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Discovery of Benzenesulfonamides with Potent Human Carbonic Anhydrase Inhibitory and Effective Anticonvulsant Action: Design, Synthesis and Pharmacological Assessment

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Abstract. We report two series of novel benzenesulfonamide derivatives acting as effective carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. The synthesized compounds were tested against human (h) isoforms hCA I, hCA II, hCA VII and hCA XII. The first series of compounds, 4-(3-(2-(4-substitued piperazin-1-yl)ethyl)ureido)benzenesulfonamides, showed low nanomolar inhibitory action against hCA II, being less effective against the other isoforms. The second series, 2-(4-substitued piperazin-1-yl)-N-(4-sulfamoylphenyl)acetamide derivatives, showed low nanomolar inhibitory activity against hCA II and hCA VII, isoforms involved in epileptogenesis. Some of these derivatives were evaluated for their anticonvulsant activity and displayed effective seizure protection against MES and scPTZ induced seizures in Swiss Albino mice. These sulfonamides were also found effective upon their oral administration to Wistar rats and inhibited MES induced seizure episodes in this animal model of the disease. Some of the new

compounds showed a long duration of action in the performed time course anticonvulsant studies, being non-toxic in subacute toxicity studies.

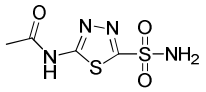
Introduction.

Epilepsy is one of the most common neurological disorders worldwide, being characterized by recurrent unprovoked seizure episodes. Globally, 50 million people are affected by epilepsy which may cause momentous disability as well as mortality, if untreated.[1] Although various antiepileptic drugs (AEDs) are used clinically to prevent or suppress seizure episode, about 25-40% of seizure patients suffer of uncontrolled seizures due to non-responsiveness to the AEDs. A seizure may lead to an abnormal, excessive, synchronous or paroxysmal discharge in a mass of neurons.[2-3] There are several targets for AEDs, such as GABA, Na^+, K^+, Cl^-, and/or Ca^{2+} ion channel, AMPA receptors, etc., with some approved drugs for all of them which are able to control seizure episodes in many epileptic patients.[4] Apart from these well-known molecular targets for epilepsy, brain carbonic anhydrases (CAs, EC 4.2.1.1) also emerged as interesting yet controversial targets to control seizures, because they are recognized to modulate several neuronal signaling mechanisms.[5-9] Several studies have showed that epileptic seizures are related to rapid changes in the ionic composition within some brain compartments, including the enhancement of extracellular potassium concentration and pH shifts within the brain. pH directly affects seizure activity, as for example the alkalosis potentiates seizure spreading, whereas acidosis generates the opposite effects.[5-7] The pH buffering of extracellular and intracellular spaces is governed by the CO_2/HCO_3^- buffer system and CAs prominently regulate this equilibrium by catalyzing the interconversion of the two species with high efficacy.[8,9] Thus, CA are considered as an interesting target for the development of potent anticonvulsant agents, also considering that some of the clinically used AEDs show powerful CA inhibitory activity.[9]

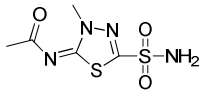
There are 15 different CA isoforms (hCA I-hCA XIV, hCA VA and VB) reported in humans, which are classified into cytosolic (hCA I-III, hCA VII and hCA XIII) membrane-bound (hCA

IV, hCA IX, hCA XII and hCA XIV) and mitochondrial (hCA VA and hCA VB) isoforms.[10,11] These isozyme contain a Zn^{2+} ion within the active site, which is crucial for the catalytic activity. The zinc ion is situated at the bottom of a half hydrophilic and half hydrophobic cleft and tetrahedrally coordinated to the three histidine residues through their imidazole nitrogens and one water molecule/hydroxide ion.[12] They are involved in many physiological processes associated with CO_2/bicarbonate equilibrium, and proficiently catalyze the interconversion between CO_2 and bicarbonate.[13] In the mammalian brain many CA isoforms are being present: CA II is widely expressed in the choroid plexus, oligodendrocytes, myelinated tracts, astrocytes as well as myelin sheaths, being involved in the physiology/pathophysiology of central nervous system (CNS), including epileptogenesis.[14,15] For example, the expression of CA II was induced in the CA1 cells after 3-12 h of exposure to kainic acid for producing status epilepticus.[16] Furthermore, some studies showed that CA II deficient mice are more resistant to seizures,[17] which is in agreement with using CA inhibition as an antiepileptic mechanism. In the past years, many studies have proved that CA VII is an isoform mainly present in the brain where it promotes depolarizing and excitatory GABAergic transmission through HCO_3^- currents. Several investigations also suggested the role of CA VII in the generation of seizures through GABAergic excitation.[18,19] Additionally, recent studies evoked significant role of CA VII in the generation of febrile seizures through the activation of $GABA_A$ receptors.[19] Intraneuronal generation of HCO_3^- by CA VII influenced GABA response, which probably plays a crucial role in hippocampal epileptiform activity.[18-20] CA XII expression has been also observed in brain besides, its overexpression in various tumors, and high levels of CA XII mRNA were found in granule cells and media amygdala.[21] A well known CA inhibitor, acetazolamide (AAZ) was approved for the treatment of epilepsy in 1954 and it is still used in partial, absence, myoclonic

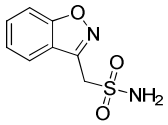
and primary generalized tonic-clonic seizures in refractory epilepsy alone, or in combination with other AEDs.[22] Some other CA inhibitors such as zonisamide (ZNS), topiramate (TPM) and methazolamide (MZA) are also used clinically for therapy of epileptic disorders (Chart 1).[23-24] Thus, the development of potent CA inhibitors, which also possess anticonvulsant activity along with enduring effectiveness is required.



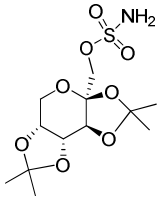
AAZ



MZA



ZNS



TPM

Chart 1: CAIs showing AED behavior.

Primary sulfonamide ($-SO_2NH_2$) group containing molecules have an incredible history in the field of CA research and many of them are successfully used clinically as antiglaucoma agents, diuretics as well as AEDs.[25-28] The X-Ray crystallographic studies have revealed that primary sulfonamide group present in these CA inhibitors binds as anion to the Zn^{2+} ion within the enzyme active site, blocking catalysis efficiently.[29-30] Therefore, sulfonamide derivatives are highly investigated for the inhibition of various CA isoforms. By incorporating this functional group in various scaffolds, many CA inhibitor classes have been developed in the last 70 years, which displayed excellent inhibitory activity and various pharmacological applications, as mentioned above.[31-32] In the present study we extend this research trend and report 2-(4-substitued piperazin-1-yl)-N-(4-sulfamoylphenyl)acetamides and 4-(3-(2-(4-substitued piperazin-

1-yl)ethyl)ureido) benzenesulfonamides as novel CA inhibitors endowed with good anticonvulsant activity.

Results and discussion

Drug Design and Synthesis. The benzenesulfonamide is a versatile scaffold to develop potent and selective CA inhibitors.[10] Numerous derivatives of benzenesulfonamide were synthesized by many investigators via appending a wide range of alkyl, aromatic, heterocycle or sugar scaffolds, or by including various linkers (such as carboxamide, urea, thiourea, sulfoxide, secondary sulfonamide etc) between the aromatic and tail fragments of the molecule.[33-36] These derivatizations bestowed a number of potent CA inhibitors including 4-(4-fluorophenylureido)benzenesulfonamide (Chart 2),[36c] in which the benzenesulfonamide group is linked to a *p*-fluorophenyl moiety via the urea linkage and this compound successfully completed Phase I clinical trials as an anti-cancer agent targeting hypoxic, metastatic tumors.[37] X-ray crystallographic studies have disclosed that the urea linker of this compound facilitates the achievement of the desired selectivity against several CA isoforms, by providing a great flexibility in order to adopt various orientations within the enzyme active site[36c, 38]. Similar findings were observed for other benzenesulfonamide derivatives in which urea was present as a flexible linker.[39] Piperazine is another important heterocyclic building block which has been extensively used to develop various clinically useful drug molecules with diverse biological activities.[40] Literature survey revealed that piperazine moiety is also widely used to develop potent CA inhibitors[41]. Recently, Congiu et al. synthesized a series of sulfonamide containing piperazinyl-ureido derivatives to evaluate their CA inhibitory effect. The results of this study provide potent piperazine containing CA inhibitors, which possessed nanomolar range affinity towards CA I, CA II, CA IX, CA XII isoform.[39]

By considering these facts along with keeping the pharmacophoric frame picture of ureido-containing sulfonamides in mind,[36c] we aimed to design novel CA inhibitors of the 4-(3-(2-(4-substitued piperazin-1-yl)ethyl)ureido)benzenesulfonamide type, in which the benzenesulfonamide moiety is linked with various substituted piperazines via ethylurea/acetamide linkers (Chart 2).

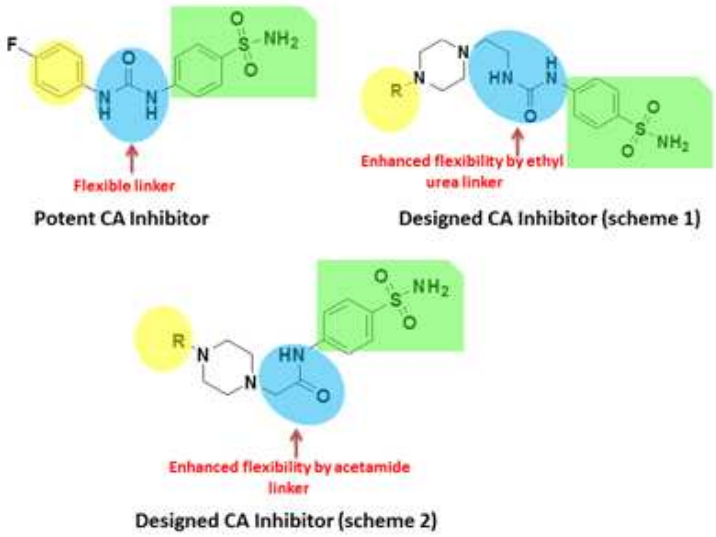
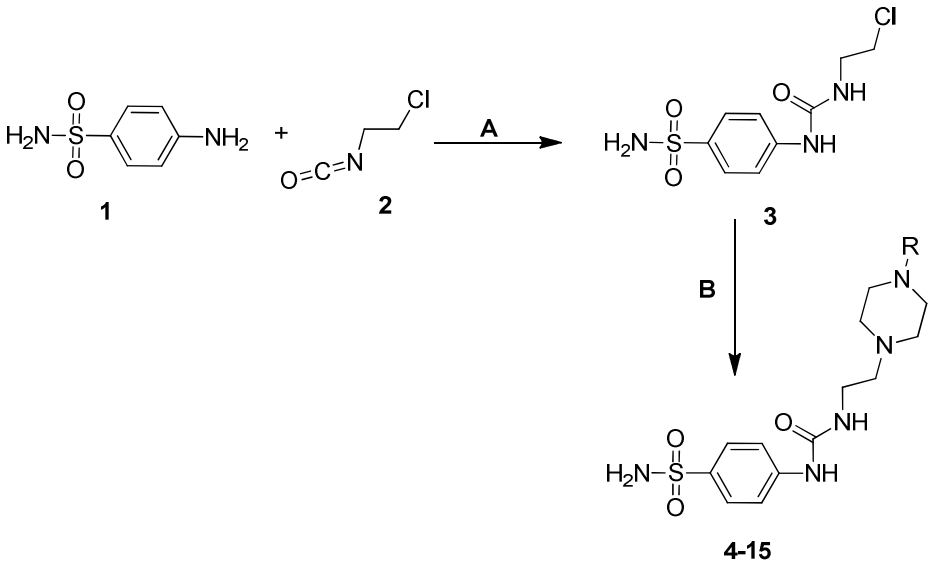
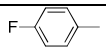
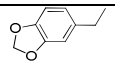
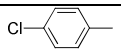
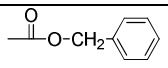
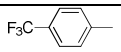
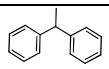
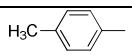
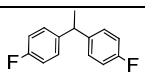


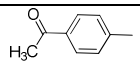
Chart 2: Designed novel CA inhibitors reported in the paper.

The introduction of the *N*-substituted piperazine as tail along with the ethylureido linker is thought to give better flexibility to adjust within the CAs active site. Furthermore, we have replaced the ethylureido linker with another flexible linker, the acetamide one, in order to obtain 2-(4-substitued piperazin-1-yl)-N-(4-sulfamoylphenyl)acetamide derivatives and study the impact of the acetamide versus ethyl ureidolinker for the CA inhibitory properties (Chart 2).

