz), 7.11 (d, 2H, Ar, J= 8.4Hz), 7.49-7.54 (m, 4H, Ar+NH_2), 7.61 (d, 2H, Ar, J= 8.4Hz), 7.94 (d, 2H, Ar, J= 7.6Hz), 8.05 (d, 2H, Ar, J= 8.4Hz), 8.34 (s, 1H, CH), 11.79 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 46.6, 46.8, 114.2, 118.1, 124.1, 126.3 (q, J=20 Hz), 127.0 (q, J=260 Hz), 136.5, 146.4, 148.7, 151.7, 152.9, 161.7; LC–MS: m/z; 532(M^{+1}). HPLC purity: 96.7 %.

4.3.3.4. (E)-4-(2-(4-(4-(2,4-dimethylphenyl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8d)

Yellow solid; yield 79%; mp: 216-218°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.24 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.93 (s, 4H, piperazine, CH_2), 3.39 (s, 4H, piperazine, CH_2), 6.95-7.06 (m, 5H, Ar), 7.51 (s, 2H, NH_2), 7.60 (d, 2H, Ar, J= 8.4Hz), 7.93 (d, 2H, Ar, J= 8.4 Hz), 8.04 (d, 2H, Ar, J= 8.4 Hz), 8.33 (s, 1H, CH), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.4, 20.3, 47.7, 51.4, 114.5, 118.6, 124.0, 125.7, 126.9, 128.2, 128.4, 131.4, 131.8, 136.5, 146.4, 148.4, 148.8, 152.2, 161.7; LC–MS: m/z; 492 (M^{+1}); HPLC purity: 98.6 %.

4.3.3.5. (E)-4-(2-(4-(4-(pyridin-2-yl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8e)

Cream solid; yield 85%; mp: 275-277°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.37(t, 4H, piperazine, CH_2, J= 4.9Hz), 3.63(t, 4H, piperazine, CH_2, J= 4.5Hz), 6.66(t, 1H, Ar, J= 3.4Hz), 6.88(d, 1H, Ar, J= 8.3Hz), 7.05(d, 2H, Ar, J= 9.1Hz), 7.50-7.61(m, 5H, Ar+NH_2), 7.93(d, 2H, Ar, J= 8.4Hz), 8.04(d, 2H, Ar, J= 8.4Hz), 8.11(d, 1H, Ar, J= 3.0Hz), 8.33(s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 44.3, 46.9, 107.2, 113.2, 114.6, 125.7, 128.2, 128.4, 136.5, 137.5, 147.5, 148.8, 159.0, 161.7; LC–MS: m/z; 465(M^{+1}); HPLC purity: 98.9 %.

4.3.3.6. (E)-4-(2-(4-(4-(pyrimidin-2-yl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8f)

Creamish solid; yield 77%; mp: 274-276°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.35-3.36 (m, 4H, piperazine, CH_2), 3.87 (t, 4H, piperazine, J= 4.9Hz), 6.65 (t, 1H, Ar, J= 4.5Hz), 7.04 (d, 2H, Ar, J= 9.1Hz), 7.51 (s, 2H, NH_2), 7.59 (d, 2H,

Ar, J= 8.4Hz), 7.93 (d, 2H, Ar, J= 8.4Hz), 8.04 (d, 2H, Ar, J= 8.4Hz), 8.33 (s, 1H, CH), 8.37 (d, 2H, Ar, J= 4.5Hz), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 42.9, 47.0, 110.3, 114.7, 124.1, 125.7, 128.2, 128.4, 136.5, 146.3, 148.7, 152.0, 157.9, 161.1, 161.7; LC–MS: m/z; 466(M^{+1}); HPLC purity: 95 %.

4.3.3.7. (E)-benzyl 4-(4-((2-(4-sulfamoylbenzoyl)hydrazono)methyl)phenyl)piperazine-1-carboxylate (8g)

Light yellow solid; yield 85%; mp: 251-253°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.26 (s, 4H, piperazine, CH_2), 3.53 (s, 4H, piperazine, CH_2), 5.10 (s, 2H, CH_2), 7.00 (d, 2H, Ar, J= 8.4Hz), 7.31-7.37 (m, 5H, Ar), 7.51 (s, 2H, NH_2), 7.59 (d, 2H, Ar, J= 8.4Hz), 7.91-7.95 (m, 2H, Ar), 8.05 (d, 2H, Ar, J= 8.4Hz), 8.33 (s, 1H, CH), 11.79 (a, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 43.1, 47.1, 66.3, 114.9, 124.3, 125.7, 127.6, 127.9, 128.3, 128.4, 128.5, 136.6, 136.8, 146.5, 148.8, 151.9, 154.4, 161.8; LC–MS: m/z; 522(M^{+1}); HPLC purity: 99.1 %.

4.3.3.8. (E)-4-(2-(4-(4-benzoylpiperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8h)

Cream solid; yield 89%; mp: 260-262°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.24-3.29 (m, 4H, piperazine, CH_2), 3.47-3.75 (m, 4H, piperazine, CH_2), 7.01 (d, 2H, Ar, J= 9.1Hz), 7.44-7.51 (m, 7H, Ar+NH_2), 7.59 (d, 2H, Ar, J= 9.1Hz), 7.93 (d, 2H, Ar, J= 7.6Hz), 8.04 (d, 2H, Ar, J= 8.4Hz), 8.33 (s, 1H, CH), 11.78 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 46.6, 47.4, 114.9, 124.3, 125.7, 127.0, 128.2, 128.4, 129.6, 135.7, 146.5, 148.7, 151.8, 161.7, 169.0; LC–MS: m/z; 492 (M^{+1}); HPLC purity: 99.6 % .

