Bioorganic & Medicinal Chemistry 19 (2011) 3221–3228

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and evaluation of new carbonic anhydrase inhibitors

Zhonghai Xiao [a,†], Ruifeng Duan [a,†], Wenyu Cui [b], Yanfang Zhang [b], Shouguo Zhang [c], Fangjin Chen [d], Yankun Zhang [a], Jiaying Liu [a], Dongxiang Zhang [a], Yuan Meng [e], Lin Wang [c,*], Hai Wang [a,b,e,*]

[a] Department of Environmental Medicine, Institute of Health and Environmental Medicine, Academy of Military Medical Sciences, Tianjin 300050, China
[b] Department of Cardiovascular Pharmacology, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China
[c] Department of Medicinal Chemistry, Institute of Radiation Medicine, Academy of Military Medical Sciences, Beijing 100850, China
[d] Department of Spinal Cord Injury Repair, Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing 100850, China
[e] Thadweik Academy of Medicine, Beijing 100039, China

ARTICLE INFO

Article history:
Received 20 January 2011
Revised 25 March 2011
Accepted 26 March 2011
Available online 2 April 2011

Keywords:
Synthesis
Acetazolamide
Human carbonic anhydrase II
Acute mountain sickness
Hypoxia

ABSTRACT

A series of new sulfamide derivatives have been synthesized, their structures were confirmed by ^{1}H NMR and ESI-MS. Some target compounds were assessed by the tool of Dock6, and inhibition effects of all the new compounds on carbonic anhydrase II have been investigated. In addition, some compounds have been investigated for their antihypoxic effects in mice. Results indicated that nine target compounds exhibit as effectively as acetazolamide and 10 compounds have more potent inhibition effects on carbonic anhydrase II than acetazolamide. Three of them (**I-8**, **I-18** and **I′-3**) can prolong markedly the survival time of mice in hypoxia, which are worth carrying out further studies.

1. Introduction

Acute mountain sickness (AMS) is a common disease at high altitude, its disease rate reaches up to 60% among Chinese people and about 42% at abroad.[1,2] The clinical symptoms are headache, cardiopalmus, dyspnea, anorexia, nausea, acratia, cyanosis and oedema et al. Prevention and treatment of AMS is an important topic. One of the carbonic anhydrase (CA) inhibitors-acetazolamide (AZA) is regarded as an efficient drug to prevent and cure AMS. In America, AZA is the unique medicine to the indication, which was approved by FDA in 1994.[3]

But the common dose is much higher (500 g everyday) and the rate of significant side-effects of AZA for preventing and treating AMS is about 64.1%,[4] the generalization and application of AZA are confined greatly. Therefore, it is important to find a new kind of safe and effective medicine to prevent and cure AMS. It has been discovered that 1,3,4-thiadiazole and sulfamide group in the molecular structure of AZA (Fig. 1) are the chief functional groups of the sulfamide CA II inhibitors.[5] To find more potent and less side-effects drug than AZA, with AZA as a lead compound and 1,3,4-thiadiazole and sulfamide group as pharmacophore, two series of new derivatives have been synthesized and evaluated. The lipophilic groups were introduced manually in order to increase compounds' inhibition effects and selectivity. Assisted by computer-aided drug design, the interactions between 10 compounds and CA II were assessed by flex-docking method. The inhibition effects on CA II of 36 new compounds were investigated, and the antihypoxic effects in mice of 10 compounds were assessed.

2. Results and discussion

2.1. Chemistry

With the 2-(*p*-toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (**3I**) or 2-benzene-sulfonamido-l,3,4-thiadiazole-5-sulfonamide (**3I′**) as a scaffold; we have synthesized a new class of AZA derivatives of such system to be tested against the cytosolic isozymes as well as the AMS-associated CA II.[6,7]

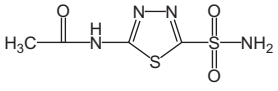
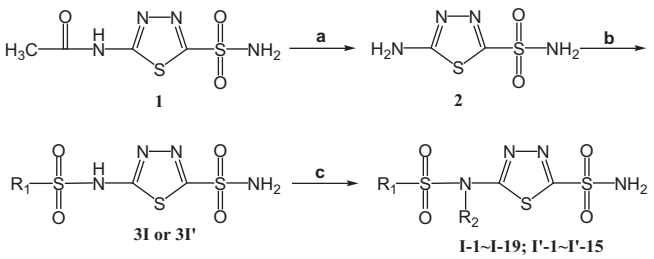


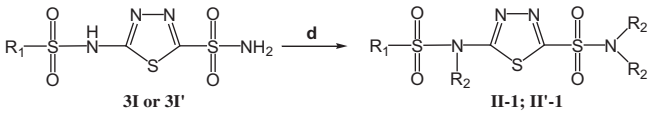
Figure 1. Structure of acetazolamide.

* Corresponding authors. Tel./fax: +86 856 10 6693 2239 (L.W.); tel.: +86 10 6818 6828; fax: +86 10 6693 1557 (H.W.).
E-mail addresses: zhonghai.x@163.com (Z. Xiao), wanglin07@sina.com (L. Wang), wh9588@yahoo.com.cn (H. Wang).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.03.061



Scheme 1. Synthesis routes for target compounds **I-1–I-19** and **I′-1–I′-15**. Reagents and conditions: (a) HCl, ethanol, reflux 4 h; (b) *p*-toluenesulfonyl chloride, acetone, 2.5 mol L^{-1} NaOH aq, 5 mol L^{-1} NaOH aq, stir 30 min at 0–5 °C, HCl, Et_2O; benzenesulfonyl chloride, acetone, 2.5 mol L^{-1} NaOH aq, 5 mol L^{-1} NaOH aq, stir 30 min at room temperature, HCl, Et_2O; (c) **3I** or **3I′**(1 mol), R_2X (X = Br or I, 1 mol), KOH (1 mol), DMF, 60–80 °C (2–4 h).



Scheme 2. Synthesis routes for target compounds **II-1** and **II′-1**. Reagents and conditions: (d) **3I** or **3I′** (1 mol), R_2X (X = Br or I, 3 mol), KOH (3 mol), DMF, 60 °C (2 h).

The synthesis of target compounds **I-1–I-19** and **I′-1–I′-15** is outlined in Scheme 1 and that of **II-1** and **II′-1** is outlined in Scheme 2. The starting compound, 2-amino-l,3,4-thiadiazole-5-sulfonamide (2)[8,9] was prepared according to the method reported by Arslan and Clapp et al. through the reaction between AZA and concentrated HCl in ethanol.