Chemistry. The general synthesis of the novel benzenesulfonamide derivatives is depicted in Scheme 1 and 2. To obtain 4-(3-(2-(4-substituedpiperazin-1-yl)ethyl) ureido) benzenesulfonamide derivatives (Scheme 1), the commercially available sulfanilamide was reacted with 2-chloroethylisocyanate to afford chloroethylurea derivative (**3**). Thereafter, the chloroethylurea derivative was reacted with *N*-substituted piperazines in the presence of sodium iodide and sodium bicarbonate to yield target compounds **4-15**.

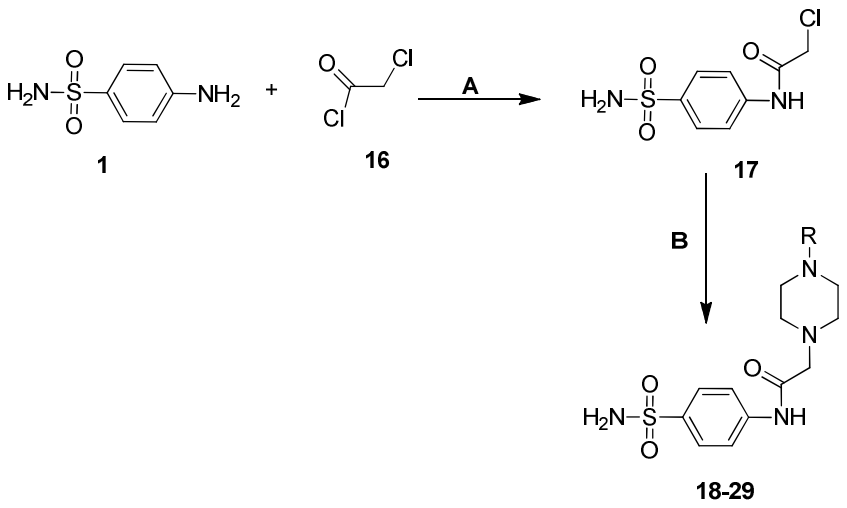


Compound No	R-Group	Compound No	R-Group
4		**10**	
5		**11**	
6		**12**	
7		**13**	
8		**14**	

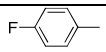
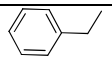
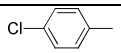
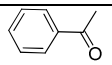
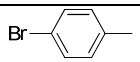
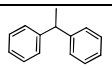
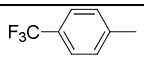
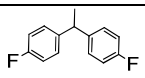
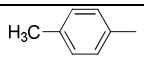
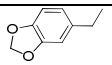
9		**15**	

Scheme 1. Preparation of compounds **4-15**. Reagent and conditions: A. Dried THF, RT, 2-3 h; B. Dried THF, NaI, $NaHCO_3$ reflux 8-10 h

Next, 2-(substituted piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamides (Scheme 2) were synthesized by utilizing sulfanilamide as a starting material. In due course, sulfanilamide and 2-chloroacetyl chloride were reacted to provide 2-chloro-N-(4-sulfamoylphenyl)acetamide (compound **17**). Compound **17** was coupled with N-substituted piperazines in a basic medium (Na_2CO_3) to provide the desired compounds **18-29**. The synthesized compounds were purified by column chromatography followed by re-crystallization. Compounds were characterized by using ^{1}H & ^{13}C nuclear magnetic resonance (NMR) and mass spectroscopy. The purity of the target compounds was assessed by reverse phase HPLC.



Compound No	R-Group	Compound No	R-Group

18		**24**	
19		**25**	
20		**26**	
21		**27**	
22		**28**	
23		**29**	

Scheme 2. Synthesis of sulfonamides **18-29**. Reagent and Conditions: A. Dried Acetone, K_2CO_3, RT, 5-8 h; B. DMF, Na_2CO_3, reflux 8-10 h

In-Vitro CA Inhibition Studies. The newly synthesized compounds (**4-14**, **18-29**) were tested for the inhibition against four CA isozymes of human origin, i.e. hCA I, hCA II, hCA VII and hCA XII (Table 1), some of which, as mentioned earlier, are involved in CNS function and epileptogenesis. The following structure-activity relationship (SAR) was observed by analyzing CA inhibition data of Table 1.

i) Most of the synthesized compounds in both series did not display potent inhibitory activity for hCA I, normally an offtarget isoform for antiepileptics.[8,10] Only the phenylpiperazine containing ethylureidobenzenesulfonamide derivative (**4**) and the phenyl (**18**), *p*-bromophenyl (**21**), 2-pyridine (**24**) as well as benzoylpiperzine (**26**) bearing sulfamoylphenylacetamide derivatives showed medium potency inhibitory inhibition (82.1-138 nM) against hCA I.

ii) The majority of the synthesized compounds in both series strongly inhibited the physiologically dominant hCA II isoform. Although, compounds **7**, **15**, **20**-**23**, **27**-**29** exhibited

satisfactory nanomolar range (22.3-50.7 nM) inhibitory activity, they were slightly weaker inhibitors compoared to the standard drug AAZ. However, the rest of the derivatives showed excellent inhibitory potency in the nanomolar/subnanomolar range (0.69-12 nM), being superior to AAZ. Particularly, in the ethylureido benzenesulfonamide series most of the compounds showed a low nanomolar range activity, except compounds **7** and **15** which contained trifluorophenyl and acetophenyl moieties in their tails, respectively. SAR studies revealed that most of the derivatives of this series were found hCA II - selective over hCA I, hCA VII and hCA XII. Thus, it may be stated that trifluorophenyl and acetophenyl present at the terminal end for this pharmacophore is not suitable to generate effective inhibitory potency for hCA II. Interestingly, thye *p*-fluorophenyl piperazine substituted derivative **5** appeared as an excellent hCA II inhibitor, which possessed sub-nanomolar activity (0.69 nM) along with high selectivity towards hCA I, hCA VII and hCA XII. Derivative **5** displayed 5000-fold selectivity over hCA I and >7246 fold selectivity over hCA VII as well as hCA XII. In the sulfamoylphenyl acetamide series, derivatives bearing phenyl (**18**), pyridine (**24**), benzyl (**25**) and benzoyl (**26**) within the tail inhibited hCA II better than AAZ.

iii) The synthesized ethylureido benzenesulfonamides were not able to strongly inhibit hCA VII. However, some of them showed medium inhibitory potency (58.5-203 nM) against this isoform (e.g., **7**, **9** and **14** which bear trifluorophenyl, pyrimidine, *p*-fluorodiphenyl group at the terminal end, respectively). Compounds belonging to the second series, with acetamide linker, showed better inhibition of hCA VII compared to series 1 compounds (which contains ethyl ureido linker). In series 2, the majority of the compounds showed good inhibitory activity against hCA VII, with inhibition constants ranging between 0.74 and 60.5 nM (Table 1). The unsubstituted phenyl derivative (**18**) displayed better inhibitory activity for hCA VII compared to AAZ.

Derivatives which contain *p*-bromophenyl (**21**), 2-pyridine (**24**), benzyl (**25**), benzoyl (**26**), diphenyl (**27**) as well as pipernoyl (**29**) groups were also highly effective inhibitors (Ki= 0.74-10.5 nM) of this isoform. Only two derivatives, **22** and **23** did not display the satisfactory inhibitory potency (Ki= 188-204 nM) in this series against hCA VII.

iv) Our results revealed that ethylureido benzenesulfonamide derivatives of the first series did not show an inhibitory effect against hCA XII and displayed Ki >50000 nM. However, two derivatives of series 2 (sulfamoylphenyl acetamides), compounds **18** and **24**, incorporating phenyl and pyridine tails, respectively, showed good inhibitory potential for hCA XII (Ki of 6.8 – 8.4 nM). Other two derivatives of this series (compound **19** and **26**) appeared as weak inhibitors. Thus, in terms of hCA XII inhibition, sulfamoylphenyl acetamides emerged as better inhibitors than ethylureido benzenesulfonamides.

Table 1. hCA I, II, VII and XII inhibition with compounds **4-15**, **18-29** with **AAZ** as standard

Compound	**K_I (nM)***			
	hCA I	**hCA II**	**hCA VII**	**hCA XII**
4	83.8±7.1	6.2±0.4	310±23	>50000
5	3490±125	0.69±0.05	>50000	>50000
6	909±64	8.8±0.6	>50000	>50000
7	8130±540	50.7±4.2	58.5±41	>50000

8	1790±97	7.9±0.5	>50000	>50000
9	3060± 169	7.9±0.3	318±21	>50000
10	860±73	5.2±0.2	>50000	>50000
11	3390±245	6.1±0.5	360±24	>50000
12	1730±106	4.3±0.3	299±18	>50000
13	3300±175	8.7±0.8	>50000	>50000
14	5900±342	9.1±0.7	203±16	>50000
15	>50000	26.3±1.4	490±35	>50000
18	138±10	3.6±0.1	0.74±0.05	6.8±0.3
19	437±24	12.0±0.9	21.0±1.2	49.9±3.3
20	527±40	22.3±1.7	161±15	>50000
21	92.3±6.3	28.9±1.0	9.4±0.8	>50000
22	2330±189	48.1±3.8	188±10.2	>50000
23	680±34	27.0±1.5	404±29	>50000
24	82.1±6.1	4.4±0.3	10.5±0.9	8.6±0.4
25	220±17	4.9±0.1	6.5±0.4	>50000
26	96.4±5.1	9.3±0.7	2.6±0.09	91.5±7.6

27	1771±107	43.2±1.8	8.9±0.7	>50000
28	245±16	16.9±1.3	64.5±4.9	>50000
29	8550±653	36.1±1.8	4.0±0.3	>50000
AAZ	250±11	12.1±0.8	2.5±0.2	5.7±0.3
TPM	250±8	10.4 ±0.9	0.9 ±0.01	3.8 ±0.2

* Mean ± standard error (from 3 different determinations).

v) The SAR studies clearly evoked that the ethylureido linker containing compounds showed selective inhibition towards hCA II exclusively, whereas the acetamide linker bearing compounds bestowed excellent inhibition for both hCA II as well as hCA VII. Thus, for inhibition of both epilepsy associated isoforms (hCA II and hCA VII), the acetamide linker bearing derivatives appeared as more appropriate to be investigated in detail in animal models of the disease.

In-Vivo Studies

Anticonvulsant activity. It is well known that isoform CA I, II, III, IV, VB, VII, VIII, X, XI, XII and XIV are present in the brain.[14-20] Several studies highlighted a crucial role of CA II and CA VII in epileptiogenesis and have shown that their inhibition either blocks or suppresses epileptic seizure spread. There is sufficient evidence implicating that numerous CA inhibitors (CAIs), which prominently inhibit CA II and CA VII possess good anticonvulsant property.[9,10]

Our *in vitro* CA inhibition study revealed that compounds **5**, **18**, **24**, **25** and **26** appeared as most potent inhibitors for CA II and CA VII (Table 1). These compounds displayed a (sub)nanomolar range inhibitory activity against both isoforms (CA II: 0.69-9.3 nM; CA VII 0.74-10.5 nM) and thus were chosen to conduct anticonvulsant activity *in-vivo*. Both AAZ and TPM are potent CAIs and are used clinically to treat several forms of epilepsy, as mentioned in the introduction. Therefore, AAZ and TPM were taken as standard drugs for *in vivo* experiments.

The AED discovery is based on the characterization of anti-epileptic activity of the new AED candidate in a variety of epileptic animal models. Even though there are many animal models of epilepsy are available, the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure tests are used regularly by most of antiepileptic drug discovery (ADD) programs and are considered as the 'gold standards' in early stages of anticonvulsant testing [42]. Most of the clinically used AEDs are protective either in both or at least one of these (primarily MES) seizure tests. Therefore, anticonvulsant activity of compounds **5**, **18**, **24**, **25** and **26** was performed using MES and scPTZ seizure tests.

Table 2: Anticonvulsant Activity in MES and scPTZ seizure test in mice

Compounds ([a]Dose in mg/kg)	MES[b] screen		scPTZ[c] screen	
	0.5 h	3.0 h	0.5 h	3.0 h
5 (30)	6/8	5/8	3/6	3/6
5 (100)	6/8	8/8	4/6	3/6
18 (30)	7/8	6/8	5/6	5/6
18 (100)	7/8	6/8	5/6	5/6
24 (30)	6/8	6/8	0/6	1/6

24 (100)	8/8	6/8	1/6	1/6
25 (30)	8/8	6/8	3/6	4/6
25 (100)	8/8	5/8	3/6	3/6
26 (30)	7/8	7/8	4/6	4/6
26 (100)	5/8	4/8	4/6	4/6
TPM (30)	8/8	8/8	3/6	3/6
TPM (100)	NT	NT	2/6	3/6
AAZ (30)	7/8	4/8	3/6	3/6
AAZ (100)	8/8	4/8	5/6	3/6

Response comments: [a]30 and 100 mg/kg of doses were administered intraperitoneally (i.p.), The animals were examined at 0.5 h and 3.0 h after injection were administered. [b]Maximal electroshock test (MES) (n=8 mice for each tested dose in the MES test); [c]subcutaneous pentylenetetrazole test (scPTZ) (n=6 mice for each tested dose in scPTZ test); NT: Not Tested.