4.3.3.9. (E)-4-(2-(4-(4-(furan-2-carbonyl)piperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8i)

Creamish solid; yield 84%; mp: 242-244°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.36 (t, 4H, piperazine, CH_2, J= 4.5Hz), 3.81 (s, 4H, piperazine, CH_2), 6.64 (t, 1H, Ar, J= 1.5Hz), 7.01-7.04 (m, 3H, Ar), 7.51 (s, 2H, NH_2), 7.60 (d, 2H, Ar, J= 9.1 Hz), 7.86 (s, 1H, Ar), 7.93 (d, 2H, Ar, J= 8.4Hz), 8.04 (d, 2H, Ar, J= 7.6Hz), 8.33 (s, 1H, CH), 11.78 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.2, 111.3, 114.7, 115.8, 124.2, 125.7, 128.2, 128.5, 136.6, 144.8, 146.5, 146.9, 148.7, 151.7, 158.3, 161.8; LC–MS: m/z; 482(M^{+1}); HPLC purity: 99.8 %.

4.3.3.10. (E)-4-(2-(4-(4-benzylpiperazin-1-yl)benzylidene)hydrazinecarbonyl)benzenesulfonamide (8j)

Yellow solid; yield 86%; mp: 228-230°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.24 (s, 4H, piperazine, CH_2), 3.36 (s, 4H, piperazine, CH_2), 3.50 (s, 2H, CH_2), 6.97 (d, 2H, Ar, J= 8.4Hz), 7.25-7.33 (m, 5H, Ar), 7.52-7.58 (m, 4H, Ar+NH_2), 7.94 (d, 2H, Ar, J= 8.4Hz, Ar), 8.05 (d, 2H, Ar, J= 8.4Hz), 8.32 (s, 1H, CH), 11.77 (s, 1H, NH), ^{13}C NMR (DMSO-d_6,

100 MHz): δ 47.1, 52.3, 62.0, 114.4, 123.8, 125.7, 126.9, 128.2, 128.4, 128.9, 136.6, 137.9, 146.4, 148.8, 152.1, 161.7; LC–MS: m/z; 478(M^{+1}); HPLC purity: 98.4 %.

4.3.3.11. (E)-4-(2-(4-(4-(benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl)benzylidene)hydrazinecarbonyl) benzenesulfonamide (8k)

Yellowish-white solid; yield 77%; mp: 230-232°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.48 (s, 4H, piperazine, CH_2), 3.24 (s, 4H, piperazine, CH_2), 3.42 (s, 2H, CH_2), 5.98 (s, 2H, CH_2), 6.77 (d, 1H, Ar, J= 8.4Hz), 6.85 (t, 2H, Ar, J= 8.3Hz), 6.97 (d, 2H, Ar, J= 8.4Hz), 7.50 (s, 2H, NH_2), 7.55 (d, 2H, Ar, J= 8.4Hz), 7.92 (d, 2H, Ar, J= 8.4Hz), 8.03 (d, 2H, Ar, J= 8.4Hz), 8.31 (s, 1H, CH), 11.75 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 47.0, 52.1, 61.6, 100.7, 107.8, 109.1, 114.3, 121.8, 123.7, 125.7, 128.1, 128.4, 131.7, 136.6, 146.1, 146.4, 147.2, 148.7, 161.7, LC–MS: m/z; 522(M^{+1}); HPLC purity: 98.9 %.

4.4. Carbonic anhydrase inhibition assay: An applied photophysics stopped-flow instrument has been employed to examine the CA catalyzed CO_2 hydration activity as protocol described in our earlier publications [13,30,33, 34]. The human isoforms hCA I, II, VII and IX were recombinant enzymes produced in our laboratory as described earlier 13,30, 33,34].

4.5. Anti-convulsant Activity: The anti-convulsant potency of novel CA inhibitors was evaluated in MES as well as sc-PTZ induced seizure animal models. Healthy adult male Swiss Albino mice (25-30g) as well as male Wistar rats (100-150g) were obtained from "Disease free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences" Hisar, Haryana, India. Animals were housed under standard animal laboratory conditions at animal house of Dr. B.R Ambedkar Center For Biomedical Research, University of Delhi, India. The test compounds were freshly prepared in 1% gum acacia, injected intraperitoneally (i.p.) to mice (0.01 ml/g body weight) and orally to rats (0.04 ml per 10 g of body weight). The pentylenetetrazole was dissolved in normal sterile saline solution and experimental protocols were earlier approved by the Institutional Animal Ethics Committee (Approval number: IAEC/ACBR/2016/PML/016) for animal care. Additionally, it was tried to minimize animal suffering during whole experimental tenure and used only necessary number of animals to acquire reliable scientific data. All animal experiments performed in the manuscript were conducted in compliance with institutional guidelines.

4.5.1. Maximal Electroshock (MES) Test: MES anti-convulsant test was performed at the doses of 30 and 100 mg/kg. Oral bioavailability studies have been also performed using MES and protection against MES induced seizures was documented at 0.25, 0.5, 1, 2 and 4 h time intervals. The seizures were provoked by using an electroconvulsometer (Techno Instruments, Lucknow, India) and the electric stimulus of 50 mA (mice) and 150 mA (rats) was delivered transauricularly for 0.2 s and complete obliteration of hind limb was documented as protection against MES induced seizures [40b, 40d, 37e].

4.5.2. Subcutaneous Pentylenetrazole (scPTZ) Test: In this test, convulsive dose of PTZ (85 mg/kg, (CD_{97}) was used to induce seizures in Swiss albino male mice. Protection against sc-PTZ induced seizure was evaluated at the doses of 30 and 100 mg/kg as describe earlier[40b,40e,37e].

4.5.3. Time course study. Time course study was performed at the dose of 30 mg/ kg (compound 8d) and the percentage protection obtained against MES induced seizure was noted at various time intervals (0.5, 1, 2, 3, 4 and 6 h) by using MES test [37e].