The preparation of the key intermediate, 2-(*p*-toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (**3I**),[10,11] was achieved through the reaction by stirring the 2-amino-l,3,4-thiadiazole-5-sulfonamide (2) with *p*-toluenesulfonyl chloride in alkaline medium for 30 min at 0–5 °C followed by acidification. 2-Benzenesulfonamido-l,3,4-thiadiazole-5-sulfonamide (**3I′**)[10,11] was achieved through the reaction by stirring the 2-amino-l,3,4-thiadiazole-5-sulfonamide (2) with benzenesulfonyl chloride in alkaline medium for 30 min at room temperature followed by acidification.

The target compounds, 2-[(*p*-toluenesulfonamido)-*N*-yl]-l,3,4-thiadiazole-5-sulfonamides **I-1–I-19**, were obtained by refluxing 2-(*p*-toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (**3I**) with different halogenated hydrocarbons in alkaline medium followed by acidification. Similarly, the derivatives 2-(benzenesulfonamido-*N*-yl)-l,3,4-thiadiazole-5-sulfonamides **I′-1–I′-15** were prepared by refluxing 2-benzene-sulfonamido-l,3,4-thiadiazole-5-sulfonamide (**3I′**) with different halogenated hydrocarbons in alkaline medium followed by acidification. The target compounds **II-1** and **II′-1** were prepared by refluxing **3I** and **3I′** with a large excess of different halogenated hydrocarbons and alkali followed by acidification.

2.2. Carbonic anhydrase inhibition

2.2.1. In vitro inhibition

Derivatives **I-1–I-19**, **I′-1–I′-15**, **II-1** and **II′-1** were investigated for their inhibitory effects on hCA II (Table 1). The reported inhibitory data for the potent CA inhibitor AZA was also mentioned for comparison. Because the inhibition rate of AZA was about 50% when the concentration of AZA was 0.3 μmol L^{-1} in the conditions of the experiments, we use the same concentration for all compounds to compare their inhibition effects on hCA II.

The results of in vitro inhibition evaluation for all compounds are shown in Table 1: (i) hCA II was inhibited by compounds **I-1–I-19**, **I′-1–I′-15** and AZA with inhibition rate in the range of 5.75–65.85%. The inhibition rate of AZA was 55.35%. The inhibition effects of ten compounds (**I-5**, **I-7**, **I-12**, **I-17**, **I-18**, **I′-1**, **I′-2**, **I′-3**, **I′-9** and **I′-11**) was higher than that of AZA (P <0.05), which showed higher activities with inhibition rate of 56.22–65.85%. The inhibition effects of nine compounds (**I-3**, **I-4**, **I-8**, **I′-4**, **I′-8**, **I′-10**, **I′-12**, **I′-13** and **I′-14**) equaled to that of AZA (P >0.05), which showed moderate activities with inhibition rate of 52.66–56.04%, while other derivatives have less inhibition effects than AZA. (ii) Derivatives **II-1** and **II′-1** showed inhibition rate of −1.75% and 0.66%, respectively. Compounds **II-1** and **II′-1** had no activities.

2.2.2. In vivo inhibition

Derivatives **I-2**, **I-7**, **I-8**, **I-12**, **I-17**, **I-18**, **I′-1**, **I′-2**, **I′-3** and **I′-8** were investigated for their antihypoxic effects on hypoxic

Table 1
Inhibition data for derivatives **I-1–I-19** and **I′-1–I′-15** and **II-1** and **II′-1** investigated in the present paper and standard sulfonamide CAIs (AZA) on hCA II ($\bar{x} \pm s$)

Compd	R_1	R_2	Inhibition rate/%	Compd	R_1	R_2	Inhibition rate/%
AZA	CH_3-CO–	H	55.35 ± 2.67	AZA	CH_3-CO–	H	55.35 ± 2.67
I-1	4-CH_3-Ph-SO_2–	*n*-C_4H_9–	51.52 ± 1.54	**I-19**	4-CH_3-Ph-SO_2–	2-CH_3O-Ph-(C_3H_6)–	48.76 ± 2.36
I-2	4-CH_3-Ph-SO_2–	Ph-CH_2–	51.83 ± 1.00	**I′-1**	Ph-SO_2–	C_2H_5–	60.83 ± 0.95***
I-3	4-CH_3-Ph-SO_2–	*n*-C_3H_7–	56.04 ± 1.35#	**I′-2**	Ph-SO_2–	*n*-C_4H_9–	60.57 ± 3.56**
I-4	4-CH_3-Ph-SO_2–	CH_3–	52.66 ± 1.82#	**I′-3**	Ph-SO_2–	*n*-C_3H_7–	58.61 ± 0.97***
I-5	4-CH_3-Ph-SO_2–	C_2H_5–	56.22 ± 0.95*	**I′-4**	Ph-SO_2–	Ph-CH_2-CH_2–	55.35 ± 3.03#
I-6	4-CH_3-Ph-SO_2–	*n*-$C_{12}H_{25}$–	38.75 ± 1.93	**I′-5**	Ph-SO_2–	4-Cl-Ph-CH_2–	20.86 ± 16.45
I-7	4-CH_3-Ph-SO_2–	*i*-C_3H_7–	60.17 ± 1.32***	**I′-6**	Ph-SO_2–	2,4-Cl-Ph-CH_2–	48.22 ± 4.10
I-8	4-CH_3-Ph-SO_2–	*i*-C_4H_9–	54.78 ± 1.09#	**I′-7**	Ph-SO_2–	CH_3–	54.62 ± 2.87#
I-9	4-CH_3-Ph-SO_2–	*n*-C_5H_{11}–	50.18 ± 3.15	**I′-8**	Ph-SO_2–	*n*-C_5H_{11}–	55.47 ± 3.68#
I-10	4-CH_3-Ph-SO_2–	4-Cl-Ph-CH_2–	12.79 ± 13.49	**I′-9**	Ph-SO_2–	*i*-C_4H_9–	57.82 ± 0.98*
I-11	4-CH_3-Ph-SO_2–	2,4-Cl-Ph-CH_2–	38.90 ± 2.30	**I′-10**	Ph-SO_2–	*n*-$C_{12}H_{25}$–	53.55 ± 3.15#
I-12	4-CH_3-Ph-SO_2–	Ph-CH_2-CH_2–	65.85 ± 1.80***	**I′-11**	Ph-SO_2–	Ph-CH_2–	58.19 ± 0.93**
I-13	4-CH_3-Ph-SO_2–	4-Br-Ph-CH_2–	5.75 ± 3.55	**I′-12**	Ph-SO_2–	4-CH_3O-Ph-CH_2–	54.57 ± 0.47#
I-14	4-CH_3-Ph-SO_2–	4-CH_3O-Ph-CH_2–	47.19 ± 1.95	**I′-13**	Ph-SO_2–	*i*-C_3H_7–	55.41 ± 1.36#
I-15	4-CH_3-Ph-SO_2–	4-Br-Ph-CH_2-CH_2–	34.77 ± 7.67	**I′-14**	Ph-SO_2–	4-Br-Ph-CH_2-CH_2–	45.75 ± 10.83
I-16	4-CH_3-Ph-SO_2–	3,4,5-CH_3O-Ph-CH_2–	49.44 ± 1.01	**I′-15**	Ph-SO_2–	3,4,5-CH_3O-Ph-CH_2–	50.52 ± 0.94
I-17	4-CH_3-Ph-SO_2–	4-CH_3-Ph-CH_2–	62.30 ± 0.49***	**II-1**	4-CH_3-Ph-SO_2–	CH_3–	−1.75 ± 1.66
I-18	4-CH_3-Ph-SO_2–	4-F-Ph-CH_2–	64.88 ± 1.25***	**II′-1**	Ph-SO_2–	Ph-CH_2–	0.66 ± 1.75