Maximal Electroshock Test: The MES test is an excellent animal model for the recognition of novel agents that block seizure spread and as such are likely to be efficacious for the management of generalized tonic–clonic seizures (GTCS) in human[43]. This anticonvulsant screening test implies the ability of new compound to inhibit the generalized (grand-mal) seizures in rodents. Table 2 summarizes the anticonvulsant activity in the MES test of tested compounds at the doses of 30 and 100 mg/kg up to 3 hour time duration. It was clearly observed that compound **5** selectively inhibited CAII (table 1) among all tested CA isoforms. In MES test, compound **5** showed 75% seizure protection at 0.5 h (30 &100 mg/kg) and 100% seizure cessation at 3 h (100 mg/kg), which suggests its fast onset and longer duration of action to

prevent seizure spread from the epileptic focus in the brain. Compound **18** displayed a significant (87.5%) seizure protection at 0.5 h and this protection was maintained up to 75% at 3 h and in quantitative evaluation it's displayed an ED_{50} value 18.0 mg/kg. The compound **24** (100 mg/kg) revealed 100% seizure protection after 0.5 has similar to standard drug AAZ but after 3 h, this compound was found more effective in blocking seizures where it protected 75% percent of animals while, AAZ only showed 50% seizure protection Similarly compounds **25** and **26** provided seizure attenuation at the low dose of 30 mg/kg in both time intervals specifying their fast onset as well as longer duration of action as compare with reference drug TPM. Compound **25** and **26** showed an ED_{50} 13.7mg/kg and 15.0 mg/kg respectively in anticonvulsant quantification study. Overall, we observed that compounds **5, 18, 24, 25** and **26** selectively inhibited CA II and CA VII isoform except **5**, which is only potent CA II inhibitor. Therefore, it may be concluded that CA II and CA VII inhibition contribute pointedly to the anticonvulsant activity by modulation of pH dependent voltage and receptor-gated ion channels.

Subcutaneous Pentylenetetrazole Test. Pentylenetetrazole (PTZ) is a γ-amino butyric acid (GABA) receptor antagonist and known to block $GABA_A$ subtype receptor in the brain. PTZ is a potent chemoconvulsant and systemic injection of PTZ produces severe seizure episodes in both mice and rats. Therefore, the PTZ test serves as a valid model for studying human generalized myoclonic and absence and is useful to identify new anticonvulsant candidate which acts by raising the seizure threshold[44]. Table 2 summarizes the anticonvulsant activity of compounds **5, 18, 24, 25** and **26** in the scPTZ test. It was perceived that all tested compounds except **24** displayed anti-PTZ effects. It is reported that $GABA_AR$-mediated excitation is dependent on the continuous replenishment of neuronal $HCO3^-$ by cytosolic CA activity. In particular, CA II and VII isoforms regulate neuronal pH and has a defined role in the generation of seizures. Thus,

administration of CA inhibitor has proven beneficial for the suppression of seizure activity in the brain. Compound **18** had shown significant protection by raising the seizure threshold at both time intervals at the lower dose of 30 mg/kg and it was better than TPM as well as AAZ. This appreciable anti-PTZ effect of compound **18** (CA II: K_i= 3.6nM; CA VII: K_i= 0.74 nM) could be explained by its good inhibition of CA VII isoform better than AAZ (CA II: K_i= 12.1 nM ; CA VII: K_i= 2.5 nM). Other active compound **26** (30 and 100 mg/kg) determined a unique pattern of seizure protection (66%) at 0.5 and 3 h. Rest compounds **5** and **25** have also shown abolition of myoclonic jerks against scPTZ induced seizures as similar with TPM and AAZ. Three compounds **18**, **25** and **26** presented good anticonvulsant effect in both MES and scPTZ seizure tests. Hence, these compounds were further evaluated for phase II anticonvulsant screening.

MES study in rats upon oral administration of test compounds. The anticonvulsant potential of compounds **18**, **25** and **26** were also evaluated in rats upon oral administration in MES test at various time intervals (0.25, 0.5, 1, 2 and 4 h). The results showed that compounds **18** significantly protect MES induce seizures after 1h of drug administration orally to rats and activity was sustained up to 4h (Table 3). Compound 25 showed significant protection from seizures up to 1h of drug administration and action was gone reduced after 1h. Thus, 1h after the compound **18** administration showed best peak time effect in this test. However, Compound **26** displayed satisfactory protection from seizure up to 4h of administration. It displayed 50 % protection at 0.25 h time interval and 75% at 4h. Studies clearly indicate that these derivatives showed reasonable protection from MES induced seizures after oral administration and this finding also proved good oral bioavailability of these compounds.

Table 3: Anti-MES activity in Rats dosed orally with test compounds **18**, **25** and **26**

Test compounds	Number of protected/tested rats vs. time, after drug administration in the MES test [a]				
	Time intervals (h) [b]				
	0.25	0.5	1	2	4
18	1/4	1/4	3/4	4/4	3/4
25	0/4	3/4	3/4	2/4	1/4
26	2/4	2/4	4/4	3/4	3/4

[a]Maximal electroshock test (number of animals protected/number of animals tested; n=4), [b] time after drug administration

Time course anticonvulsant studies. To investigate the duration of anticonvulsant activity of compounds **18**, **25** and **26**, a time course study was performed in mice through MES test. In this test seizure protection capability of these compounds was analyzed up to 6h after compounds administration. The obtained results indicate these compounds to be highly effective in protecting mice from MES induced seizures over a long duration of time (Table 4). Compound **18** showed 87.5% protection up to 1h and 75.0-62 % protection in next 2-6h. Although, compound **25** sustained the activity 100-62.5 % in first 3 h and 87.5-62% in next 3h. However, compound **26** showed less variable active with progressive time interval and it displayed 75-77.5 % protection in first 3h and no variation was observed in next 3h and constantly displayed 75% protection in this time interval. Thus, these CA inhibitors successfully protected MES induced seizures for longer duration (up to 6h) with an excellent degree of protection.

Table 4: Time course anticonvulsant activity of compounds **18**, **25** and **26** in MES Test with eight Swiss Albino mice each time

Test compounds	% Protection in MES seizure test vs. time [a,b]					
	0.5 h	1 h	2 h	3 h	4 h	6 h
18*	87	87	62	75	75	62
25*	100	75	62	75	87	62
26*	87	87	75	87	75	75

[a] The test compounds were administered i.p. to Swiss Albino mice (n=8 in each group), [b] time after drug administration. *p<0.0001 compared to control group [the control group was treated with vehicle only (1% acacia gum in normal sterile solution) and showed zero percent seizure protection at all time intervals].

Sub-acute toxicity studies. To assess the safety profile of compounds **18**, **25** and **26** a sub-acute toxicity study was conducted in normal, healthy Wistar rats at the dose of 200mg/kg/bwt. The results of this study revealed that animals of the control group and treated groups were found healthy and no signs of toxicity as well as no deaths were observed during whole experimental period. Animals pre-treated with compounds **18**, **25** and **26** did not display any considerable alteration in hematological parameters as compared to control animals (Table 5). Thus, compounds **18**, **25** and **26** did not produce any toxicity related to hematological alteration. In addition, treatment of these compounds did not significantly changed the basic level of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein and total bilirubin compared to control group (Table 6).

No alteration of these liver function associated enzymes with compounds **18**, **25** and **26** indicates these new chemical entities did not produce any significant liver toxicity to experimental rats. Moreover, treatment of these compounds did not sow major changes in renal biomarkers such as creatinine, uric acid and urea as compared to control group and these results indicative compounds had not exerted any renal toxicity (Table 7). Overall toxicology data clearly indicate that these new chemical entities did not have the toxic nature associated with normal body function, liver function and renal function. These primarily toxicological studies proved these derivatives as safe and potent CA inhibitors, which endowed with excellent anticonvulsant activity.

Table 5: Hematological parameters after oral administration of vehicle and compounds 18, 25 and 26 for 14 days in Wistar rats

Parameters	Control(vehicle, *po*) ± SD	Compound **18** (200mg/kg, *po*) ± SD	Compound **25** (200mg/kg, *po*) ± SD	Compound **26** (200mg/kg, *po*) ± SD
Hb (g/dl)	13 ± 1.8	13 ± 0.4	13 ± 0.6	12 ± 0.4
TLC (10^3/ml)	6 ± 0.8	7 ± 0.5	8 ± 1.2	7 ± 0.7
Neutrophils (%)	25 ± 3.2	25 ± 1.7	22 ± 3.7	24 ± 3.1
Lymphocytes (%)	74 ± 3.2	71 ± 2.1	73 ± 3.3	72 ± 3.5
Eosinophils (%)	2 ± 1.0	2 ± 0.5	2 ± 0.5	2 ± 0.5
Monocytes (%)	3 ± 1.0	2 ± 0.5	2 ± 0.5	2 ± 0.5
Basophils (%)	0	0	0	0
RBC (mill/mm^3)	7 ± 0.2	8 ± 0.6	7 ± 0.4	7 ± 0.1

Platelet count (thou/mm^3)	1209 ± 97.0	1074 ± 221.8	1065 ± 125.3	1085 ± 127.6

The data is represented as Mean ± standard deviation; n=6 in each group.

Table 6: Liver function test (LFT) after oral administration of vehicle and compounds **18**, **25** and **26** for 14 days in rats

Parameters	Control(vehicle, *po*) ± SD	Compound **18** (200mg/kg, *po*) ± SD	Compound **25** (200mg/kg, *po*) ± SD	Compound **26** (200mg/kg, *po*) ± SD
SGOT (U/l)	79 ± 1.7	78 ± 1.7	79 ± 1.44	78 ± 2.2
SGPT (U/l)	61 ± 4.7	59 ± 13.2	55 ± 10.3	60 ± 14.3
Alkaline Phosphatase (U/l)	122 ± 3.0	125 ± 3.4	122 ± 6.1	123 ± 3.0
Total bilirubin(mg/dl)	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Total protein (g/dl)	6 ± 0.3	6 ± 0.29	6 ± 0.1	6 ± 0.3

The data is represented as Mean ± standard deviation; n=6 in each group.

Table 7: Kidney function test (KFT) after oral administration of vehicle and compounds **18**, **25** and **26** for 14 days in rats

Parameters	Control(vehicle, *po*) ± SD	Compound **18** (200mg/kg, *po*) ± SD	Compound **25** (200mg/kg, *po*) ± SD	Compound **26** (200mg/kg, *po*) ± SD
Blood urea (mg/dl)	44 ± 1.6	43 ± 1.3	43 ± 2.7	41 ± 2.0
Creatinine (mg/dl)	0.5 ± 0.03	0.5 ± 0.01	0.5 ± 0.02	0.5 ± 0.03
Uric acid (mg/dl)	2 ± 0.7	2 ± 1.3	2 ± 0.3	2 ± 0.1
Calcium (mg/dl)	9 ± 0.4	9 ± 0.1	9 ± 0.3	9 ± 0.1
Phosphorus (mg/dl)	8 ± 0.1	8 ± 0.4	8 ± 0.5	7 ± 0.2
Na^+ (mEq/l)	144 ± 1.5	146 ± 2.0	148 ± 1.2	146 ± 1.7
K^+ (mEq/l)	5 ± 0.3	5 ± 0.3	5 ± 0.2	5 ± 0.2
Cl^- (mEq/l)	110 ± 1.4	107 ± 2.7	111 ± 0.9	107 ± 3.6

The data is represented as Mean ± standard deviation; n=6 in each group.

Conclusions. We have designed and synthesized two novel series of benzenesulfonamide derivative which behave as potent CA inhibitors, using two diverse linkers and substituted piperazines as tails. The SAR studies revealed that the acetamide linker-containing compounds (**18-29**) showed low nanomolar range inhibitory activity against hCA II and hCA VII, although, another series of compounds which had ethylurea linker (**4-15**) strongly inhibited only isoform hCA II. Compounds **5**, **18**, **24**, **25** and **26** displayed the most potent inhibitory action against hCA

II and hCA VII isoforms. Among these potent CAIs, compounds **18**, **25** and **26** showed excellent protection against MES and scPTZ induced seizures in mice. In the time course anticonvulsant studies, these compounds displayed long duration action and successfully opposed MES induced seizure up to 6 h (62-75% protection). Additionally, compounds **18**, **25** and **26** also inhibited MES induced seizures in rats upon oral administration. In sub-acute toxicity, studies these novel CA inhibitors did not produce any significant toxicity in rats. Thus, overall, the *in vivo* studies clearly indicate that the novel benzene sulfonamide derivatives **18**, **25** and **26** appeared as potent and safe CA inhibitors, being endowed with excellent anticonvulsant potential and may be considered as suitable leads for further development of anticonvulsant with an alternative mechanism of action, brain CA inhibition.