4.6. Neurotoxicity-minimal motor impairment (MMI): An acute neurotoxicity (NT) study was performed using the standard rotarod test. The rotarod instrument bears a rotating rod of 3.2 cm diameter (Techno Rotarod system, Techno Electronics, Lucknow, India). Prior to experiment, animals were trained on the accelerating rotating rod at 25 r.p.m and the mice, which fails from rotating rod for a extend period of one minute, were removed from the experiment. 30 mg/kg dose was provided to animals through i.p injection and neurotoxicity was appraised. Inability of an animal to retain on rotating rod was considered as neurological impairment and the test compound was proven as neurotoxic, if the treated animal falls from rotating rod 3 times in 3 min time period [40e].

4.7. Toxicological Studies: In this experiment, test compound and vehicle were administered orally each day to healthy adult male Wistar rats up to 14 days. During the whole experimental period, daily observation was done to identify signs of toxicity such as, outgrowth of tissue, necrosis, and altered behavior. After the stipulated period, the animal was anesthetized through anesthetic ether, and blood was taken out via cardiac puncture. The blood samples were carefully labeled and analyzed according to our pervious reported method [37e] .

4.8. Statistical analysis

Statistical analysis was executed using the GraphPad Prism 5 software (La Jolla, USA). The data of neurotoxicity assay (rotarod test) were represented as Mean ± S.E.M and analyzed by two way repeated measures ANOVA followed by Bonferroni post-test. The P value less than 0.05 was considered as statistically significant.

Acknowledgements. C.B. Mishra is grateful to Ministry of Health and Family Welfare, New Delhi, India [Grant no: V.25011/458-HRD/2016-HR] and S. Kumari is thankful to the Department of Biotechnology New Delhi, India for providing financial assistance. M. Tiwari is thankful to the University of Delhi, Delhi, India for providing the financial help to carry out this research work. The University Science Instrumentation Center (USIC), University of Delhi is highly acknowledged for NMR spectral data. Work from Supuran laboratory was supported by two EU grants (Dynano and Metoxia).

Supplementary Material

NMR (^{1}H & ^{13}C) spectra, HPLC chromatogram and PAINS screening result of synthesized compounds are provided in supplementary material.

References

[1] R.S. Fisher, B.W. van Emde, W. Blume, C. Elger, P. Genton, P. Lee, J. Jr. Engel, Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE), Epilepsia. 46 (2005) 470-472.

[2] W. Loscher, H. Klitgaard, R.E. Twyman, D. Schmidt, New avenues for anti-epileptic drug discovery and development, Nat. Rev. Drug. Discov. 12 (2013) 757-776.

[3] S. Kumari, C.B. Mishra, M. Tiwari, Polypharmacological drugs in the treatment of epilepsy: the comprehensive review of marketed and new emerging molecules, Curr. Pharm. Des. 22 (2016) 3212-3225.

[4] W. Loscher, D. Schmidt, Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma, Epilepsia. 52 (2011) 657-678.

[5] (a) A.M. Aribi, J.L. Stringer, Effects of antiepileptic drugs on extracellular pH regulation in the hippocampal CA1 region in vivo, Epilepsy Res. 49 (2002) 143-151;

(b) J.C. Chen, M. Chesler, pH transients evoked by excitatory synaptic transmission are increased by inhibition of extracellular carbonic anhydrase, Proc. Natl. Acad. Sci. USA. 89 (1992) 7786-7790;

(c) Z.Q. Xiong, P. Saggau, J.L. Stringer, Activity-dependent intracellular acidification correlates with the duration of seizure activity, J. Neurosci. 20 (2000) 1290-1296;

(d) U. Bonnet, M. Wiemann, D. Bingmann, CO_2/ HCO_3(-)-withdrawal from the bath medium of hippocampal slices: biphasic effect on intracellular pH and bioelectric activity of CA3-neurons, Brain Res. 796 (1998) 161-170.

[6] (a) A. Casini, J, Antel, F. Abbate, A. Scozzafava, S. David, H. Waldeck, S. Schafer, C.T. Supuran, Carbonic anhydrase inhibitors: SAR and X-ray crystallographic study for the interaction of sugar sulfamates/sulfamides with isozymes I, II and IV, Bioorg. Med. Chem. Lett. 13 (2003) 841-845;

(b) A. Di Fiore, G. De Simone, V. Alterio, V. Riccio, J.Y. Winum, F. Carta, C.T. Supuran, The anticonvulsant sulfamide JNJ-26990990 and its S, S-dioxide analog strongly inhibit carbonic anhydrases: solution and X-ray crystallographic studies, Org. Biomol. Chem. 14 (2016) 4853-4858;

(c) C.T. Supuran, Drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors, Expert. Opin. Drug Metab. Toxicol. 12 (2016) 423-431.

[7] (a) V. Alterio, A. Di Fiore, K. D'Ambrosio, C.T. Supuran, G. De Simone, Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms?, Chem. Rev. 112 (2012) 4421-4468;

(b) C.T. Supuran, Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators, Nat. Rev. Drug. Discovery 7 (2008) 168-181;

(c) M.J. Smeulders, T.R. Barends, A. Pol, A. Scherer, M.H. Zandvoort, A. Udvarhelyi, A.F. Khadem, A. Menzel, J. Hermans, R.L. Shoeman, H.J. Wessels, L.P. van den Heuvel, L. Russ, I. Schlichting, M.S. Jetten, H.J. Op den Camp, Evolution of a new enzyme for carbon disulphide conversion by an acidothermophilic archaeon, Nature 478 (2011) 412-416.