* P <0.05.
** P <0.01.
*** P <0.001.
\# P >0.05 versus AZA.

Table 2
Survival time of mice for some derivatives investigated in the present paper

Compd	Time (s)	Compd	Time (s)
Blank control	2199 ± 217	**I-17**	2512 ± 302$^{\Delta}$
Positive control	2726 ± 530$^{\Delta\Delta}$	**I-18**	2860 ± 392$^{\Delta\Delta,**}$
I-2	2582 ± 426$^{\Delta}$	**I′-1**	2716 ± 444
I-7	2426 ± 385	**I′-2**	2977 ± 322$^{\Delta\Delta}$
I-8	3486 ± 489$^{\Delta\Delta,**}$	**I′-3**	3135 ± 592$^{\Delta\Delta,**}$
I-12	2441 ± 358	**I′-8**	2716 ± 369$^{\Delta\Delta}$

** P <0.01 versus positive control.
$^{\Delta}$ P <0.05.
$^{\Delta\Delta}$ P <0.01 versus blank control.

tolerance in mice. The reported data for AZA was also mentioned for comparison.

The following should be noted regarding data presented in Table 2: The tolerance time of the blank control group was 2199 s, and that of the positive control group was 2726 s. The survival time of the target compound group (**I-2**, **I-8**, **I-17**, **I-18**, **I′-2**, **I′-3** and **I′-8**) and the positive control group (AZA) was all higher than that of the blank control group. The survival time of the target compound group (**I-8**, **I-18** and **I′-3**) was higher than that of the positive control group markedly, and these three compounds were worth carrying out further study.

2.3. Structure–activity relationships

The structure–activity relationships of derivatives **I-1**–**I-19**, **I′-1**–**I′-15**, **II-1** and **II′-1** were analyzed as shown that:

- When R_1 is the same, R_2 different:
  (1) For compounds group **I**: When R_2 is an aryl, the carbon chains connected with nitrogen are much shorter and the *para* orientation substituents are much smaller, the activities are much stronger; there is no activity if the carbon chains are too long or the *para* orientation substituents are too bulky; when R_2 is an alkyl and the lengths of carbon chains are 2–4, the activities are much better than others, the excess long carbon chains are disadvantageous to the compounds' activities.
  (2) For compounds group **I′**: The most compounds have no activity when R_2 is an aryl. The compounds' activities are strong when R_2 is an alkyl, the lengths of carbon chains don't influence the activities.
- When R_2 is the same, R_1 different:
  (1) When R_2 is an alkyl, compounds group **I** and **I′** all have activities, the activities of compounds group **I′** are stronger than that of I, which is consistent with that of CADD.
  (2) When R_2 is an aryl, the activities of compounds group **I** is stronger than that of **I′**, and three compounds' activities are stronger than that of AZA.
- The compound **II-1** and **II′-1** have no activity. All these results demonstrate the sulfamide group R-SO2NH2 is an essential group for inhibition of enzyme activity.

2.4. Docking studies

We tried to predict such activity by docking the synthesized compound **3I**, **3I′**, **I-1**, **I-3**, **I-4**, **I-5**, **I-9**, **I′-1**, **I′-2**, **I′-3**, **I′-8**, **I′-9** and AZA into hCA II active site using the tool of Dock6.[12,13]

The X-ray crystallographic structure of human carbonic anhydrase II complexed with brinzolamide (hCA II PDB: 1A42)[14] was used as the model to discover the inhibitor of catalytic site. The flex-docking procedures were performed for each ligand and CA II. The grid energy of ligand–enzyme complex (E_{grid}), the interaction electrostatic (E_{ele}), and van der Waals (E_{vdw}) energies between the ligand and the enzyme (E_{vdw}) were calculated (Table 3).[15]

Table 3
Grid energy of binding and other interaction energies between ligands and CA II (kcal/mol)

Compound	E_{grid}	E_{vdw}	E_{ele}
Az	−38.29	−23.81	−14.48
3I	−43.95	−28.44	−15.51
I-1	−56.26	−41.38	−14.88
I-2	−52.77	−36.29	−16.48
I-3	−49.29	−38.15	−11.14
I-4	−50.84	−38.63	−12.21
I-7	−52.39	−39.62	−12.77
3I′	−46.57	−29.58	−16.99
I′-1	−50.14	−37.61	−12.53
I′-3	−50.18	−34.56	−15.62
I′-7	−48.03	−30.40	−17.63
I′-11	−51.53	−36.15	−15.38
I′-13	−39.66	−32.50	−7.16

The energy data showed a rough correlation between the binding grid energy (E_{grid}) values of target compounds and their inhibitory effects on hCA II. With the highest activity, the compound **I-1** showed the highest E_{grid} values of −56.26 kcal/mol. Regarding the electrostatic and van der Waals energy values, all derivatives showed high E_{vdw} values indicating the importance of this types of interactions for enzyme binding.

The following notes were observed regarding the docked structures (Fig. 2): (i) The amino group of target compounds replace the hydroxyl ion/water molecule coordinated to zinc atom in the native enzyme. The zinc ion remains in its stable tetrahedral geometry being coordinated, in addition to the amino group, with imidazole nitrogens of His94, His96 and His119. (ii) The Zn–N bond distance is 1.99 Å, which is comparable with the reported data (1.95–2.10 Å).[16] Although there is no clear relationship between the Zn–N bond distance of the docked compounds and their activities, it was noted that compound **I-1** showed the shorter bond distance consistent with its higher inhibitory activity.