Experimental protocol

Chemistry. All the chemicals, reagents and solvents were procured from Sigma Aldrich (St. Louis, MO, USA), S.D Fine Chemicals (India) and Merck (Darmstadt, Germany). The homogeneity and purity of the compounds was checked by Thin layer chromatography (TLC), performed on commercially available silica gel (Kieselgel 60, F_{254}) coated aluminium sheets (Merck) by using methanol: chloroform (5:95) as the solvent system. The visualization on TLC was done by both ultra-violet (UV) light (λ= 254 nm) and iodine indicator. All the compounds were purified by column chromatography by using silica gel 100-200 mesh (Merck). Melting points were determined in open capillary tubes in a Hicon melting point apparatus (Hicon, India). The nuclear magnetic resonance (NMR) spectra were obtained on high resolution Jeol-400MHz NMR spectrophotometer (USA) DMSO-d_6 using Tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) were expressed in parts per million relative to TMS and the following abbreviations were used to describe the peak patterns when appropriate: s, (singlet); d,

(doublet); t, (triplet); m, (multiplet); brs, (broad singlet); dd, (double doublet). The coupling constant (*J*) values are given in hertz (Hz). Mass spectra were recorded using electrospray ionization liquid chromatography–mass spectrometry (ESI-LC/MS) on an Agilent 6310 Triple Quadrupole Mass Spectrometer instrument and the purity of final compounds was analyzed by reverse phase Shimadzu HPLC (Kyoto, Japan) coupled with a photodiode array detector (PDA) and C-18 column. All compounds reported here showed more than 97% HPLC purity. Samples were dissolved in acetonitrile and methanol (50:50), and an injection volume of 25 μL was used. Methanol + acetonitrile used as gradient mobile phase and flow rate was 1mL/min. All compounds reported here showed more than 97% HPLC purity.

Synthesis of 4-(3-(2-(4-substituedpiperazin-1-yl)ethyl)ureido)benzene sulfonamide (Series 1)

General procedure for the synthesis of 4-(3-(2-chloroethyl)ureido)benzene sulfonamide (3)

4-aminobenzenesulfonamide and 2-chloroethylisocyanate were taken in equimolar ratios (10 mM each) in 25 ml of dried THF, Na_2CO_3 and NaI were added to the reaction mixture and stirred at room temperature for 2-3 h. Progress of the reaction was monitored by TLC and the obtained crude solid product at the end of the reaction was filtered and washed twice with petroleum ether. The product was then washed with hot water and dried in oven at 50^0C to afford pure white colored compound **3**.

4-(3-(2-chloroethyl)ureido)benzenesulfonamide (3)

White solid; yield 92%; mp 170-172^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 3.42 (q, 2H, CH_2, J=5.8 Hz), 3.65 (t, 2H, CH_2), 6.54 (t, 1H, NH,), 7.16 (s, 2H, NH_2), 7.53 (d, 2H, Ar-H, J=9.1 Hz), 7.66 (d, 2H, Ar-H, J=8.4 Hz), 9.05 (s, 1H, NH). LC–MS: m/z; 277 (M^{+1}).

General procedure for the synthesis of 4-(3-(2-(4-substituedpiperazin-1-yl)ethyl) ureido)benzenesulfonamide derivatives (4-15)

The synthesis of target compounds **(4-15)** was accomplished by reacting compound **3** with various substituted piperazine in an equimolar ratio of 10 mM each. Firstly, compound **3** was fully dissolved in dried DMF (20-25 ml) and then piperazine was added portion wise. Then after, anhydrous sodium carbonate (15 mM) was added to the reaction mixture and heated at a reflux temperature of 90-100^{0}C for 8-10 h. After completion, the reaction mixture was diluted with water and the product was isolated with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and concentrated on rotatory evaporator. The obtained crude product was washed with petroleum and all compounds **(4-15)** were purified by column chromatography using chloroform and methanol (98:02) as eluent.

4-{3-[2-(4-Phenyl-piperazin-1-yl)-ethyl]-ureido}-benzenesulfonamide (4)

White solid; yield 80%; mp 182-184^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (t, 2H, CH_2, J= 5.6 Hz), 2.44 (s, 4H, piperazine), 3.07 (s, 4H. piperazine), 3.20 (q, 2H, CH_2, J=5.8 Hz), 6.20 (t, 1H, NH, J=5.3 Hz), 6.70 (t, 1H, Ar-H, J=7.2 Hz), 6.86 (d, 2H, Ar-H, J=7.6 Hz), 7.09-7.16 (m, 4H, Ar-H+NH_2), 7.48 (d, 2H, Ar-H, J=8.4 Hz), 7.60 (d, 2H, Ar-H, J=9.1 Hz), 9.01 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 48.1, 52.6, 57.2, 115.3, 116.7, 118.8, 126.8, 128.9, 135.9, 143.7, 151.0, 154.8. LC–MS: m/z; 403 (M^+). HPLC purity: 99.8%.

4-(3-{2-[4-(4-Fluoro-phenyl)-piperazin-1-yl]-ethyl}-ureido)-benzenesulfonamide (5)

White solid; yield 89%; mp 208-210^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (t, 2H, CH_2, J= 6.0 Hz), 2.49 (t, 4H, piperazine, J=5.3 Hz), 3.02 (t, 4H. piperazine, J=5.07 Hz), 3.19 (q, 2H, CH_2, J=5.5 Hz), 6.19 (t, 1H, NH, J=5.3 Hz), 6.86-6.90 (m, 2H, Ar-H), 6.95-7.00 (m, 2H, Ar-H), 7.09 (s,

2H, NH_2), 7.47 (d, 2H, Ar-H, J=8.4 Hz), 7.60 (d, 2H, Ar-H, J=8.4 Hz), 8.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 48.9, 52.6, 57.1, 115.1, 115.3, 116.7, 117.0, 126.7, 135.9, 143.7, 147.9, 154.7, 157.1. LC–MS: m/z; 421 (M^+). HPLC purity: 98.2%.

4-(3-{2-[4-(4-Chloro-phenyl)-piperazin-1-yl]-ethyl}-ureido)-benzenesulfonamide (6)

White solid; yield 92%; mp 168-170^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (t, 2H, CH_2, J= 6.3 Hz), 2.50 (s, 4H, piperazine), 3.08 (s, 4H. piperazine), 3.20 (q, 2H, CH_2, J=6.8 Hz), 6.20 (t, 1H, NH, J=5.6 Hz), 6.89 (d, 2H, A-H, J=8.4 Hz), 7.10 (s, 2H, NH_2), 7.16 (d, 2H, Ar-H, J=9.1 Hz), 7.48 (d, 2H, Ar-H, J=9.1 Hz), 7.60 (d, 2H, Ar-H, J=8.4 Hz), 9.01 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.1, 47.9, 52.5, 57.2, 116.6, 116.7, 122.1, 126.7, 128.6, 135.9, 143.7, 149.8, 154.8. LC–MS: m/z; 437 (M^+). HPLC purity: 98.7%.

4-(3-{2-[4-(4-Trifluoromethyl-phenyl)-piperazin-1-yl]-ethyl}-ureido)-benzene sulfonamide (7)

White solid; yield 69%; mp 196-198^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (t, 2H, CH_2, J=6.12 Hz), 2.49 (s, 4H, piperazine), 3.16-3.22 (m, 6H, piperazine +CH_2), 6.20 (t, 1H, NH, J= 5.3 Hz), 7.00 (d, 2H, Ar-H, J=8.4 Hz), 7.09 (s, 2H, NH_2), 7.42-7.48 (m, 4H, Ar-H), 7.59 (d, 2H, Ar-H, J=8.4 Hz), 8.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.1, 46.9, 52.3, 57.1, 114.1, 116.6, 126.1, 126.7, 135.9, 143.7, 153.2, 154.7. LC–MS: m/z; 471(M^+). HPLC purity: 99.8%.

4-{3-[2-(4-*p*-Tolyl-piperazin-1-yl)-ethyl]-ureido}-benzenesulfonamide (8)

White solid; yield 77%; mp 204-206^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.18 (s, 3H, CH_3), 2.44 (t, 2H, CH_2, J= 6.1 Hz), 2.53 (s, 4H, piperazine), 3.07 (s, 4H. piperazine), 3.25 (q, 2H, CH_2, J=5.5 Hz), 6.25 (t, 1H, NH, J=4.9 Hz), 6.80 (d, 2H, Ar-H, J=8.4 Hz), 7.00 (d, 2H, Ar-H, J=8.4 Hz), 7.15 (s, 2H, NH_2), 7.53 (d, 2H, Ar-H, J=8.4 Hz), 7.66 (d, 2H, Ar-H, J=8.4 Hz), 9.06 (s, 1H, NH). ^{13}C

NMR (DMSO-d_6): 20.0, 36.2, 48.6, 52.6, 57.2, 115.5, 116.6, 126.7, 127.5, 129.3, 135.9, 143.7, 148.9, 154.7. LC–MS: m/z; 417 (M^+). HPLC purity: 99.4%.

4-{3-[2-(4-Pyrimidin-2-yl-piperazin-1-yl)-ethyl]-ureido}-benzenesulfonamide (9)

White solid; yield 58%; mp 214-216^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.41-2.49 (m, 6H, piperazine+ CH_2), 3.25 (q, 2H, CH_2, J=5.7 Hz), 3.73 (s, 4H, piperazine), 6.23 (t, 1H, NH, J= 4.9 Hz), 6.60 (t, 1H, Ar-H, J=5.1 Hz), 7.15 (s, 2H, NH_2), 7.54 (d, 2H, Ar-H, J=8.3 Hz), 7.66 (d, 2H, Ar-H, J=9.1 Hz), 8.32 (d, 2, Ar-H, J= 4.5 Hz), 9.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 43.3, 52.4, 57.2, 110.1, 116.7, 126.7, 135.9, 143.7, 154.7, 157.9, 161.2. LC–MS: m/z; 405 (M^+). HPLC purity: 99.5%.

4-{3-[2-(4-Benzyl-piperazin-1-yl)-ethyl]-ureido}-benzenesulfonamide (10)

White solid; yield 74%; mp 206-208 ^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.35-2.42 (m, 9H, piperazine+ CH_2), 3.15-3.20 (m, 3H, piperazine), 3.44 (s, 2H, CH_2), 6.17 (t, 1H, NH, J= 4.9 Hz), 7.14 (s, 2H, NH_2), 7.21-7.32 (m, 5H, Ar-H), 7.52 (d, 2H, Ar-H, J=8.4 Hz), 7.65 (d, 2H, Ar-H, J=8.4 Hz), 9.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 48.6, 52.5, 57.1, 62.0, 116.7, 126.7, 126.9, 128.2, 128.9, 135.9, 137.9, 143.7, 154.7. LC–MS: m/z; 417 (M^+). HPLC purity: 99.4%.

4-{3-[2-(4-Benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-ethyl]-ureido}-benzene sulfonamide (11)

White solid; yield 82%; mp 200-202^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.27-2.42 (s, 10 H, piperazine +CH_2), 3.19 (q, 2H, CH_2, J=5.5 Hz), 3.36 (s, 2H, CH_2), 5.97 (s, 2H, CH_2), 6.19 (t, 1H, NH, J=4.5 Hz), 6.71-6.74 (m, 1H, Ar-H), 6.82-6.83 (m, 2H, Ar-H), 7.14 (s, 2H, NH_2), 7.51 (d, 2H,

Ar-H, J=8.3 Hz), 7.64 (d, 2H, Ar-H, J=7.6 Hz), 9.05 (s, 1H, NH). LC–MS: m/z; 461 (M^+). HPLC purity: 99.2%.

4-{2-[3-(4-Sulfamoyl-phenyl)-ureido]-ethyl}-piperazine-1-carboxylic acid benzyl ester (12)

White solid; yield 73%; mp 176-178^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.34-2.41 (m, 6H, piperazine+ CH_2), 3.21 (q, 2H, CH_2, J=5.5 Hz), 3.40 (s, 4H, piperazine), 5.06 (s, 2H, CH_2), 6.23 (t, 1H, NH, J= 4.9 Hz), 7.15 (s, 2H, NH_2), 7.29-7.38 (m, 5H, Ar-H), 7.52 (d, 2H, Ar-H, J=9.1 Hz), 7.65 (d, 2H, Ar-H, J=9.1 Hz), 9.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 43.5, 52.3, 57.1, 66.2, 116.7, 126.8, 127.6, 127.8, 128.4, 135.9, 136.9, 143.7, 154.4, 154.7. LC–MS: m/z; 461 (M^+). HPLC purity: 99.9%.