[8] (a) J.L. Rummer, D.J. McKenzie, A. Innocenti, C.T. Supuran, C.J. Brauner, Root effect hemoglobin may have evolved to enhance general tissue oxygen delivery, Science 340 (2013) 1327-1329;

(b) C.T. Supuran, Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnologic use for CO_2 capture, J. Enzyme Inhib. Med. Chem. 28 (2013) 229-230;

(c) D. Neri, C.T. Supuran, Interfering with pH regulation in tumors as a therapeutic strategy, Nat. Rev. Drug. Discovery. 10 (2011) 767-777;

(d) K.S. Smith, C. Jakubzick, T.S. Whittam, J.G. Ferry, Carbonic anhydrase is an ancient enzyme widespread in prokaryotes, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 15184-15189.

[9] (a) C.B. Mishra, S. Kumari, A. Angeli, S.M. Monti, M. Buonanno, A. Prakash, M. Tiwari, C.T. Supuran, Design, synthesis and biological evaluation of N-(5-methyl-isoxazol-3-yl/ 1,3,4-thiadiazol-2-yl)-4-(3 substitutedphenylureido) benzenesulfonamides as human carbonic anhydrase isoenzymes I, II, VII and XII inhibitors, J. Enzyme. Inhib. Med. Chem. 31 (2016) 174-179;

(b) C.T. Supuran, How many carbonic anhydrase inhibition mechanisms exist? J. Enzyme Inhib. Med. Chem. 31 (2016), 345-360;

(c) F. Cottier, W. Leewattanapasuk, L.R. Kemp, M. Murphy, C.T. Supuran, O. Kurzai, F.A. Muhlschlegel, Carbonic anhydrase regulation and CO_2 sensing in the fungal pathogen Candida glabrata involves a novel Rca1p ortholog, Bioorg. Med. Chem. 21 (2013) 1549-1554;

(d) C. Schlicker, R.A. Hall, D. Vullo, S. Middelhaufe, M. Gertz, C.T. Supuran, F.A. Muhlschlegel, C. Steegborn, Structure and inhibition of the CO_2-sensing carbonic anhydrase Can2 from the pathogenic fungus Cryptococcus neoformans. J. Mol. Biol. 385 (2009) 1207-1220.

[10] (a) D. Filippi, M. Sciaky, N. Limozin, G. Laurent, Anhydrase carbonique du système nerveux central du rat, Isolement et proprietes. Biochimie, 60 (1978) 99-102;

(b) L.F. Agnati, B. Tinner, W.A. Staines, K. Vaananen, K. Fuxe, On the cellular localization and distribution of carbonic anhydrase II immunoreactivity in the rat brain, Brain Res. 676 (1995) 10-24.

[11] S. Parkkila, An Overview of the Distribution and Function of Carbonic Anhydrase in Mammals. In The Carbonic Anhydrases: New Horizons; Chegwidden, R., Ed.; Birkauser Verlag: Basel, Switzerland, (2000) 79-93.

[12] P. Halmi, S. Parkkila, J. Honkaniemi, Expression of carbonic anhydrases II, IV, VII, VIII and XII in rat brain after kainic acid induced status epilepticus, Neurochem. Int. 48 (2006) 24-30.

[13] (a) T. Masuzawa, F. Sato, The enzyme histochemistry of the choroid plexus, Brain 106 (1983) 55-99;

(b) L. Velisek, S.L. Moshe, W. Cammer, Developmental changes in seizure susceptibility in carbonic anhydrase II-deficient mice and normal littermates, Dev. Brain Res. 72 (1993) 321-324;

(c) G. De Simone, A. Scozzafava, C.T. Supuran, Which carbonic anhydrases are targeted by the antiepileptic sulfonamides and sulfamates?, Chem. Biol. Drug. Des. 74 (2009) 317-321.

[14] M.M. Lakkis, K.S. Oshea, R.E. Tashian, Differential expression of the carbonic anhydrase genes for CA VII (Car7) and CA-RP VIII (Car8) in mouse brain, J. Histochem. Cytochem. 45 (1997) 657-662.

[15] (a) E. Ruusuvuori, K. Kaila, Carbonic Anhydrases and Brain pH in the Control of Neuronal Excitability. In Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications; S. Frost, R. McKenna, Eds.; Springer: New York, (2014) 271-290;

(b) E. Ruusuvuori, A.K. Huebner, I. Kirilkin, A.Y. Yukin, P. Blaesse, M. Helmy, H.J. Kang, M. El Muayed, J.C. Hennings, J. Voipio, N. Sestan, C.A. Hubner, K. Kaila, Neuronal carbonic anhydrase VII provides GABAergic excitatory drive to exacerbate febrile seizures, EMBO J. 32 (2013) 2275-2286.

[16] (a) E. Ruusuvuori, H. Li, K. Huttu, J.M. Palva, S. Smirnov, C. Rivera, K. Kaila, J.Voipio, Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells, J. Neurosci. 24 (2004) 2699-2707;

(b) C. Rivera, J. Voipio, K. Kaila, Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII, J. Physiol. 562 (2005) 27-36;

(c) I. Nishimori, Acatalytic CAs: Carbonic Anhydrase Related Proteins. In Carbonic Anhydrase: Its Inhibitors and Activators; C.T. Supuran, A. Scozzafava, J., Conway, Eds.; CRC Press: Boca Raton, FL, (2004) 25-43.

[17] (a) M. Chesler, Regulation and modulation of pH in the brain, Physiol. Rev. 83 (2003) 1183-1221;

(b) J.R. Casey, S. Grinstein, J. Orlowski, Sensors and regulators of intracellular pH, Nat. Rev. Mol. Cell. Biol. 11 (2010) 50-61;

(c) E. Ruusuvuori, I. Kirilkin, N. Pandya, K. Kaila, Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism, J. Neurosci. 30 (2010) 15638-15642;

(d) M. Pasternack, J. Voipio, K. Kaila, Intracellular carbonic anhydrase activity and its role in GABA-induced acidosis in isolated rat hippocampal pyramidal neurons, Acta Physiol. Scand. 148 (1993) 229-231;

(e) R.H. Williams, L.T. Jensen, A. Verkhratsky, L. Fugger, D. Burdakov, Control of hypothalamic orexin neurons by acid and CO_2, Proc. Natl. Acad. Sci. U. S. A 104 (2007) 10685-10690.