3. Conclusion

The synthesis of a new series of 1,3,4-thiadiazole-2-sulfamide derivatives was reported here. The compounds have been tested for their inhibitory effects on hCA II. The results revealed that some derivatives were very effective inhibitors for hCA II. Some compounds have been investigated for their antihypoxic effects, the survival time of mice administered some derivatives was much longer apparently than that of AZA. In addition, the binding mode of the tested compounds inside the hCA II active site was predicted using a docking technique initially. And structure–activity relationship study is useful theoretical and experimental evidence to optimize the compound's structure and find new drugs for the prevention and treatment of AMS.

4. Experimental

4.1. General

Melting points were recorded in open capillaries with electrothermal melting point apparatus (RY-1, China) and are uncorrected. ^{1}H NMR (400 MHz) spectra were recorded on 'JNM-ECA-400' (Japan) with DMSO-d_6 or $CDCl_3$ as a solvent, TMS as an internal reference and the chemical shifts are expressed in parts

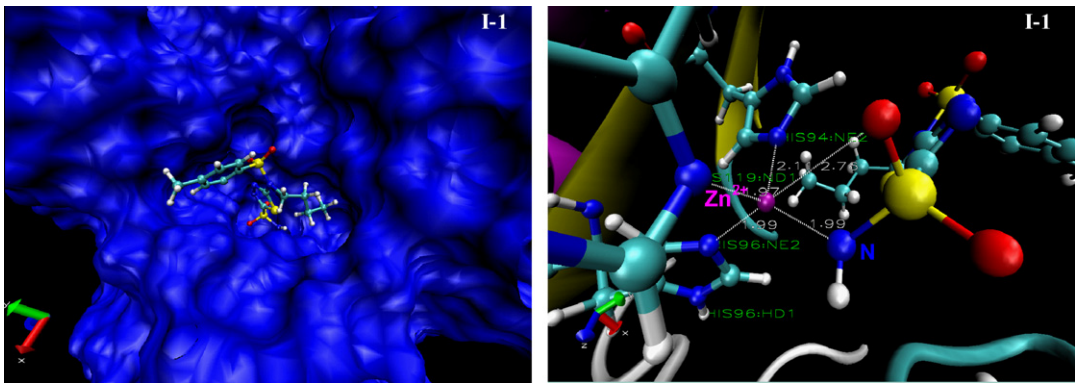


Figure 2. 3D docked structure of **I-1** (ball and stick) at hCA II active site. The purple ball is represented catalytically critical Zn(II), and the Zn–N (–SO_2NH_2) bond distance is 1.99 Å. The figure has been generated by using 'Linux' operating system installed on the Xeon with a 2.6 MHz processor and 1G RAM. The X-ray crystallographic structure of hCA II complexed with brinzolamide (hCA II PDB: 1A42) was obtained from the Protein Data Bank.

per million (δ, ppm). Mass spectra (MS) were performed on 'LCQ Advantage MAX 10 spectrometer' (Finnigan, America). Elemental analyses were performed on 'Carlo Erbal 106' (Italy). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 GF_{245} precoated sheets 20 × 20 cm, layer thickness 0.2 mm (Qing Dao Hai Yang, China) and were visualized by UV-lamp at wavelength (λ) 254 nm. All chemicals and solvents were analytical reagents, and the solvents were distilled before use.

4.2. Chemistry

4.2.1. Synthesis of 2-amino-l,3,4-thiadiazole-5-sulfonamide (2)[8,9]

A mixture of AZA (12.15 g, 0.055 mol), 12 mL of concentrated HC1 and 180 mL of anhydrous ethanol was heated in 80 °C oil bath for 4 h. After a part of ethanol had evaporated, the obtained suspension was allowed to cool slowly. The solid was filtered and crystallized from water to give the analytical sample (86.0%); mp: 216–219 °C; ^{1}H NMR (DMSO-d_6) δ: 7.81 (s, 2H, H_2N-thiadiazole), 8.05 (s, 2H, –SO_2NH_2); ESI-MS, *m/z*: $[M+H]^+$ (calcd) 180.90 (180.98), $[M-H]^-$ (calcd) 178.88 (178.98).

4.2.2. Synthesis of 2-(*p*-toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (3I)[10,11]

With stirring at 0–5 °C, 2 mL 5 mol L^{-1} sodium hydroxide (0.01 mol) and a solution of *p*-toluenesulfonyl chloride (2.28 g, 0.012 mol) in 3 mL of acetone were added simultaneously to a solution of 2-amino-l,3,4-thiadiazole-5-sulfonamide (1.80 g, 0.01 mol) in 4 mL 2.5 mol L^{-1} sodium hydroxide (0.01 mol). The reaction mixture, which became quite warm due to exothermic heat of reaction, was recooled to 0–5 °C and stirred for an additional 30 min. The solution was then extracted with 15 mL of ether and the aqueous phase was separated, treated with activated charcoal, filtered and acidified with concentrated hydrochloric acid to pH 3. On cooling and stirring, the product was separated slowly from this solution as a finely divided white solid, the crude product was recrystallized from 80 mL of hot water to give the white floccular crystal. Mp: 254–255 °C (0.70 g, 10.6%). ^{1}H NMR (DMSO-d_6) δ: 2.38(s, 3H, –CH_3), 7.38–7.40 (d, 2H; *J* = 8.1 Hz; H-3, 5 of phenyl ring), 7.71–7.73 (d, 2H; *J* = 8.4 Hz; H-2, 6 of phenyl ring), 8.50 (s, 2H, –SO_2NH_2), 13.40 (s, 1H, *H*N-thiadiazole). Anal. Calcd for $C_9H_{10}N_4O_4S_3$: C, 32.33; H, 3.01; N, 16.75. Found: C, 32.52; H, 2.86; N, 17.04. ESI-MS, *m/z*: $[M-H]^-$ (calcd) 332.81 (332.99).

4.2.3. Synthesis of 2-benzenesulfonamido-l,3,4-thiadiazole-5-sulfonamide (3I′)[10,11]

The procedure was same to the above except that a reaction temperature of room temperature was used and *p*-toluenesulfonyl chloride was replaced by benzenesulfonyl chloride (2.10 g, 0.012 mol). The crude product was recrystallized from 60 mL of hot water to give the white floccular crystal. Mp: 230–232 °C (0.27 g, 15.5%). Anal. Calcd for $C_8H_8N_4O_4S_3$: C, 29.99; H, 2.52; N, 17.49. Found: C, 30.10; H, 2.36; N, 18.02. ESI-MS, *m/z*: $[M-H]^-$ (calcd) 318.80 (318.97).