4-{3-[2-(4-Benzhydryl-piperazin-1-yl)-ethyl]-ureido}-benzenesulfonamide (13)

White solid; yield 77%; mp 210-212^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.27-2.40 (m, 10H, piperazine +CH_2), 3.19 (s, 2H, CH_2), 4.27 (s, 1H, CH), 6.20 (brs, 1H, NH), 7.14-7.19 (m, 4H, Ar-H+NH_2), 7.28 (t, 4H, Ar-H, J=7.6 Hz), 7.40 (d, 4H, Ar-H, J=7.6 Hz), 7.50 (d, 2H, Ar-H, J=8.4 Hz), 7.64 (d, 2H, Ar-H, J=8.4 Hz), 9.05 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.1, 51.2, 52.7, 57.1, 75.0, 116.7, 126.7, 126.8, 127.5, 128.5, 135.9, 142.7, 143.7, 154.7. LC–MS: m/z; 493 (M^+). HPLC purity: 98.3%.

4-[3-(2-{4-[Bis-(4-fluoro-phenyl)-methyl]-piperazin-1-yl}-ethyl)-ureido]benzene sulfonamide (14)

White solid; yield 64%; mp 225-227^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.17-2.41 (m, 10H, piperazine +CH_2), 3.17 (q, 2H, CH_2, J=6.0 Hz), 4.34 (s, 1H, CH), 6.16 (t, 1H, NH, J=4.9 Hz), 7.09-716 (m, 6H, Ar-H+NH_2), 7.39-7.42 (m, 4H, Ar-H), 7.50 (d, 2H, Ar-H, J=9.1 Hz), 7.64 (d,

2H, Ar-H, J=9.1 Hz), 9.02 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 36.2, 51.2, 52.8, 57.1, 73.0, 115.2, 115.4, 116.7, 126.8, 129.3, 129.4, 135.9, 138.7, 143.7, 154.7, 159.8, 162.2. LC–MS: m/z; 529 (M^+). HPLC purity: 99.6%.

4-(3-{2-[4-(4-Acetyl-phenyl)-piperazin-1-yl]-ethyl}-ureido)-benzenesulfonamide (15)

White solid; yield 65%; mp 216-218 ^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (s, 2H, CH_3), 2.46-2.51 (m, 6H, piperazine+ CH_2), 3.21 (q, 2H, CH_2, J=5.5 Hz), 3.28 (s, 4H, piperazine), 6.21 (t, 1H, NH, J= 5.3 Hz), 6.92 (d, 2H, Ar-H, J=9.1 Hz), 7.10 (s, 2H, NH_2), 7.48 (d, 2H, Ar-H, J=9.1 Hz), 7.61 (d, 2H, Ar-H, J=8.4 Hz), 7.74 (d, 2H, Ar-H, J=9.1 Hz), 9.00 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 26.1, 36.2, 46.6, 52.3, 57.1, 113.0, 116.7, 126.6, 126.7, 130.0, 135.9, 143.7, 153.8, 154.7, 195.6. LC–MS: m/z; 445 (M^+). HPLC purity: 99.6%.

Synthesis of 2-(4-substituedpiperazin-1-yl)-*N*-(4-sulfamoylphenyl) acetamides (Series 2)

General procedure for the synthesis of 2-chloro-*N*-(4-sulfamoylphenyl)acetamide (112)

An equimolar ratio of 4-aminobenzenesulfonamide and 2-chloroacetylchloride were stirred in dried acetone in the presence of anhydrous potassium carbonate (equimolar ratio) at room temperature for about 5-8 h. The precipitate that appeared was filtered and washed with acetone twice. Then after, the precipitate was again washed with petroleum ether twice and dried at 50^{0}C in the oven to give pure white colored product **17**.

2-Chloro-*N*-(4-sulfamoylphenyl)acetamide (17)

White solid; yield 88% ; mp 200-202^{0}C; δ ^{1}H NMR(DMSO-d_6,400 MHz): 4.29 (s, 2H, CH_2), 7.28 (s, 2H, NH_2), 7.74 (q, 4H, Ar-H, J=8.5 Hz), 10.60 (s, 1H, NH). LC–MS: m/z; 249 (M^{+1}).

General procedure for the synthesis of 2-(4-substituedpiperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamides (18-29)

The key intermediate **17** (10 mmole) was firstly dissolved in dried DMF (25 ml), then after substituted piperazine (10 mmole) was added portion wise. Equal amount of anhydrous sodium carbonate (10 mmole) was also added to the reaction mixture. The reaction mixture was heated at a reflux temperature of 90-100^{0}C for 8-10 h. After completion, the reaction mixture was poured in ice-cold water and the product was extracted out with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and concentrated on rotatory evaporator. The crude products (**18-29**) were washed with petroleum ether and dried in oven. All crude products were purified by column chromatography by using chloroform and methanol (98/02) as eluent to afford piperazine-sulfamoyl acetamides (**18-29**).

2-(4-Phenylpiperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (18)

White solid; yield 78% ; mp 180-182^{0}C; δ ^{1}H NMR(DMSO-d_6,400 MHz): 2.66 (s, 4H, piperazine), 3.19-3.22 (m, 6H, piperazine + CH_2), 6.76 (t, 1H, Ar-H, J=7.2 Hz), 6.93 (d, 2H, Ar-H, J=7.6 Hz), 7.20 (t, 2H, Ar-H, J=6.8 Hz), 7.26 (s, 2H, NH_2), 7.75 (d, 2H, Ar-H, J=9.1 Hz), 7.81 (d, 2H, Ar-H, J=9.1 Hz), 10.1 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 48.1, 52.6, 61.6, 115.4, 118.8, 119.0, 126.6, 128.9, 138.5, 141.5, 151.0, 168.8. LC–MS: *m/z*; 374 (M^+), HPLC purity: 97.6%.

2-(4-(4-Fluorophenyl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (19)

White solid; yield 73%; mp 220-222^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.66 (s, 4H, piperazine), 3.13 (s, 4H, piperazine), 3.22 (s, 2H, CH_2), 6.92-6.96 (m, 2H, Ar-H), 7.03 (t, 2H, Ar-H, J=8.7 Hz), 7.26 (s, 2H, NH_2), 7.75 (d, 2H, Ar-H, J=9.1 Hz), 7.81 (d, 2H, Ar-H, J=8.4 Hz), 10.0 (s, 1H, NH).

^{13}C NMR (DMSO-d_6): 48.8, 52.6, 61.6, 115.1, 115.3, 117.1, 119.0, 126.6, 138.5, 141.5, 147.9, 154.8, 157.1, 168.8. LC–MS: *m/z*; 393 (M^{+1}), HPLC purity: 98.8%.

2-(4-(4-Chlorophenyl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (20)

White solid; yield 84%; mp 225-227^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.65 (s, 4H, piperazine), 3.18-3.22 (m, 6H, piperazine +CH2), 6.94 (d, 2H, Ar-H, J=9.1 Hz), 7.21 (d, 2H, Ar-H, J=8.4 Hz), 7.25 (s, 2H, NH2), 7.74 (d, 2H, Ar-H, J=9.2 Hz), 7.80 (d, 2H, Ar-H, J=8.4 Hz), 10.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 47.8, 52.4, 61.5, 116.8, 119.0, 122.3, 126.6, 128.6, 138.5, 141.5, 149.7, 168.8. LC–MS: *m/z*; 408 (M^+), HPLC purity: 98.2%.

2-(4-(4-Bromophenyl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (21)

White solid; yield 79%; mp 210-212^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.65 (s, 4H, piperazine), 3.22-3.18 (m, 6H, piperazine +CH_2), 6.89 (d, 2H, Ar-H, J=9.1 Hz), 7.25 (s, 2H, NH_2), 7.33 (d, 2H, Ar-H, J=9.1 Hz), 7.76 (d, 2H, Ar-H, J=8.4 Hz), 7.80 (d, 2H, Ar-H, J=8.4 Hz), 10.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 47.7, 52.4, 61.5, 109.9, 117.3, 119.0, 126.6, 131.4, 138.4, 141.5, 150.1, 168.8. LC–MS: *m/z*; 453 (M^{+1}), HPLC purity: 97.6%.

***N*-(4-Sulfamoylphenyl)-2-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)acetamide (22)**

White solid; yield 81%; mp 212-214^{0}C; δ ^{1}H NMR(DMSO-d_6,400 MHz): 2.66 (s, 4H, piperazine), 3.24 (s, 2H, CH_2), 3.33 (s, 4H, piperazine), 7.06 (d, 2H, Ar-H, J=8.3 Hz), 7.26 (s, 2H, NH_2), 7.49 (d, 2H, Ar-H, J=9.1 Hz), 7.75 (d, 2H, Ar-H, J= 9.1 Hz), 7.81 (d, 2H, Ar-H, J=9.1 Hz), 10.0 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 46.8, 52.3, 61.4, 114.2, 119.0, 123.6, 126.1, 126.6, 138.5, 141.5, 153.2, 168.8. LC–MS: *m/z*; 442 (M^+), HPLC purity: 98.8%.

***N*-(4-Sulfamoylphenyl)-2-(4-(*p*-tolyl)piperazin-1-yl)acetamide (23)**

White solid; yield 67% ; mp 216-218^{0}C; δ ^{1}H NMR(DMSO-d_6,400 MHz): 2.18 (s, 3H, CH_3), 2.65 (s, 4H, piperazine), 3.12 (s, 4H, piperazine), 3.21 (s, 2H, CH_2), 6.83 (d, 2H, Ar-H, J=9.1 Hz), 7.01 (d, 2H, Ar-H, J=8.4 Hz), 7.25 (s, 2H, NH_2), 7.75 (d, 2H, Ar-H, J=9.9 Hz), 7.81 (d, 2H, Ar-H, J=6.8 Hz), 10.0 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 20.7, 48.5, 52.7, 61.7, 115.7, 119.0, 126.6, 127.6, 129.3, 138.5, 141.5, 148.9, 168.8. LC–MS: *m/z*; 388 (M^+), HPLC purity: 98.8%.

2-(4-(Pyridin-2-yl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (24)

White solid; yield 70%; mp 162-164^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.60 (t, 4H, piperazine, J=4.5 Hz), 3.22 (s, 2H, CH_2), 3.53 (t, 4H, piperazine, J=4.5 Hz), 6.61-6.64 (m, 1H, Ar-H), 6.81 (d, 1H, Ar-H, J=8.4 Hz), 7.25 (s, 2H, NH_2), 7.49-7.54 (m, 1H, Ar-H), 7.75 (d, 2H, Ar-H, J=9.1 Hz), 7.81 (d, 2H, Ar-H, J=8.4 Hz), 8.09-8.10 (m, 1H, Ar-H), 10.1 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 44.5, 52.4, 61.6, 107.1, 113.0, 119.0, 126.6, 137.5, 138.5, 141.5, 147.5, 159.0, 168.8. LC–MS: *m/z*; 375 (M^+), HPLC purity: 98.5%.

2-(4-Benzylpiperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (25)

White solid; yield 77%; mp 168-170^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.39 (s, 4H, piperazine), 2.51 (s, 4H, pipearzine), 3.11 (s, 2H, CH_2), 3.43 (s, 2H, CH2), 7.18-7.30 (m, 7H, Ar-H+NH_2), 7.73 (q, 4H, Ar-H, J=8.8 Hz), 9.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 52.4, 52.7, 61.8, 62.0, 118.9, 126.6, 126.9, 128.1, 128.8, 138.2, 138.4, 141.5, 168.9. LC–MS: *m/z*; 389 (M^{+1}), HPLC purity: 99.8%.

2-(4-Benzoylpiperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (26)

White solid; yield 65%; mp 243-245^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.58 (s, 4H, piperazine), 3.22 (s, 2H, CH_2), 3.38 (s, 2H, piperazine), 3.68 (s, 2H, piperazine), 7.25 (s, 2H, NH_2), 7.36-7.44

(m, 5H, Ar-H), 7.73-7.80 (m, 4H, Ar-H), 10.1 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 41.4, 47.0, 52.6, 61.3, 119.0, 126.6, 126.9, 128.4, 129.5, 135.8, 138.5, 141.5, 168.7, 169.0. LC–MS: *m/z*; 403 (M^{+1}), HPLC purity: 99.3%.

2-(4-Benzhydrylpiperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (27)

White solid; yield 53%; mp 223-225^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.30 (s, 4H, piperazine), 2.52 (s, 4H, piperazine), 3.08 (s, 2H, CH_2), 4.21 (s, 1H, CH), 7.10 (t, 2H, Ar-H, J=7.2 Hz), 7.18-7.23 (m, 6H, Ar-H+NH_2), 7.35 (d, 4H, Ar-H, J=7.6 Hz), 7.68 (q, 4H, Ar-H, J=8.3 Hz), 9.94 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 51.3, 52.8, 61.6, 75.1, 118.9, 126.6, 126.8, 127.5, 128.5, 138.4, 141.5, 142.9, 168.8. LC–MS: *m/z*; 464 (M^+), HPLC purity: 99.7%.