[18] T. Taira, S. Smirnov, J. Voipio, K. Kaila, Intrinsic proton modulation of excitatory transmission in rat hippocampal slices, Neuroreport. 4 (1993) 93-96.

[19] J.A. Gottfried, M. Chesler, Endogenous H^+ modulation of NMDA receptormediated EPSCs revealed by carbonic anhydrase inhibition in rat hippocampus, J. Physiol., 478 (1994), 373-378.

[20] (a) W.G. Reiss, K.S. Oles, Acetazolamide in the treatment of seizures, Ann. Pharmacother. 30 (1996) 514-519;

(b) F. Katayama, H. Miura, S. Takanashi, Long-term effectiveness and side effects of acetazolamide as an adjunct to other anticonvulsants in the treatment of refractory epilepsies, Brain Dev. 24 (2002) 150-154;

(c) S. Parkkila, A.K. Parkkila, J. Kivela, Role of Carbonic Anhydrase and Its Inhibitors in Gastroenterology, Neurology and Nephrology. In Carbonic Anhydrase: Its Inhibitors and Activators; C.T. Supuran, A. Scozzafava, J. Conway, Eds.; CRC Press: Boca Raton, FL, (2004) 283-301.

[21] (a) I.E. Leppik, Zonisamide: chemistry, mechanism of action, and pharmacokinetics, Seizure 13 (2004) S5-S9;

(b) M. Bialer, S.I. Johannessen, H.J. Kupferberg, R.H. Levy, P. Loiseau, E. Perucca, Progress report on new antiepileptic drugs: a summary of the Fifth Eilat Conference (EILAT V), Epilepsy Res. 43 (2001) 11-58;

(c) A.I. Herrero, N. Del Olmo, J.R. Gonzalez-Escalada, J.M. Solis, Two new actions of topiramate: inhibition of depolarizing GABA (A)-mediated responses and activation of a potassium conductance, Neuropharmacology 42 (2002) 210-220.

[22] (a) M.A. Ilies, B. Masereel, S. Rolin, A. Scozzafava, G. Campeanu, V. Cimpeanu, C.T. Supuran, Carbonic anhydrase inhibitors: aromatic and heterocyclic sulfonamides incorporating adamantyl moieties with strong anticonvulsant activity. Bioorg. Med. Chem. Lett. 12 (2004) 2717-2726;

(b) T. Leniger, J. Thone, M. Wiemann, Topiramate modulates pH of hippocampal CA3 neurons by combined effects on carbonic anhydrase and Cl^-/HCO_3^- exchange. Br. J. Pharmacol. 142 (2004) 831-842.

[23] W.B. Cammer, L.P. Brion, Carbonic Anhyrdase in the Nervous System. The Carbonic Anhydrases New Horizons; Birkauser Verlag, Basel, (2000) 475-489.

[24] (a) A. Di Fiore, C. Capasso, V. De Luca, S.M. Monti, V. Carginale, C.T. Supuran, A. Scozzafava, C. Pedone, M. Rossi, G. De Simone, X-ray structure of the first extremo-α-carbonic anhydrase', a dimeric enzyme from the thermophilic bacterium Sulfurihydrogenibium yellowstonense YO3AOP1, Acta Crystallogr. D. Biol. Crystallogr. 69 (2013) 1150-1159;

(b) S.Z. Fisher, L. Govindasamy, N. Boyle, M.A. McKenna, D.N. Silverman, G.M. Blackburn, R. McKenna, X-ray crystallographic studies reveal that the incorporation of spacer groups in carbonic anhydrase inhibitors causes alternate binding modes, Acta Cryst. 62 (2006) 618-622.

[25] (a) D. Vullo, C.T. Supuran, A. Scozzafava, G. De Simone, S.M. Monti, V. Alterio, F. Carta, Kinetic and X-ray crystallographic investigations of substituted 2-thio-6-oxo-1,6 dihydropyrimidine-benzenesulfonamides acting as carbonic anhydrase inhibitors, Bioorg. Med. Chem. 24 (2016) 3643-3648;

(b) G. De Simone, S.M. Monti, V. Alterio, M. Buonanno, V. De Luca, M. Rossi, V. Carginale, C.T. Supuran, C. Capasso, A. Di Fiore, Crystal structure of the most catalytically effective carbonic anhydrase enzyme known, SazCA from the thermophilic bacterium Sulfurihydrogenibium azorense, Bioorg. Med. Chem. Lett. 25 (2015) 2002-2006.

[26] G. De Simone, V. Alterio, C.T. Supuran, Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors, Expert Opin. Drug Discovery 8 (2013) 793-810.

[27] (a) R.L. Arechederra, A. Waheed, W.S. Sly, C.T. Supuran, S.D. Minteer, Effect of sulfonamides as selective carbonic anhydrase VA and VB inhibitors on mitochondrial metabolic energy conversion, Bioorg. Med. Chem. 21 (2013) 1544-1548;

(b) E. Masini, F. Carta, A. Scozzafava, C.T. Supuran, Antiglaucoma carbonic anhydrase inhibitors: A patent review, Expert Opin. Ther. Pat. 23 (2013) 705-716;

(c) C.T. Supuran, Carbonic anhydrase inhibitors, Bioorg. Med. Chem. Lett. 20 (2010) 3467-3474.