4.2.4. General procedure for the synthesis of 2-[(*p*-toluenesulfonamido)-*N*-yl]-l,3,4-thiadiazole-5-sulfonamides (I-1–I-19)

2-(*p*-Toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (**3I**) (0.16 g, 0.5 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF, 1 mL) with stirring at room temperature. Potassium hydroxide (0.03 g, 0.5 mmol) was suspended in DMF (1 mL) then added to the above solution, potassium hydroxide was dissolved slowly. Then a mixture of halogenated hydrocarbons (0.6 mmol) and DMF (1 mL) was added to the reaction mixture and heated to 60–80 °C in an oil-bath for 1–3 h (controlled by TLC). The solvent was evaporated to dryness in vacuum, the residue was flaxen solid. The compound **I-1–I-19** was obtained by chromatographic fractionation.

4.2.4.1. 2-[(*p*-Toluenesulfonamido)-*N*-(*n*-butyl)]-l,3,4-thiadiazole-5-sulfonamide (I-1). Yield, 51.3%; mp: 136–138 °C; ^{1}H NMR ($CDCl_3$) δ: 0.85–0.89 (t, 3H; *J* = 7.3 Hz; CH_3–CH_2–CH_2–CH_2–N<), 1.23–1.29 (m, 2H; *J* = 7.6 Hz; CH_3–CH_2–CH_2–CH_2–N<), 1.70–1.75 (m, 2H; *J* = 7.6 Hz; CH_3–CH_2–CH_2–CH_2–N<), 2.42 (s, 3H, CH_3–Ph-SO_2–), 4.13–4.17 (t, 2H; *J* = 7.3 Hz; CH_3–CH_2–CH_2–CH_2–N<), 5.69 (s, 2H, –SO_2–NH_2), 7.29–7.31 (d, 2H; *J* = 7.9 Hz; H-3, 5 of phenyl ring), 7.76–7.78 (d, 2H; *J* = 8.4 Hz; H-2, 6 of phenyl ring). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 391.04 (391.06), $[M-H]^-$ (calcd) 388.97 (389.04).

4.2.4.2. 2-[(*p*-Toluenesulfonamido)-*N*-benzyl]-l,3,4-thiadiazole-5-sulfonamide (I-2). Yield, 70.8%; mp: 179–181 °C; ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3-Ph-SO_2-), 5.34 (s, 2H, Ph-CH_2-N<), 7.19–7.22 (m, 2H; *J* = 7.6 Hz; *Ph*-CH_2-N<), 7.29–7.32 (m, 3H; *J* = 6.8 Hz; *Ph*-CH_2-N<), 7.36–7.38 (d, 2H; *J* = 8.9 Hz; CH_3-*Ph*-SO_2-), 7.68–7.70 (d, 2H; *J* = 8.4 Hz; CH_3-*Ph*-SO_2-), 8.61 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 424.94 (425.04), $[M-H]^-$ (calcd) 422.93 (423.03). Anal. Calcd for $C_{16}H_{16}N_4O_4S_3$: C, 45.27; H, 3.80; N, 13.20. Found: C, 45.54; H, 3.50; N, 13.24.

4.2.4.3. 2-[(*p*-Toluenesulfonamido)-*N*-(*n*-propyl)]-l,3,4-thiadiazole-5-sulfonamide (I-3). Yield, 71.8%; mp: 161–163 °C; ^{1}H NMR ($CDCl_3$) δ: 0.86–0.90 (t, 3H; *J* = 7.4 Hz; CH_3–CH_2–CH_2–N<), 1.77–1.80 (m, 2H; *J* = 7.4 Hz; CH_3–CH_2–CH_2–N<), 2.42 (s, 3H, CH_3-Ph-SO_2-), 4.10–4.14 (t, 2H; *J* = 7.4 Hz; CH_3–CH_2–CH_2–N<), 5.69 (s,

2H, $-SO_2-NH_2$), 7.29–7.31 (d, 2H; J = 8.1 Hz; H-3, 5 of phenyl ring), 7.76–7.78 (d, 2H; J = 8.1 Hz; H-2, 6 of phenyl ring). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 377.01 (377.04), $[M-H]^-$ (calcd) 374.94 (375.03).

4.2.4.4. 2-[(*p*-Toluenesulfonamido)-*N*-methyl]-l,3,4-thiadiazole-5-sulfonamide (I-4). Yield, 69.0%; mp: 205–208 °C; ^1H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3-Ph-SO_2-), 3.71 (s, 3H, CH_3-N<), 7.39–7.41 (d, 2H; J = 8.4 Hz; H-3, 5 of phenyl ring), 7.74–7.76 (d, 2H; J = 8.1 Hz; H-2, 6 of phenyl ring), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 348.97 (349.01), $[M-H]^-$ (calcd) 346.91 (346.99).

4.2.4.5. 2-[(*p*-Toluenesulfonamido)-*N*-ethyl]-l,3,4-thiadiazole-5-sulfonamide (I-5). Yield, 60.8%; mp: 200–203 °C; ^1H NMR (DMSO-d_6) δ: 1.24–1.28 (t, 3H; J = 7.3 Hz; CH_3-CH_2-N<), 2.38 (s, 3H, CH_3-Ph-SO_2-), 4.10–4.16 (q, 2H; J = 7.3 Hz; CH_3–CH_2–N<), 7.39–7.41 (d, 2H; J = 8.1 Hz; H-3, 5 of phenyl ring), 7.74–7.76 (d, 2H; J = 8.1 Hz; H-2, 6 of phenyl ring), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 363.02 (363.03), $[M-H]^-$ (calcd) 360.92 (361.01).