2-(4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (28)

White solid; yield 86%; mp 180-182^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.34 (s, 4H, piperazine), 2.54 (s, 4H, piperazine), 3.15 (s, 2H, CH_2), 4.36 (s, 1H, CH), 7.11 (t, 4H, Ar-H, J=9.3 Hz), 7.25 (s, 2H, NH_2), 7.42 (t, 4H, Ar-H, J=8.4 Hz), 7.78-7.72 (m, 4H, Ar-H), 10.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 51.1, 52.8, 61.6, 73.0, 115.2, 115.4, 118.9, 126.6, 129.3, 129.4, 138.4, 138.7, 141.5, 159.8, 162.2, 168.8. LC–MS: *m/z*; 500 (M^+), HPLC purity: 99.2%.

2-(4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-*N*-(4-sulfamoylphenyl)acetamide (29)

White solid; yield 85%; mp 163-165^{0}C; δ ^{1}H NMR (DMSO-d_6,400 MHz): 2.40 (brs, 4H, piperazine), 2.51 (s, 4H, piperazine), 3.13 (s, 2H, CH_2), 3.37 (s, 2H, CH_2), 5.97 (s, 2H, CH_2), 6.73 (d, 1H, Ar-H, J=7.6 Hz), 6.82 (d, 2H, Ar-H, J=7.6 Hz), 7.25 (s, 2H, NH_2), 7.76 (q, 4H, Ar-H, J=9.1 Hz), 10.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 52.2, 52.7, 61.7, 61.8, 100.7, 107.8, 109.0, 118.9,

121.9, 126.6, 132.0, 138.4, 141.5, 146.1, 147.2, 168.9. LC–MS: *m/z*; 432 (M^+), HPLC purity: 99.4%.

Carbonic anhydrase inhibition assay. An applied photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as buffer, and 20 mM Na_2SO_4 for maintaining constant the ionic strength (this anion is not inhibitory and has a K_i>200mM against these enzymes), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each measurement three traces of the initial 5–10% of the reaction have been used for determining the initial velocity (which was the mean of the three traces), working with 10-fold decreasing inhibitor concentrations ranging between 0.1nM and 10–100 mM (depending on the inhibitor potency, but at least five points at different inhibitor concentrations were employed for determining the inhibition constants). The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.1 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using the Cheng–Prusoff equation, and represent the mean from three independent experiments. The human isoforms hCA I, II, VII and XII were recombinant enzymes produced as described earlier in our laboratory.[13,30,33-34]

Statistics. The standard (s_N) deviation for the *in vitro/in vivo* experiments has been calculated using the following equation:

$$s_N = [(1/N) \cdot \sum(x_i - x_m)^2)]^{1/2}$$

Where N = number of experiments; x_i = are the observed values in each set of experiments and x_m = the mean value of these values. Thus, we always evaluated the square root of the sample variance, which is the average of the squared deviations about the sample mean.

Anticonvulsant Activity: The anticonvulsant activity of the synthesized compounds was evaluated in the maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (scPTZ) seizure tests. Healthy adult male Swiss Albino mice (25-30g) and male Wistar rats (100-150g) were procured from " Disease free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences" Hisar, Haryana, India and were kept under standard animal laboratory conditions (25-30^{0}C; 12 h light/dark cycle) at Dr. B.R Ambedkar Center For Biomedical Research, University of Delhi, India. After a week of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups and each mice/rat was used only one time for the experiments. In general, the tested compounds were freshly suspended in 1% acacia gum and administered intraperitoneally (i.p.) to Swiss albino mice in a volume of 0.01 ml/g body weight and in a volume of 0.04 ml per 10 g of body weight orally to Wistar albino rats. In sub-acute toxicity studies, the control group animals were administered with vehicle (1% acacia gum in normal sterile solution) only. Topiramate (TPM) and Acetazolamide (AAZ) were also suspended in 1% acacia gum solution and used as reference drugs for comparison. The pentylenetetrazole solution at convulsant dose was dissolved in normal sterile saline solution. All the experimental protocols were prior approved by the Institutional Animal Ethics Committee (Approval number:

IAEC/ACBR/2016/PML/016) for animal care. Moreover, all efforts were made to minimize animal suffering and to use only the number of animals essential to produce consistent scientific data. Groups of eight animals were used for each dose of administered drug. The control group also included 8 animals.

Maximal Electroshock (MES) Test: In this anticonvulsant screening test eight animals (n=8) was taken in each group. The test compounds (**5**, **18**, **24**, **25** & **26**), TPM and AAZ were injected (i.p.) at the doses of 30 and 100 mg/kg to Swiss albino mice 0.5 or 3 h prior to seizure induction. In oral bioavailablity studies, three compounds **18**, **25** and **26** (30 mg/kg, per oral) were tested and four (n=4) Wistar rats were taken for each time interval (0.25, 0.5, 1.0, 2.0 & 4.0 h). The seizures were induced by 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. Transauricular stimulation preferably activates the brain stem region that leads to elicitation of severe tonic convulsions in rodents. The endpoint was the tonic extension of the hind limbs. In the control groups the procedure caused immediate hind limb tonic extension and the complete abolition of hind limb extensor phase was taken considered as protection[40e, 40f].

Subcutaneous Pentylenetrazole (scPTZ) Test: The pentylenetetrazole (PTZ) was injected subcutaneously (sc) at the convulsive dose of 85 mg/kg (CD_{97}) and this dose produced clonic convulsions lasting for at least five seconds, with accompanying loss of the righting reflex. A number of six animals (n=6) were taken for each group. The test compounds (**5**, **18**, **24**, **25** & **26**) and reference drugs were administered (i.p.) at the doses of 30 and 100 mg/kg in Swiss albino mice and then after, PTZ was administered 0.5 and 3 h after injections of test compounds and observation were carried out for 30 min. The mice were placed in a clear rectangular plastic cage**,**

permitting a full view of the animal's seizure episodes. The number of clonic seizures, tonic seizures and deaths were noted carefully. A threshold convulsion was an episode of clonic seizures lasting for at least 5 seconds and the absence of this threshold convulsion during the experimental time period of 0.5 and 3 h was interpreted as the compound's ability to protect against PTZ-induced seizure[40b, 40e].

Anticonvulsant Quantification Studies (ED_{50} Determination). Anticonvulsant quantification study (ED_{50}) was determined for selected compounds **18**, **25** and **26**. Eight Swiss albino mice in each group were used in this study and the effective dose (ED_{50}) of each compounds was evaluated by the MES test. Animals were administered in varying doses of test compounds **18**, **25** and **26** until at least two points were established between the dose level of 0% protection and of 100% protection against MES-induced tonic extension (Table S1, supplementary material). The dose of drug required to produce the desired endpoint in 50% of animals, i.e. ED_{50} values with their 95% confidence intervals were calculated according to Litchfield and Wilcoxon.[44c]

Time course study. The in-vivo time course studies were carried out on Swiss albino mice and eight (n=8) animals were taken in each group. The test compounds (**18**, **25** and **26**) were administered i.p. at a fixed dose of 30 mg/kg and the percentage of seizure protection were accessed at varying time intervals (0.5, 1, 2, 3, 4 and 6 h) by employing MES test.

Toxicological Studies: Three compounds **18**, **25** and **26** were evaluated for their in-vivo toxicity in sub-acute toxicity studies. The healthy adult male Wistar rats were taken as experimental animals (n=6 in each group) and daily administered orally with test compounds (**18**, **25** & **26**) and vehicle for 14 days. During the experimental period, the animals were observed daily to detect any signs of

toxicity such as inflammation, outgrowth of tissue, skin and fur quality and any necrosis. After the stipulated period, at day 15, each animal was anesthetized by anesthetic ether, and blood was collected by cardiac puncture. The blood samples were placed separately in sterilized heparinized and non-heparinized tubes for further hematological as well as biochemical evaluations. Hematological parameters such as red blood cell (RBC), Total leukocyte count (TLC), differential leukocyte count, (DLC), platelet count and hemoglobin (Hb) were estimated by using automated hematological analyzer machine (Nihon Kohden Japan).The clear serum was separated from the non-heparinized blood at 37^0 C and was assayed for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total protein (TP), albumin (Alb), globulin (Glob), total billirubin (TBil), Blood urea, creatinine (Cr), uric acid, calcium, phosphorus, sodium, potassium and chlorine by biochemical auto-analyzer (Agappe, India).

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Supporting information. Spectral and HPLC data for the reported compounds as well as a table showing the calculation of the ED_{50} values are available free of charge on internet.

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Abbreviations. DMF, Dimethylformamide; THF, Tetrahydrofuran; AAZ, Acetazolamide; K_I, inhibition constant; CA, carbonic anhydrase; CAI, CA inhibitor; MES, maximal electroshock seizure; scPTZ, subcutaneous pentylenetetrazole

References.

1. WHO data available from http://www.who.int/mediacentre/factsheets/fs999/en/, February 3, 2016.

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

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

4. Meldrum, B. S.; Rogawski, M. A. Molecular targets for antiepileptic drug development. *Neurotherapeutics* **2007**, *4*, 18–61.

5. Bonnet, U.; Wiemann, M.; Bingmann, D. CO_2/HCO3(-)-withdrawal from the bath medium of hippocampal slices: biphasic effect on intracellular pH and bioelectric activity of CA3-neurons. *Brain Res*. **1998**, *796*, 161-170.

6. Velisek, L.; Dreier, J. P.; Stanton, P. K.; Heinemann, U.; Moshe, S. L. Lowering of extracellular pH suppresses low-Mg(2+)-induces seizures in combined entorhinal cortex-hippocampal slices. *Exp. Brain. Res*. **1994**, *101*, 44-52.

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

8. (a) Aribi, A. M.; Stringer, J. L. Effects of antiepileptic drugs on extracellular pH regulation in the hippocampal CA1 region in vivo. *Epilepsy Res*. **2002**, *49*, 143-151; (b) Chen, J. C.; Chesler,

M. pH transients evoked by excitatory synaptic transmission are increased by inhibition of extracellular carbonic anhydrase. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7786-7790.

9. (a) Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schafer, S.; Supuran, C. T. 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.* **2003***, 13*, 841-845; (b) Di Fiore, A.; De Simone, G.; Alterio, V.; Riccio, V.; Winum, J.Y.; Carta, F.; Supuran, C.T. The anticonvulsant sulfamide JNJ-26990990 and its S,S-dioxide analog strongly inhibit carbonic anhydrases: solution and X-ray crystallographic studies. *Org. Biomol. Chem.* **2016**, *14*, 4853-4858; (c) Supuran, C.T. Drug interaction considerations in the therapeutic use of carbonic anhydrase inhibitors. *Expert Opin. Drug Metab. Toxicol*. **2016**, *12*, 423-431.

10. (a) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms? *Chem. Rev*. **2012**, *112*, 4421−4468; (b) Supuran, C. T. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. *Nat. Rev. Drug Discovery* **2008**, *7*, 168−181; (c) Supuran, C.T. How many carbonic anhydrase inhibition mechanisms exist? *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 345-360.

11. (a) Rummer, J. L.; McKenzie, D. J.; Innocenti, A.; Supuran, C. T.; Brauner, C. J. Root effect hemoglobin may have evolved to enhance general tissue oxygen delivery. *Science* **2013**, *340*, 1327−1329; (b) Supuran, C.T. Carbonic anhydrases: from biomedical applications of the inhibitors and activators to biotechnologic use for CO_2 capture. *J. Enzyme Inhib. Med. Chem*. **2013**, *28*, 229−230; (c) Neri, D.; Supuran, C.T. Interfering with pH regulation in tumors as a therapeutic strategy. *Nat. Rev. Drug Discovery* **2011**, *10*, 767−777; (d) Smith, K. S.; Jakubzick, C.; Whittam, T. S.; Ferry, J.G. Carbonic anhydrase is an ancient enzyme widespread in

prokaryotes. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 15184−15189; (e) Supuran, C.T. Structure-based drug discovery of carbonic anhydrase inhibitors. *J. Enzyme Inhib. Med. Chem*. **2012**, *27*, 759−772.

12. (a) Supuran, C.T. Structure and function of carbonic anhydrases. *Biochem. J*. **2016,** *473*, 2023–2032; (b) Aggarwal, M.; Boone, C. D.; Kondeti, B.; McKenna, R. Structural annotation of human carbonic anhydrases. *J. Enzyme Inhib. Med. Chem*. **2013**, *28*, 267−277; (c) Xu, Y., Feng, L., Jeffrey, P.D., Shi, Y. Morel, F.M. Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms. *Nature* **2008***, 452*, 56–61.