[28] (a) M. Aggarwal, R. McKenna, Update on carbonic anhydrase inhibitors: a patent review, (2008-2011), Expert. Opin. Ther. Pat. 22 (2012) 903-915;

(b) M. Aggarwal, B. Kondeti, R. McKenna, Anticonvulsant/antiepileptic carbonic anhydrase inhibitors: a patent review, Expert. Opin. Ther. Pat. 23 (2013) 717-724;

(c) A. Thiry, J.M. Dogne, C.T. Supuran, B. Masereel, Anticonvulsant sulfonamides/sulfamates/sulfamides with carbonic anhydrase inhibitory activity: drug design and mechanism of action, Curr. Pharm. Des. 14 (2008) 661-671;

(d) F. Fabrizi, F. Mincione, T. Somma, G. Scozzafava, F. Galassi, E. Masini, F. Impagnatiello, C.T. Supuran, A new approach to antiglaucoma drugs: carbonic anhydrase inhibitors with or without NO donating moieties, Mechanism of action and preliminary pharmacology, J. Enzyme Inhib. Med. Chem. 27 (2012) 138-147.

[29] (a) A. Thiry, J.M. Dogne, B. Masereel, C.T. Supuran, Carbonic anhydrase inhibitors as anticonvulsant agents, Curr. Top. Med. Chem. 7 (2007) 855-864;

(b) F. Carta, C.T. Supuran, A. Scozzafava, Novel therapies for glaucoma: a patent review 2007-2011, Expert. Opin. Ther. Pat. 22 (2012) 79-88;

(c) F. Carta, C.T. Supuran, Diuretics with carbonic anhydrase inhibitory action: A patent and literature review (2005-2013), Expert. Opin. Ther. Pat. 23 (2013) 681-691.

[30] (a) M. Ferraroni, L. Lucarini, E. Masini, M. Korsakov, A. Scozzafava, C.T. Supuran, M. Krasavin, 1, 3-Oxazole-based selective picomolar inhibitors of cytosolic human carbonic anhydrase II alleviate ocular hypertension in rabbits: Potency is supported by X-ray crystallography of two leads, Bioorg. Med. Chem. 25 (2017) 4560-4565;

(b) N. Karal, A. Akdemir, F. Goktaş, P. Eraslan, A. Angeli, M. Kızılırmak, C.T. Supuran, Novel sulfonamide-containing 2-indolinones that selectively inhibit tumor-associated alpha carbonic anhydrases, Bioorg. Med. Chem. 25 (2017) 3714-3718;

(c) S. Akocak, N. Lolak, A. Nocentini, G. Karakoc, A. Tufan, C.T. Supuran, Synthesis and biological evaluation of novel aromatic and heterocyclic bis-sulfonamide Schiff bases as carbonic anhydrase I, II, VII and IX inhibitors, Bioorg. Med. Chem. 25 (2017) 3093-3097;

(d) M. Bozdag, A.M. Alafeefy, A.M. Altamimi, F. Carta, C.T. Supuran, D. Vullo, Synthesis of new 3-(2-mercapto-4-oxo-4H-quinazolin-3-yl)-benzenesulfonamides with strong inhibition properties against the tumor associated carbonic anhydrases IX and XII, Bioorg. Med. Chem. 25 (2017) 2782-2788;

(e) F. Carta, D. Vullo, S.M. Osman, Z. AlOthman, C.T. Supuran, Synthesis and carbonic anhydrase inhibition of a series of SLC-0111 analogs, Bioorg. Med. Chem. 25 (2017) 2569-2576.

[31] (a) D. Bibi, H. Mawasi, A. Nocentini, C.T. Supuran, B. Wlodarczyk, R.H. Finnell, M. Bialer, Design and Comparative Evaluation of the Anticonvulsant Profile, Carbonic-Anhydrase Inhibition and Teratogenicity of Novel Carbamate Derivatives of Branched Aliphatic Carboxylic Acids with 4-Aminobenzensulfonamide, Neurochem. Res. 42 (2017) 1972-1982;

(b) E. Bruno, M.R. Buemi, L. De Luca, S. Ferro, A.M. Monforte, C.T. Supuran, D. Vullo, G. De Sarro, E. Russo, R. Gitto, In Vivo Evaluation of Selective Carbonic Anhydrase Inhibitors as Potential Anticonvulsant Agents, ChemMedChem. 11 (2016) 1812-1818;

(c) N. Hen, M. Bialer, B. Yagen, A. Maresca, M. Aggarwal, A.H. Robbins, R. McKenna, A. Scozzafava, C.T. Supuran, Anticonvulsant 4-aminobenzenesulfonamide derivatives with branched-alkylamide moieties: X-ray crystallography and inhibition studies of human carbonic anhydrase isoforms I, II, VII, and XIV, J. Med. Chem. 54 (2011) 3977-3981.

[32] (a) C.T. Supuran, Structure-based drug discovery of carbonic anhydrase inhibitors, J Enzyme Inhib Med Chem. 27 (2012) 759-772;

(b) C.T. Supuran, Advances in structure-based drug discovery of carbonic anhydrase inhibitors, Expert. Opin. Drug Discov. 12 (2017) 61-88.

[33] (a) H.I. Gul, E. Mete, S.E. Eren, H. Sakagami, C. Yamali, C.T. Supuran, Designing, synthesis and bioactivities of 4-[3-(4-hydroxyphenyl)-5-aryl-4, 5-dihydro-pyrazol-1-yl]benzenesulfonamides, J. Enzyme Inhib. Med. Chem. 23 (2016) 1-7;

(b) E. Mete, B. Comez, Gul. Inci, I. Gulcin, C.T. Supuran, Synthesis and carbonic anhydrase inhibitory activities of new thienyl-substituted pyrazoline benzenesulfonamides, J. Enzyme Inhib. Med. Chem. 19 (2016) 1-5;

(c) M. Bozdag, A.M. Alafeefy, F. Carta, M. Ceruso, A.S. Al-Tamimi, A.A. Al-Kahtani, F.A.S. Alasmary, C.T. Supuran, Synthesis 4-[2-(2-mercapto-4-oxo-4H-quinazolin-3-yl)-ethyl]- benzenesulfonamides with subnanomolar carbonic anhydrase II and XII inhibitory properties, Bioorg. Med. Chem. 24 (2016) 4100-4107.