4.2.4.6. 2-[(*p*-Toluenesulfonamido)-*N*-(*n*-lauryl)]-l,3,4-thiadiazole-5-sulfonamide (I-6). Yield, 26.1%; mp: 96–99 °C; ^1H NMR ($CDCl_3$) δ: 0.86–0.90 (t, 3H; J = 6.8 Hz; CH_3-$(CH_2)_9$-CH_2-CH_2-N<), 1.20–1.30 (m, 18H, CH_3-$(CH_2)_9$-CH_2-CH_2-N<), 1.72–1.75 (m, 2H; J = 6.8 Hz; CH_3-$(CH_2)_9$-CH_2-CH_2-N<), 2.42 (s, 3H, CH_3-Ph-SO_2-), 4.12–4.16 (t, 2H; J = 7.3 Hz; CH_3-$(CH_2)_9$-CH_2-CH_2-N<), 5.81 (s, 2H, $-SO_2-NH_2$), 7.28–7.30 (d, 2H; J = 8.1 Hz; H-3, 5 of phenyl ring), 7.75–7.77 (d, 2H; J = 8.1 Hz; H-2, 6 of phenyl ring). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 503.15 (503.18), $[M-H]^-$ (calcd) 501.05 (501.17).

4.2.4.7. 2-[(*p*-Toluenesulfonamido)-*N*-(*i*-propyl)]-l,3,4-thiadiazole-5-sulfonamide (I-7). Yield, 45.2%; mp: 186–188 °C; ^1H NMR (DMSO-d_6) δ: 1.32–1.33 (d, 6H; J = 6.7 Hz; $(CH_3)_2$-CH-N<), 2.38 (s, 3H, CH_3-Ph-SO_2-), 4.77–4.81 (m, 1H; J = 6.6 Hz; $(CH_3)_2$-CH-N<), 7.39–7.41 (d, 2H; J = 8.1 Hz; H-3, 5 of phenyl ring), 7.74–7.76 (d, 2H; J = 8.4 Hz; H-2, 6 of phenyl ring), 8.57 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 376.98 (377.04), $[M-H]^-$ (calcd) 374.93 (375.03).

4.2.4.8. 2-[(*p*-Toluenesulfonamido)-*N*-(*i*-butyl)]-l,3,4-thiadiazole-5-sulfonamide (I-8). Yield, 51.3%; mp: 182–184 °C; ^1H NMR (DMSO-d_6) δ: 0.79–0.81 (d, 6H; J = 6.7 Hz; $(CH_3)_2$-CH-CH_2-N<), 2.03–2.10 (m, 1H; J = 6.9 Hz; $(CH_3)_2$-CH-CH_2-N<), 2.38 (s, 3H, CH_3-Ph-SO_2-), 3.95–3.97 (d, 2H; J = 7.0 Hz; $(CH_3)_2$-CH-CH_2-N<), 7.38–7.40 (d, 2H; J = 8.1 Hz; H-3, 5 of phenyl ring), 7.72–7.74 (d, 2H; J = 8.4 Hz; H-2, 6 of phenyl ring), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 391.02 (391.06), $[M-H]^-$ (calcd) 388.94 (389.04).

4.2.4.9. 2-[(*p*-Toluenesulfonamido)-*N*-(*n*-pentyl)]-l,3,4-thiadiazole-5-sulfonamide (I-9). Yield, 59.4%; mp: 149–152 °C; ^1H NMR (DMSO-d_6) δ: 0.73–0.77 (t, 3H; J = 7.3 Hz; CH_3–CH_2–CH_2–CH_2–CH_2–N<), 1.08–1.22 (m, 4H, CH_3–CH_2–CH_2–CH_2–CH_2–N<), 1.64–1.70 (m, 2H; J = 7.2 Hz; CH_3–CH_2–CH_2–CH_2–CH_2–N<), 2.38 (s, 3H, CH_3-Ph-SO_2-), 4.09–4.13 (t, 2H; J = 6.9 Hz; CH_3–CH_2–CH_2–CH_2–CH_2–N<), 7.38–7.40 (d, 2H; J = 7.8 Hz; H-3, 5 of phenyl ring), 7.72–7.74 (d, 2H; J = 8.4 Hz; H-2, 6 of phenyl ring), 8.58(s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 405.04 (405.07), $[M-H]^-$ (calcd) 402.96 (403.06).

4.2.4.10. 2-[(*p*-Toluenesulfonamido)-*N*-(4-chlorobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-10). Yield, 44.8%; mp: 220–224 °C; ^1H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3-Ph-SO_2-), 5.34 (s, 2H, Cl-Ph-CH_2-), 7.22–7.24 (d, 2H; J = 8.7 Hz; H-3, 5 of Cl-*Ph*-CH_2-), 7.34–7.36 (d, 2H; J = 8.7 Hz; H-2, 6 of Cl-*Ph*-CH_2-), 7.35–7.37 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.65–7.67 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 458.93 (459.00), $[M-H]^-$ (calcd) 456.91 (456.99).

4.2.4.11. 2-[(*p*-Toluenesulfonamido)-*N*-(2,4-dichlorobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-11). Yield, 69.0%; mp: 125–126 °C; ^1H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3-Ph-SO_2-), 5.39 (s, 2H, $(Cl)_2$-Ph-CH_2-), 7.30–7.32 (d, 2H, H-5, 6 of $(Cl)_2$-*Ph*-CH_2-), 7.36–7.66 (m, 4H, CH_3-*Ph*-SO_2-), 7.95 (s, 1H, H-3 of $(Cl)_2$-*Ph*-CH_2-), 8.60 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 492.86 (492.96), $[M-H]^-$ (calcd) 490.87 (490.95).

4.2.4.12. 2-[(*p*-Toluenesulfonamido)-*N*-phenethyl]-l,3,4-thiadiazole-5-sulfonamide (I-12). Yield, 87.6%; mp: 148–150 °C; ^1H NMR (DMSO-d_6) δ: 2.40 (s, 3H, CH_3-Ph-SO_2-), 3.00–3.03 (t, 2H; J = 7.0 Hz; Ph-CH_2-CH_2-N<), 4.36–4.39 (t, 2H; J = 7.0 Hz; Ph-CH_2-CH_2-N<), 7.04–7.16 (m, 5H, *Ph*-CH_2-CH_2-N<), 7.38–7.40 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.62–7.64 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.61 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 439.02 (439.06), $[M-H]^-$ (calcd) 436.94 (437.04).

4.2.4.13. 2-[(*p*-Toluenesulfonamido)-*N*-(4-bromobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-13). Yield, 80.3%; mp: 235–238 °C; ^1H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3-Ph-SO_2-), 5.32 (s, 2H, Br-Ph-CH_2-N<), 7.15–7.37 (m, 4H, Br-*Ph*-CH_2-N<), 7.48–7.66 (m, 4H, CH_3-*Ph*-SO_2-), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 502.85 (502.95), $[M-H]^-$ (calcd) 500.85 (500.94).