13. (a) Mishra, C. B.; Kumari, S.; Angeli, A.; Monti, S. M.; Buonanno, M.; Prakash, A.; Tiwari, M.; Supuran, C. T. 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*. **2016** *31*, Suppl. 2, 174-179; (b) Borras, J.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C.T. Carbonic anhydrase inhibitors. Part 73. Synthesis of water-soluble, topically effective intraocular pressure lowering aromatic/ heterocyclic sulfonamides containing 8-quinoline-sulfonyl moieties: is the tail more important than the ring ? *Bioorg. Med. Chem*. **1999**, *7*, 2397-2406; (c) Cottier, F.; Leewattanapasuk, W.; Kemp, L. R.; Murphy, M.; Supuran, C. T.; Kurzai, O.; Mühlschlegel, F. A. Carbonic anhydrase regulation and CO_2 sensing in the fungal pathogen *Candida glabrata* involves a novel Rca1p ortholog. *Bioorg. Med. Chem.* **2013**, *21*, 1549-1554; (d) Schlicker, C.; Hall, R. A.; Vullo, D.; Middelhaufe, S.; Gertz, M.; Supuran, C. T.; Mühlschlegel, F. A.; Steegborn, C. Structure and inhibition of the CO_2-sensing carbonic anhydrase Can2 from the pathogenic fungus *Cryptococcus neoformans*. *J. Mol. Biol.* **2009**, 385, 1207−1220.

14. (a) Filippi, D.; Sciaky, M.; Limozin, N.; Laurent, G. Anhydrase carbonique du système nerveux central du rat. Isolement et propriétés. *Biochimie* **1978**, *60*, 99-102; (b) Agnati, L. F.; Tinner, B.; Staines, W. A.; Vaananen, K.; Fuxe, K. On the cellular localization and distribution of carbonic anhydrase II immunoreactivity in the rat brain. *Brain Res*. **1995**, *676*, 10-24.

15. Parkkila, S. An overview of the distribution and function of carbonic anhydrase in mammals. In *The Carbonic Anhydrases New Horizons*; Chegwidden, R., Ed., Birkäuser Verlag: Basel, Boston, Berlin, **2000**; pp. 79-93.

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

17. (a) Masuzawa, T.; Sato, F. The enzyme histochemistry of the choroid plexus. *Brain* **1983**, *106*, 55-99; (b) Velisek, L.; Moshe, S. L.; Cammer, W. Developmental changes in seizure susceptibility in carbonic anhydrase II-deficient mice and normal littermates. *Dev. Brain Res*. **1993**, *72*, 321-324; (c) De Simone, G.; Scozzafava, A.; Supuran, C.T. Which carbonic anhydrases are targeted by the antiepileptic sulfonamides and sulfamates?. *Chem. Biol. Drug. Des*. **2009**, *74*, 317–321.

18. (a) Ruusuvuori, E.; Kaila, K. Carbonic anhydrases and brain pH in the control of neuronal excitability. In *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications*; Frost, S., McKenna, R., Eds.; Springer, **2014**; pp 271–290; (b) Ruusuvuori, E.; Huebner, A. K.; Kirilkin, I.; Yukin, A. Y.; Blaesse, P.; Helmy, M.; Kang, H. J.; El Muayed, M.; Hennings, J. C.; Voipio, J.; Šestan, N.; Hübner, C. A.; Kaila, K. Neuronal carbonic anhydrase VII provides GABAergic excitatory drive to exacerbate febrile seizures. *EMBO J.* **2013**, *32*, 2275.

19. (a) Ruusuvuori, E.; Li, H.; Huttu, K.; Palva, J. M.; Smirnov, S.; Rivera, C.; Kaila, K.; Voipio, J. Carbonic anhydrase isoform VII acts as a molecular switch in the development of synchronous gamma-frequency firing of hippocampal CA1 pyramidal cells. *J. Neurosci*. **2004**, *24*, 2699-2707; (b) Rivera, C.; Voipio, J.; Kaila, K. Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. *J. Physiol*. **2005**, *562*, 27-36; (c) Nishimori, I. Acatalytic CAs: Carbonic anhydrase related Proteins. In *Carbonic Anhydrase: Its Inhibitors and Activators*; Supuran, C.T., Scozzafava, A., Conway, J. Eds.; CRC Press: Boca Raton, **2004**; pp. 25-43.

20. (a) Chesler, M. Regulation and modulation of pH in the brain. *Physiol. Rev*. **2003**, *83*, 1183–1221; (b) Casey, J. R.; Grinstein, S.; Orlowski, J. Sensors and regulators of intracellular pH. *Nat. Rev. Mol. Cell. Biol*. **2010**, *11*, 50–61; (c) Ruusuvuori, E.; Kirilkin, I.; Pandya, N.; Kaila, K. Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism. *J. Neurosci*. **2010**, *30*, 15638–15642; (d) Pasternack, M.; Voipio, J.; Kaila, K. Intracellular carbonic anhydrase activity and its role in GABA-induced acidosis in isolated rat hippocampal pyramidal neurones. *Acta Physiol Scand*. **1993**, *148*, 229–231; (e) Williams, R. H.; Jensen, L. T.; Verkhratsky, A.; Fugger, L.; Burdakov, D. Control of hypothalamic orexin neurons by acid and CO_2. *Proc. Natl. Acad. Sci. U.S.A*. **2007**, *104*, 10685–10690.

21. (a) Vullo, D.; Innocenti, A.; Nishimori, I.; Pastorek, J.; Scozzafava, A.; Pastorekova, S.; Supuran, C. T. Carbonic anhydrase inhibitors: Inhibition of the transmembrane isozyme XII with sulfonamides-a new target for the design of antitumor and antiglaucoma drugs? *Bioorg. Med. Chem. Lett*. **2005**, *15*, 963-969; (b) Vullo, D.; Voipio, J.; Innocenti, A.; Rivera, C.; Ranki, H.; Scozzafava, A.; Kaila, K.; Supuran, C.T. Carbonic anhydrase inhibitors. Inhibition of the human

cytosolic isozyme VII with aromatic and heterocyclic sulfonamides. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 971-976.

22. (a) Reiss, W. G.; Oles, K. S. Acetazolamide in the treatment of seizures. *Ann. Pharmacother*. **1996**, *30*, 514–519; (b) Katayama, F.; Miura, H.; Takanashi, S. Long-term effectiveness and side effects of acetazolamide as an adjunct to other anticonvulsants in the treatment of refractory epilepsies. *Brain Dev*. **2002**, *24*, 150-154; (c) Parkkila, S.; Parkkila, A. K.; Kivela, J. Role of carbonic anhydrase and its inhibitors in gastroenterology, neurology and nephrology. In *Carbonic Anhydrase: Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J. Eds.; CRC Press: Boca Raton, **2004**; pp. 283-301.

23. (a) Leppik, I. E. Zonisamide: chemistry, mechanism of action, and pharmacokinetics. *Seizure* **2004**, *13*, S5-9; (b) Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P.; Perucca, E. Progress report on new antiepileptic drugs: a summary of the Fifth Eilat Conference (EILAT V). *Epilepsy Res*. **2001**, *43*, 11-58; (c) Herrero, A. I.; Del Olmo, N.; Gonzalez-Escalada, J. R.; Solis, J. M. Two new actions of topiramate: inhibition of depolarizing GABA (A)-mediated responses and activation of a potassium conductance. *Neuropharmacology* **2002**, *42*, 210-220.

24.(a) Ilies, M. A.; Masereel, B.; Rolin, S.; Scozzafava, A.; Campeanu, G.; Cimpeanu, V.; Supuran, C. T. Carbonic anhydrase inhibitors: aromatic and heterocyclic sulfonamides incorporating adamantyl moieties with strong anticonvulsant activity. *Bioorg. Med. Chem. Lett*. **2004**, *12*, 2717-2726; (b) Leniger, T.; Thone, J.; Wiemann, M. Topiramate modulates pH of hippocampal CA3 neurons by combined effects on carbonic anhydrase and Cl^-/HCO_3^- exchange. *Br. J. Pharmacol*. **2004**, *142*, 831-842.

25. (a) De Simone, G.; Alterio, V.; Supuran, C. T. Exploiting the hydrophobic and hydrophilic binding sites for designing carbonic anhydrase inhibitors. *Expert Opin. Drug Discovery* **2013**, *8*, 793−810; (b) Scozzafava, A.; Supuran, C.T.; Carta, F. Anti-obesity carbonic anhydrase inhibitors: A literature and patent review. *Expert Opin. Ther. Pat*. **2013**, *23*, 725−735; (c) Supuran, C.T. Advances in structure-based drug discovery of carbonic anhydrase inhibitors. *Expert Opin. Drug Discov*ery **2017**, *12*, 61-88.

26. (a) Arechederra, R. L.; Waheed, A.; Sly, W. S.; Supuran, C.T.; Minteer, S. D. Effect of sulfonamides as selective carbonic anhydrase VA and VB inhibitors on mitochondrial metabolic energy conversion. *Bioorg. Med. Chem*. **2013**, *21*, 1544−1548; (b) Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C.T. Antiglaucoma carbonic anhydrase inhibitors: A patent review. *Expert Opin. Ther. Pat*. **2013**, *23*, 705−716; (c) Supuran, C. T. Carbonic anhydrase inhibitors. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3467−3474.

27. (a) Aggarwal, M.; McKenna, R. Update on carbonic anhydrase inhibitors: a patent review (2008−2011). *Expert Opin. Ther. Pat*. **2012**, *22*, 903−915; (b) Aggarwal, M.; Kondeti, B.; McKenna, R. Anticonvulsant/ antiepileptic carbonic anhydrase inhibitors: a patent review. *Expert Opin. Ther. Pat*. **2013**, *23*, 717−724; (c) Thiry, A.; Dognè, J. M.; Supuran, C. T.; Masereel, B. Anticonvulsant sulfonamides/sulfamates/sulfamides with carbonic anhydrase inhibitory activity: drug design and mechanism of action. *Curr. Pharm. Des*. **2008**, *14*, 661−671; (d) Fabrizi, F.; Mincione, F.; Somma, T.; Scozzafava, G.; Galassi, F.; Masini, E.; Impagnatiello, F.; Supuran, C. T. 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*. **2012**, *27*, 138−147.

28. (a) Thiry, A.; Dognè, J. M.; Masereel, B.; Supuran, C. T. Carbonic anhydrase inhibitors as anticonvulsant agents. *Curr. Top. Med. Chem*. **2007**, *7*, 855−864; (b) Carta, F.; Supuran, C. T.; Scozzafava, A. Novel therapies for glaucoma: a patent review 2007−2011. *Expert Opin. Ther. Pat*. **2012**, *22*, 79−88; (c) Carta, F.; Supuran, C. T. Diuretics with carbonic anhydrase inhibitory action: A patent and literature review (2005−2013). *Expert Opin. Ther. Pat*. **2013**, *23*, 681−691.

29. (a) Ilies, M.A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M.T.; Pastorekova, S.; Supuran, C.T. Carbonic anhydrase inhibitors. Inhibition of tumor-associated isozyme IX with halogenosulfanilamide and halogenophenylaminobenzolamide derivatives. *J. Med. Chem.* **2003**, *46*, 2187-2196; b) Supuran, C.T.; Ilies, M.A.; Scozzafava, A. Carbonic anhydrase inhibitors. Part 29. Interaction of isozymes I, II and IV with benzolamide-like derivatives. *Eur. J. Med. Chem*. **1998**, *33*, 739-752.

30. (a) Vullo, D.; Supuran, C.T.; Scozzafava, A.; De Simone, G.; Monti, S. M.; Alterio, V.; Carta, F. Kinetic and X-ray crystallographic investigations of substituted 2-thio-6-oxo-1,6 dihydropyrimidine-benzenesulfonamides acting as carbonic anhydrase inhibitors. *Bioorg. Med. Chem*. **2016**, *24*, 3643-3648; (b) De Simone, G.; Monti, S. M.; Alterio, V.; Buonanno, M.; De Luca, V.; Rossi, M.; Carginale, V.; Supuran, C. T.; Capasso, C.; Di Fiore, A. Crystal structure of the most catalytically effective carbonic anhydrase enzyme known, SazCA from the thermophilic bacterium Sulfurihydrogenibium azorense. *Bioorg. Med. Chem. Lett*. **2015,** *25*, 2002-2006; (c) Di Fiore, A.; Capasso, C.; De Luca, V.; Monti, S. M.; Carginale, V.; Supuran, C. T.; Scozzafava, A.; Pedone, C.; Rossi, M.; De Simone, G. 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*. **2013**, *69*, 1150-1159.

31. (a) Ilies, M.; Supuran, C. T.; Scozzafava, A.; Casini, A.; Mincione, F.; Menabuoni, L.; Caproiu, M. T.; Maganue, M.; Banciu, M. D. Carbonic anhydrase inhibitors: Sulfonamides incorporating furan, thiophene- and pyrrole-carboxamido groups possess strong topical intraocular pressure lowering properties as aqueous suspensions. *Bioorg. Med. Chem.* **2000**, *8*, 2145-2155; (b) Scozzafava, A.; Mastrolorenzo, A.; Supuran C.T. Arylsulfonyl-N, N-diethyl-dithiocarbamates: A novel class of antitumor agents. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1887-1891; (c) Renzi, G.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors: Topical sulfonamide antiglaucoma agents incorporating secondary amine moieties. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 673-676; (d) Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors: Synthesis of N-morpholylthiocarbonylsulfenylamino aromatic/heterocyclic sulfonamides and their interaction with isozymes I, II and IV. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1117-1120; (e) Güzel, Ö.; Maresca, A.; Scozzafava, A.; Salman, A.; Balaban, A.T.; Supuran, C.T. Carbonic anhydrase inhibitors. Synthesis of 2,4,6-trimethylpyridinium derivatives of 2-(hydrazinocarbonyl)-3-aryl-1H-indole-5-sulfonamides acting as potent inhibitors of the tumor-associated isoform IX and XII. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2931–2934.