[34] (a) N.M. Abdel Gawad, N.H. Amin, M.T. Elsaadi, F.M. Mohamed A. Angeli, V. De Luca, C. Capasso, C.T. Supuran, Synthesis of 4-(thiazol-2-ylamino)-benzenesulfonamides with carbonic anhydrase I, II and IX inhibitory activity and cytotoxic effects against breast cancer cell lines, Bioorg. Med. Chem. 24 (2016) 3043-3051;

(b) C.T. Supuran, S. Kalinin, M. Tanç, P. Sarnpitak, P. Mujumdar, S.A. Poulsen, M. Krasavin, Isoform-selective inhibitory profile of 2-imidazoline-substituted benzene sulfonamides against a panel of human carbonic anhydrases, J .Enzyme Inhib. Med. Chem. 24 (2016) 1-6;

(c) H.I. Gul, M. Tugrak, H. Sakagami, P. Taslimi, I. Gulcin, C.T. Supuran, Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3H-indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides, J Enzyme Inhib Med Chem. 31 (2016) 1619-1624;

(d) W.M. Eldehna, M. Fares, M. Ceruso, H.A. Ghabbour, S.M. Abou-Seri, H.A. Abdel-Aziz, D.A. Abou El Ella, Amido/ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII, Eur. J. Med. Chem. 110 (2016) 59-266;

(e) C.L. Lomelino, B.P. Mahon, R. McKenna, F. Carta, C.T. Supuran, Kinetic and X-ray crystallographic investigations on carbonic anhydrase isoforms I, II, IX and XII of a thioureido analog of SLC-0111, Bioorg. Med. Chem. 24 (2016) 976-981.

[35] (a) B. Cornelio, M. Laronze-Cochard, M. Ceruso, M. Ferraroni, G.A. Rance, F. Carta, A.N. Khlobystov, A. Fontana, C.T. Supuran, J. Sapi, 4-Arylbenzenesulfonamides as human carbonic anhydrase Inhibitors (hCAIs): Synthesis by Pd nanocatalyst-mediated Suzuki−Miyaura reaction, enzyme inhibition, and X-ray crystallographic studies, J. Med. Chem. 59 (2016) 721-732;

(b) G. La Regina, A. Coluccia, V. Famiglini, S. Pelliccia, L. Monti, D. Vullo, E. Nuti, V. Alterio, G. De Simone, S.M. Monti, P. Pan, S. Parkkila, C.T. Supuran, A. Rossello, R. Silvestri, Discovery of 1, 1′-biphenyl-4-sulfonamides as a new class of potent and selective carbonic anhydrase XIV inhibitors, J. Med. Chem. 58 (2015) 8564-8572;

(c) G.M. Rankin, D. Vullo, C.T. Supuran, S.A. Poulsen, Phosphate chemical probes designed for location specific inhibition of intracellular carbonic anhydrases, J. Med. Chem. 58 (2015) 7580-7590;

(d) B.L. Wilkinson, A. Innocenti, D. Vullo, C.T. Supuran, S.A. Poulsen, Inhibition of Carbonic Anhydrases with Glycosyltriazole Benzene Sulfonamides, J. Med. Chem. 51 (2008) 1945-1953;

(e) A. Casini, A. Scozzafava, F. Mincione, L. Menabuoni, M.A. Ilies, C.T. Supuran, Carbonic anhydrase inhibitors: water-soluble 4-sulfamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects, J. Med. Chem. 43 (2000) 4884-4892.

[36] (a) M. Bozdag, M. Pinard, F. Carta, E. Masini, A. Scozzafava, R. McKenna, C.T. Supuran, A class of 4-sulfamoylphenyl-ω-aminoalkyl ethers with effective carbonic anhydrase inhibitory action and antiglaucoma effects, J. Med. Chem. 57 (2014) 9673-9686;

(b) A. Innocenti, A. Casini, M.C. Alcaro, A.M. Papini, A. Scozzafava, C.T. Supuran, Carbonic anhydrase inhibitors: the first on-resin screening of a 4-sulfamoylphenylthiourea library, J. Med. Chem. 47 (2004) 5224-5229;

(c) F. Pacchiano, F. Carta, P.C. McDonald, Y. Lou, D. Vullo, A. Scozzafava, S. Dedhar, C.T. Supuran, C. T. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis, J. Med. Chem. 54 (2011) 1896-1902.

[37] (a) S. Biswas, R. McKenna, C.T. Supuran, Effect of incorporating a thiophene tail in the scaffold of acetazolamide on the inhibition of human carbonic anhydrase isoforms I, II, IX and XII, Bioorg. Med. Chem. Lett. 23 (2013) 5646-5649;

(b) S. Carradori, A. Mollica, M. Ceruso, M. DAscenzio, C. De Monte, P. Chimenti, R. Sabia, A. Akdemir, C.T. Supuran, New amide derivatives of Probenecid as selective inhibitors of carbonic anhydrase IX and XII: biological evaluation and molecular modelling studies, Bioorg. Med. Chem. 23 (2015) 2975-2981;

(c) M. DAscenzio, S. Carradori, D. Secci, D. Vullo, M. Ceruso, A. Akdemir, C.T. Supuran, Selective inhibition of human carbonic anhydrases by novel amide derivatives of probenecid: synthesis, biological evaluation and molecular modelling studies, Bioorg. Med. Chem. 22 (2014) 3982-3988;

(d) S. Angapelly, P.V.S. Ramya, A. Angeli, S. Del Prete, C. Capasso, M. Arifuddin, C.T. Supuran, Development of sulfonamides incorporating phenylacrylamido functionalities as carbonic anhydrase isoforms I, II, IX and XII inhibitors, Bioorg. Med. Chem. 25 (2017) 5726-5732;

(e) C.B. Mishra, S. Kumari, A. Angeli, S.M. Monti, M. Buonanno, M. Tiwari, C.T. Supuran, Discovery of Benzenesulfonamides with Potent Human Carbonic Anhydrase Inhibitory and Effective Anticonvulsant Action: Design, Synthesis, and Pharmacological Assessment, J. Med Chem. 60 (2017) 2456-2469.