4.2.4.14. 2-[(*p*-Toluenesulfonamido)-*N*-(4-methoxylbenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-14). Yield, 66.7%; mp: 225–227 °C; ^1H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3-Ph-SO_2-), 3.72 (s, 3H, CH_3O-Ph-CH_2-N<), 5.26 (s, 2H, CH_3O-Ph-CH_2-N<), 6.83–6.85 (d, 2H; J = 8.7 Hz; H-3, 5 of CH_3O-*Ph*-SO_2-), 7.15–7.17 (d, 2H; J = 8.7 Hz; H-2, 6 of CH_3O-*Ph*-SO_2-), 7.37–7.39 (d, 2H; J = 8.4 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.70–7.71 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.59 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 454.95 (455.05), $[M-H]^-$ (calcd) 452.95 (453.04).

4.2.4.15. 2-[(*p*-Toluenesulfonamido)-*N*-(4-bromophenethyl)]-l,3,4-thiadiazole-5-sulfonamide (I-15). Yield, 65.9%; mp: 195–198 °C; ^1H NMR (DMSO-d_6) δ: 2.41 (s, 3H, CH_3-Ph-SO_2-), 2.99–3.03 (t, 2H; J = 6.8 Hz; Br-Ph-CH_2–CH_2–N<), 4.38–4.41 (t, 2H; J = 6.8 Hz; Br-Ph-CH_2–CH_2–N<), 6.99–7.01 (d, 2H; J = 8.4 Hz; H-3, 5 of Br-*Ph*-CH_2–CH_2–N<), 7.27–7.29 (d, 2H; J = 8.4 Hz; H-2, 6 of Br-*Ph*-CH_2–CH_2–N<), 7.39–7.41 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2–), 7.61–7.63 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.61 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 516.90(516.97), $[M-H]^-$ (calcd) 514.88(514.95).

4.2.4.16. 2-[(*p*-Toluenesulfonamido)-*N*-(3,4,5-trimethoxylbenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-16). Yield, 85.6%; mp: 82–85 °C; ^1H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3-Ph-SO_2-), 3.57 (s, 9H, $(CH_3O)_3$-Ph-CH_2-N<), 5.26 (s, 2H, $(CH_3O)_3$-Ph-CH_2-N<), 6.51 (s, 2H, H-2, 6 of $(CH_3O)_3$-*Ph*-CH_2-N<), 7.36–7.38 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.72–7.74 (d, 2H; J = 8.4 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.61 (s, 2H, $-SO_2-NH_2$). ESI-MS, *m/z*: $[M+Na]^+$ (calcd) 536.96 (537.05), $[M-H]^-$ (calcd) 512.97 (513.06).

4.2.4.17. 2-[(*p*-Toluenesulfonamido)-*N*-(4-methylbenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-17). Yield, 77.1%; mp: 192–195 °C; ^1H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3-Ph-CH_2-N<), 2.39 (s, 3H, CH_3-Ph-SO_2-), 5.29 (s, 2H, CH_3-Ph-CH_2-N<), 7.09 (s, 4H, CH_3-*Ph*-CH_2-N<), 7.36–7.38 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.68–7.70 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.59

(s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 438.92 (439.06), $[M-H]^-$ (calcd) 436.95 (437.04).

4.2.4.18. 2-[(*p*-Toluenesulfonamido)-*N*-(4-fluorobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I-18). Yield, 58.8%; mp: 199–201 °C; ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3-Ph-SO_2-), 5.33 (s, 2H, F-Ph-CH_2-N<), 7.11–7.16 (t, 2H; *J* = 8.8 Hz; H-3, 5 of F-*Ph*-CH_2-N<), 7.26–7.29 (q, 2H, H-2, 6 of F-*Ph*-CH_2-N<), 7.36–7.38 (d, 2H; *J* = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.68–7.70 (d, 2H; *J* = 7.9 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 442.97 (443.03), $[M-H]^-$ (calcd) 440.94 (441.02).

4.2.4.19. 2-[(*p*-Toluenesulfonamido)-*N*-(2-methoxylphenylpropyl)]-l,3,4-thiadiazole-5-sulfonamide (I-19). Yield, 55.6%; mp: 111–113 °C; ^{1}H NMR (DMSO-d_6) δ: 1.91–1.95 (m, 2H; *J* = 7.4 Hz; CH_3O-Ph-CH_2-CH_2-CH_2-N<), 2.36 (s, 3H, CH_3-Ph-SO_2-), 3.71 (s, 3H, CH_3O-Ph-CH_2-CH_2-CH_2-N<), 4.09–4.13 (t, 2H; *J* = 7.0 Hz; CH_3O-Ph-CH_2-CH_2-CH_2-N<), 6.78–7.17 (m, 4H, CH_3O-*Ph*-CH_2-CH_2-CH_2-N<), 7.37–7.39 (d, 2H; *J* = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.72–7.74 (d, 2H; *J* = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-), 8.58 (s, 2H, –SO_2–NH_2). ^{1}H NMR ($CDCl_3$) δ: 2.04–2.11 (m, 2H; *J* = 7.3 Hz; CH_3O-Ph-CH_2-CH_2-CH_2-N<), 2.40 (s, 3H, CH_3-Ph-SO_2-), 2.57–2.61 (t, 2H; *J* = 7.4 Hz; CH_3O-Ph-CH_2-CH_2-CH_2-N<), 4.15–4.18 (t, 2H; *J* = 7.1 Hz; CH_3O-Ph-CH_2-CH_2-CH_2-N<), 5.61 (s, 2H, –SO_2–NH_2), 6.80–7.20 (m, 4H, CH_3O-*Ph*-CH_2-CH_2-CH_2-N<), 7.26–7.28 (d, 2H; *J* = 7.5 Hz; H-3, 5 of CH_3-*Ph*-SO_2–), 7.75–7.77 (d, 2H; *J* = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2–). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 483.07 (483.08), $[M-H]^-$ (calcd) 481.02 (481.07).

4.2.5. General procedure for the synthesis of 2-(benzenesulfonamido-*N*-yl)-l,3,4-thiadiazole-5-sulfonamides (I′-1–I′-15)

0.16 g of 2-benzenesulfonamido-l,3,4-thiadiazole-5-sulfonamide (**3I′**) (0.5 mmol) was dissolved into DMF (1 mL) with stirring at room temperature. The suspending liquid of 0.03 g of potassium hydroxide (0.5 mmol) and 1 mL of DMF was added to the above solutions, potassium hydroxide was dissolved slowly. Then a mixture of halogenated hydrocarbons (0.6 mmol) and 1 mL of DMF was added to the reaction mixture and heated to 60–80 °C in an oil-bath for 1–4 h (monitored by TLC). The solvent was evaporated to dryness in vacuum, the residue was flaxen solid. The compound **I′-1–I′-15** was obtained by chromatographic fractionation.