32. (a) Brzozowski, Z.; Slawinski, J.; Innocenti, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Regioselective synthesis of novel 1-substituted 1,4-dihydro-4-oxo-3 pyridinesulfonamides and their inhibition of the human cytosolic isozymes I and II and transmembrane cancer-associated isozymes IX and XII. *Eur. J. Med. Chem*. **2010**, *45*, 3656-3661; (b) Brzozowski, Z.; Slawinski, J.; Saczewski, F.; Innocenti, A.; Supuran, C.T. Carbonic anhydrase inhibitors: Synthesis and inhibition of the human cytosolic isozymes I and II and transmembrane isozymes IX, XII (cancer-associated) and XIV with 4-substituted 3-pyridinesulfonamides. *Eur. J. Med. Chem*. **2010**, *45*, 2396-2404; (c) Akdemir, A.; Güzel-

Akdemir, Ö.; Scozzafava, A.; Capasso, C.; Supuran, C.T. Inhibition of tumor-associated human carbonic anhydrase isozymes IX and XII by a new class of substituted-phenylacetamido aromatic sulfonamides. *Bioorg. Med. Chem.* **2013**, *21*, 5228–5232; (d) Thiry, A.; Rolin, S.; Vullo, D.; Frankart, A.; Scozzafava, A.; Dogne, J.; Wouters, J.; Supuran, C. T.; Masereel, B. Indanesulfonamides as carbonic anhydrase inhibitors and anticonvulsant agents: Structureeactivity relationship and pharmacological evaluation. *Eur. J. Med. Chem.* **2008**, *43*, 2853-2860; (e) Leitans, J.; Sprudza, A.; Tanc, M.; Vozny, I.; Zalubovskis, R.; Tars, K.; Supuran, C. T. 5-Substituted-(1, 2, 3-triazol-4-yl) thiophene-2-sulfonamides strongly inhibit human carbonic anhydrases I, II, IX and XII: Solution and X-ray crystallographic studies. *Bioorg. Med. Chem.* **2013**, *21*, 5130–5138.

33. (a) Nishimori, I.: Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C.T. Carbonic anhydrase inhibitors. The mitochondrial isozyme VB as a new target for sulfonamide and sulfamate inhibitors. *J. Med. Chem.* **2005**, *48*, 7860-7866; (b) Mete, E.; Comez, B.; Inci, Gul.; Gulcin, I.; Supuran, C. T. Synthesis and carbonic anhydrase inhibitory activities of new thienyl-substituted pyrazoline benzenesulfonamides. *J. Enzyme Inhib. Med. Chem.* **2016,** *19*, 1-5; (c) Bozdag, M.; Alafeefy, A. M.; Carta, F.; Ceruso, M.; Al-Tamimi, A. S.; Al-Kahtani, A. A.; Alasmary, F. A. S.;. Supuran, C. T. 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.* **2016**, *24*, 4100–4107; (d) Vullo, D.; Supuran, C. T.; Scozzafava, A.; Simone, G. D.; Monti, S. M.; Alterio, V.; Carta, F. Kinetic and X-ray crystallographic investigations of substituted 2-thio-6-oxo-1,6-dihydropyrimidine–benzenesulfonamides acting as carbonic anhydrase inhibitors. *Bioorg. Med. Chem.* **2016**, *24* 3643–3648.

34. (a) Abdel Gawad, N. M.; Amin, N. H.; Elsaadi, M. T.; Mohamed F. M.; Angeli, A.; De Luca, V.; Capasso, C.; Supuran, C. T. 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.* **2016**, *24*, 3043–3051; (b) Supuran, C. T.; Kalinin, S.; Tanç, M.; Sarnpitak, P.; Mujumdar P.; Poulsen, S. A.; Krasavin, M. Isoform-selective inhibitory profile of 2-imidazoline-substituted benzene sulfonamides against a panel of human carbonic anhydrases. *J. Enzyme Inhib. Med. Chem*. **2016**, *24*, 1-6; (c) Gul, H. I.; Tugrak, M.; Sakagami, H.; Taslimi, P.; Gulcin, I.; Supuran, C. T. 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*. **2016**, *31*, 1619-1624; (d) Eldehna, W. M.; Fares, M.; Ceruso, M.; Ghabbour, H. A.; Abou-Seri, S. M.; Abdel-Aziz, H. A.; Abou El Ella, D. A. Amido/ ureidosubstituted benzenesulfonamides-isatin conjugates as low nanomolar/subnanomolar inhibitors of the tumor-associated carbonic anhydrase isoform XII. *Eur. J. Med. Chem*. **2016**, *110*, 259-266; (e) Lomelino, C. L.; Mahon, B. P.; McKenna, R.; Carta, F.; Supuran, C. T. 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.* **2016**, *24*, 976–981.

35. (a) Cornelio, B.; Laronze-Cochard, M.; Ceruso, M.; Ferraroni, M.; Rance, G. A.; Carta, F.; Khlobystov, A. N.; Fontana, A.; Supuran, C. T.; Sapi, J. 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*. **2016**, *59*, 721−732; (b) La Regina, G.; Coluccia, A.; Famiglini, V.; Pelliccia, S.; Monti, L.; Vullo, D.; Nuti, E.; Alterio, V.; De Simone, G.; Monti, S. M.; Pan, P.; Parkkila, S.; Supuran, C. T.; Rossello, A.; Silvestri, R. Discovery of 1, 1′-biphenyl-4-sulfonamides as a new class of potent

and selective carbonic anhydrase XIV inhibitors. *J. Med. Chem*. **2015**, *58*, 8564−8572; (c) Rankin, G. M.; Vullo, D.; Supuran, C. T.; Poulsen, S. A. Phosphate chemical probes designed for location specific inhibition of intracellular carbonic anhydrases. *J. Med. Chem*. **2015**, *58*, 7580-7590; (d) Wilkinson, B. L.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S. A. Inhibition of Carbonic anhydrases with glycosyltriazole benzene sulfonamides. *J. Med. Chem*. **2008**, *51*, 1945–1953; (e) Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. Carbonic anhydrase inhibitors: water-soluble 4-sulfamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects. *J. Med. Chem*. **2000**, *43*, 4884-4892.

36. (a) Bozdag, M.; Pinard, M.; Carta, F.; Masini, E.; Scozzafava, A.; McKenna, R.; Supuran CT. A class of 4-sulfamoylphenyl-ω-aminoalkyl ethers with effective carbonic anhydrase inhibitory action and antiglaucoma effects. *J. Med. Chem.* **2014**, *57*, 9673−9686; (b) Innocenti, A.; Casini, A.; Alcaro, M. C.; Papini, A. M.; Scozzafava, A.; Supuran, C. T. Carbonic anhydrase inhibitors: the first on-resin screening of a 4-sulfamoylphenylthiourea library. *J. Med. Chem*. **2004**, *47*, 5224-5229; (c) Pacchiano, F.; Carta, F.; McDonald, P. C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; 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*. **2011**, *54*, 1896–1902; (d) Hen, N.; Bialer, M.; Yagen, B.; Maresca, A.; Aggarwal, M.; Robbins, A.H.; McKenna, R.; Scozzafava, A.; Supuran, C. T. 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*. **2011**, *54*, 3977–3981.

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

38. Pacchiano, F.; Aggarwal, M.; Avvaru, B. S.; Robbins, A. H.; Scozzafava, A.; McKenna, R.; Supuran, C.T. Selective hydrophobic pocket binding observed within the carbonic anhydrase II active site accommodate different 4-substituted-ureido-benzenesulfonamides and correlate to inhibitor potency. *Chem. Commun*. **2010**, *44*, 8371-8373.

39. Congiu, C.; Onnis, V.; Deplano, A.; Balboni, G.; Dedeoglu, N.; Supuran, C. T .Synthesis of sulfonamides incorporating piperazinyl-ureido moieties and their carbonic anhydrase I, II, IX and XII inhibitory activity. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 3850–3853.

40. (a) Mishra, C. B.; Manral, A.; Kumari, S.; Saini, V.; Tiwari, M. 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.* **2016**, *24*, 3829–3841; (b) Mishra, C. B.; Kumari, S.; Tiwari, M. 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*. **2016**, *39*, 603–617; (c) Mishra, C. B.; Kumari, S.; Manral, A.; Prakash, A.; Saini, V.; Lynn, A. M.; Tiwari, M. 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*. **2016,** *125*, 736-750; (d) Kumari, S.; Mishra, C. B.; Tiwari, M. 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*. **2016**, *68*, 250-258; (e) Kumari, S.; Mishra, C. B.; Tiwari, M. 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.* **2015**, *25*, 1092–1099; (f) Shaquiquzzaman, M.; Verma, G.; Marella, A.; Akhter, M.; Akhtar, W.; Khan, M. F.; Tasneem, S.; Alam, M. M. Piperazine scaffold: A remarkable tool in generation of diverse pharmacological agents. *Eur. J. Med. Chem*. **2015**, *102*, 487-529.

41. (a) Turkmen, H.; Durgun, M.; Yilmaztekin, S.; Emul, M.; Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C.T. Carbonic anhydrase inhibitors. Novel sulfanilamide/acetazolamide derivatives obtained by the tail approach and their interaction with the cytosolic isozymes I and II, and the tumor-associated isozyme IX. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 367-372; (b) Abdülkadir Coban, T.; Beydemir, S.; Gülcin, I.; Ekinci, D.; Innocenti, A.; Vullo, D.; Supuran, C. T. Sildenafil is a strong activator of mammalian carbonic anhydrase isoforms I-XIV. *Bioorg. Med. Chem*. **2009**, *17*, 5791-5795; (c) Sławiński, J.; Szafrański, K.; Vullo, D.; Supuran, C. T. Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine-3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII. *Eur. J. Med. Chem*. **2013**, *69*, 701-10.

42. Rogawski, M. A. Molecular targets versus models for new antiepileptic drug discovery. *Epilepsy Res*. **2006**, *68*, 22-28.

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

44. (a) Huang, Q. R.; Bell Horner, L. C.; Mohammad, I. D.; Covey, F. D.; Drewe, J. A.; Dillon, G. H. Pentylenetetrazole-induced inhibition of recombinant-aminobutyric acid type A (GABAA) receptors: mechanism and site of action. *J. Pharm. Exp. Ther.* **2001**, *298*, 986–995; (b) Loscher, W.; Honack, D.; Fassbender, P. C.; Nolting, B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. *Epilepsy Res.* **1991**, *8*, 171–189; (c) Litchfeld, J.T. Jr.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99-113.

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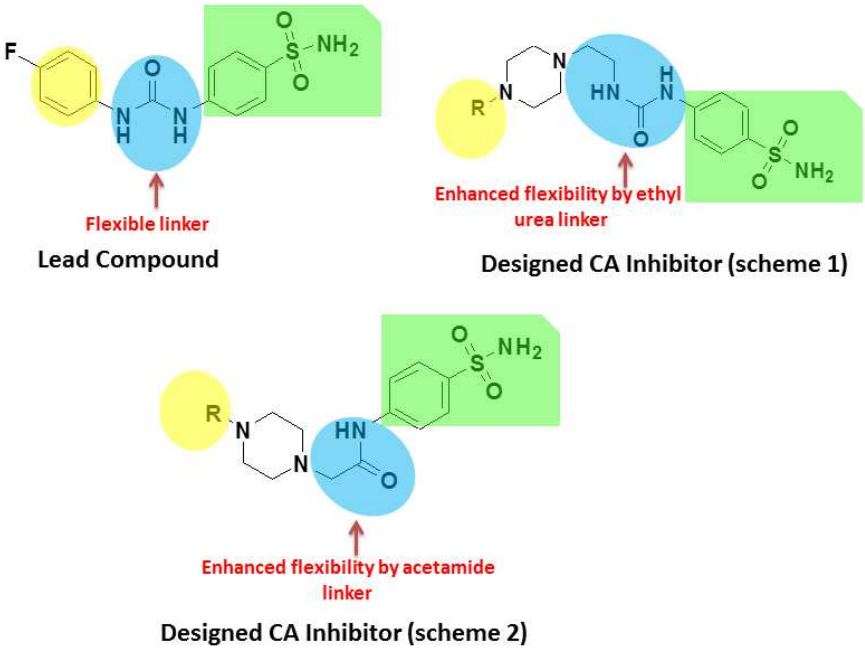
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