[38] Y. Lou, P.C. McDonald, A. Oloumi, S. Chia, C. Ostlund, A. Ahmadi, A. Kyle, U. Auf dem Keller, S. Leung, D. Huntsman, B. Clarke, B.W. Sutherland, D. Waterhouse, M. Bally, C. Roskelley, C.M. Overall, A. Minchinton, F. Pacchiano, F. Carta, A. Scozzafava, N. Touisni, J.Y. Winum, C.T. Supuran, S. Dedhar, Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors, Cancer Res. 71 (2011) 3364-3376.

[39] (a) M. Shaquiquzzaman, G. Verma, A. Marella, M. Akhter, W. Akhtar, M.F. Khan, S. Tasneem, M.M. Alam,. Piperazine scaffold: A remarkable tool in generation of diverse pharmacological agents, Eur. J. Med. Chem. 102 (2015) 487-529.

(b) Mishra CB, Kumari S, Tiwari M. Thiazole: a promising heterocycle for the development of potent CNS active agents, Eur J Med Chem. 92 (2015) 1-34.

[40] (a) C.B. Mishra, A. Manral, S. Kumari, V. Saini, M. Tiwari, Novel indadinone derivatives as multitarget-directed agent with cholinesterase inhibition, anti-β-amyloid aggregation, antioxidant and neuroprotection properties against Alzheimer's disease, Bioorg. Med. Chem. 24 (2016) 3829-3841;

(b) C.B. Mishra, S. Kumari, M. Tiwari, Design and synthesis of some new 1-phenyl-3/4-[4-(aryl/heteroaryl/alkyl-piperazine1-yl)-phenyl-ureas as potent anticonvulsant and antidepressant agents, Arch. Pharm. Res. 39 (2016) 603-617;

(c) C.B. Mishra, S. Kumari, A. Manral, A. Prakash, V. Saini, A.M. Lynn, M. Tiwari, Design, synthesis, in-silico and biological evaluation of novel donepezil derivatives as multi-target-directed ligands for the treatment of Alzheimer's disease, Eur. J. Med. Chem. 125 (2016) 736-750;

(d) S. Kumari, C.B. Mishra, M. Tiwari, Pharmacological evaluation of novel 1-[4-(4-benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-phenyl]-3-phenyl-urea as potent anticonvulsant and antidepressant agent, Pharmacol Rep. 68 (2016) 250-258;

(e) S. Kumari, C.B. Mishra, M. Tiwari, Design, synthesis and pharmacological evaluation of N-[4-(4-(alkyl/ aryl/heteroaryl)-piperazin-1-yl)-phenyl]-carbamic acid ethyl ester derivatives as novel anticonvulsant agents, Bioorg. Med. Chem. Lett. 25 (2015) 1092-1099.

[41] A.K. Rathi, R. Syed, H.S. Shin, R.V. Patel, Piperazine derivatives for therapeutic use: a patent review (2010-present), Expert. Opin. Ther. Pat. 26 (2016) 777-797.

[42] M. Marcinkowska, M. Kotanska, A. Zagorska, J. Sniecikowska, M. Kubacka, A. Siwek, A. Bucki, M. Pawlowski, M. Bednarski, J. Sapa, M. Starek, M. Dabrowska, M. Kolaczkowski, Synthesis and biological evaluation of N-arylpiperazine derivatives of 4,4-dimethylisoquinoline-1,3(2H,4H)-dione as potential antiplatelet agents, J Enzyme Inhib Med Chem. 3 (2018) 536-545.

[43] A.O. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules, Sci Rep. 7: 42717 (2017) 1-13.

[44] D. Lagorce, O. Sperandio, J.B. Baell, M.A. Miteva, B.O. Villoutreix, FAF-Drugs3: a web server for compound property calculation and chemical library design, Nucleic Acids Res. 43 (2015) W201-W207.

[45] M.A. Rogawski, Molecular targets versus models for new antiepileptic drug discovery, Epilepsy. Res. 68 (2006) 22-28.

[46] M.M.C. Branco, G.L. Alves, I.V. Figueiredo, A.C. Falco, M.M. Caramona, The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods, Find. Exp. Clin. Pharmacol. 31 (2009) 101-106.

[47] (a) Q.R. Huang, L.C. Bell Horner, I.D. Mohammad, F.D. Covey, J.A. Drewe, G.H. Dillon, Pentylenetetrazole-induced inhibition of recombinant-aminobutyric acid type A (GABAA) receptors: mechanism and site of action, J. Pharm. Exp. Ther. 298 (2001) 986-995;

(b) W. Loscher, D. Honack, P.C. Fassbender, B. Nolting, The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models, Epilepsy Res. 8 (1991) 171-189.

Research Highlights

- Two novel series of benzenesulfonamide derivatives have been designed and synthesized as potent carbonic anhydrase inhibitors.
- Synthesized effective hCA II and hCA VII inhibitors (**4g, 4i** and **8d**) were evaluated for in vivo anti-convulsant activity.
- Compounds **4g**, **4i** and **8d** have shown good anti-convulsant activity in MES and sc-PTZ seizure tests.
- Compound **8d** displayed long duration of anti-convulsant action in time course study.
- Compound **8d** at 30 mg/kg, bwt was found orally active as a anti-convulsant agent.