4.2.5.1. 2-(Benzenesulfonamido-*N*-ethyl)-l,3,4-thiadiazole-5-sulfonamide (I′-1). Yield, 74.6%; mp: 152–155 °C; ^{1}H NMR (DMSO-d_6) δ: 1.25–1.28 (t, 3H; *J* = 7.2 Hz; CH_3–CH_2–N<), 4.12–4.18 (q, 2H; *J* = 7.3 Hz; CH_3–CH_2–N<), 7.58–7.88 (m, 5H, *Ph*-SO_2-), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 349.01(349.01), $[M-H]^-$ (calcd) 346.93 (346.99). Anal. Calcd for $C_{10}H_{12}N_4O_4S_3$: C, 34.47; H, 3.47; N, 16.08. Found: C, 35.37; H, 3.44; N, 16.26.

4.2.5.2. 2-[Benzenesulfonamido-*N*-(*n*-butyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-2). Yield, 83.9%; mp: 178–181 °C; ^{1}H NMR (DMSO-d_6) δ: 0.76–0.80 (t, 3H; *J* = 7.3 Hz; CH_3–CH_2–CH_2–CH_2–N<), 1.16–1.17 (m, 2H; *J* = 7.3 Hz; CH_3–CH_2–CH_2–CH_2–N<), 1.63–1.67 (m, 2H; *J* = 7.2 Hz; CH_3–CH_2–CH_2–CH_2–N<), 4.11–4.15 (t, 2H; *J* = 6.9 Hz; CH_3–CH_2–CH_2–CH_2–N<), 7.58–7.87 (m, 5H, *Ph*-SO_2–), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 377.03 (377.04), $[M-H]^-$ (calcd) 374.95 (375.03).

4.2.5.3. 2-[Benzenesulfonamido-*N*-(*n*-propyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-3). Yield, 68.8%; mp: 150–152 °C; ^{1}H NMR (DMSO-d_6) δ: 0.78–0.80 (t, 3H; *J* = 3.6 Hz; CH_3–CH_2–CH_2–N<), 1.67–1.73 (m, 2H; *J* = 7.2 Hz; CH_3–CH_2–CH_2–N<), 4.08–4.12 (t, 2H; *J* = 6.8 Hz; CH_3–CH_2–CH_2–N<), 7.39–7.87 (m, 5H, *Ph*-SO_2-), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 363.00 (363.03), $[M-H]^-$ (calcd) 360.92 (361.01).

4.2.5.4. 2-(Benzenesulfonamido-*N*-phenethyl)-l,3,4-thiadiazole-5-sulfonamide (I′-4). Yield, 78.1%; mp: 152–154 °C; ^{1}H NMR (DMSO-d_6) δ: 3.00–3.04 (t, 2H; *J* = 7.0 Hz; Ph-CH_2–CH_2–N<), 4.37–4.41 (t, 2H; *J* = 7.2 Hz; Ph-CH_2–CH_2–N<), 7.02–7.15 (m, 5H, *Ph*-CH_2–CH_2–N<), 7.57–7.76 (m, 5H, *Ph*-SO_2–), 8.61 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 425.01 (425.04), $[M-H]^-$ (calcd) 422.94 (423.03).

4.2.5.5. 2-[Benzenesulfonamido-*N*-(4-chlorobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-5). Yield, 74.6%; mp: 198–199 °C; ^{1}H NMR (DMSO-d_6) δ: 5.36 (s, 2H, Cl-Ph-CH_2–N<), 7.23–7.25 (m, 2H; *J* = 9.0 Hz; H-3, 5 of Cl-*Ph*-CH_2–N<), 7.34–7.36 (m, 2H; *J* = 9.0 Hz; H-2, 6 of Cl-*Ph*-CH_2–N<), 7.55–7.80 (m, 5H, *Ph*-SO_2-), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 444.88 (444.99), $[M-H]^-$ (calcd) 442.87 (442.97).

4.2.5.6. 2-[Benzenesulfonamido-*N*-(2,4-dichlorobenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-6). Yield, 50.6%; mp: 125–128 °C; ^{1}H NMR (DMSO-d_6) δ: 5.40 (s, 2H, $(Cl)_2$-Ph-CH_2–N<), 7.32–7.56 (m, 3H, $(Cl)_2$-*Ph*-CH_2–N<), 7.58–7.79 (m, 5H, *Ph*-SO_2–), 8.59 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 478.84 (478.95), $[M-H]^-$ (calcd) 476.86 (476.93).

4.2.5.7. 2-(Benzenesulfonamido-*N*-methyl)-l,3,4-thiadiazole-5-sulfonamide (I′-7). Yield, 89.5%; mp: 194–196 °C; ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H, CH_3-N<), 7.58–7.89 (m, 5H, *Ph*-SO_2–), 8.61 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 334.95 (334.99), $[M-H]^-$ (calcd) 332.90 (332.98).

4.2.5.8. 2-[Benzenesulfonamido-*N*-(*n*-pentyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-8). Yield, 66.7%; mp: 137–139 °C; ^{1}H NMR (DMSO-d_6) δ: 0.72–0.76 (t, 3H, *J* = 7.2 Hz; CH_3–$(CH_2)_2$–CH_2–CH_2–N<), 1.09–1.23 (m, 4H, *J* = 7.4 Hz; CH_3–C$H_2)_2$–CH_2–CH_2–N<), 1.63–1.70 (m, 2H, *J* = 7.2 Hz; CH_3–$(CH_2)_2$–CH_2–CH_2–N<), 4.11–4.14 (t, 2H, *J* = 6.8 Hz; CH_3–$(CH_2)_2$–CH_2–CH_2–N<), 7.58–7.87 (m, 5H, *Ph*-SO_2–), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 391.01 (391.06), $[M-H]^-$ (calcd) 388.94 (389.04).

4.2.5.9. 2-[Benzenesulfonamido-*N*-(*i*-butyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-9). Yield, 58.2%; mp: 125–130 °C; ^{1}H NMR (DMSO-d_6) δ: 0.78–0.80 (d, 6H, *J* = 6.7 Hz; $(CH_3)_2$–CH–CH_2–N<), 2.02–2.09 (m, 1H, *J* = 6.8 Hz; $(CH_3)_2$–C*H*–CH_2–N<), 3.96–3.98 (d, 2H, *J* = 7.3 Hz; $(CH_3)_2$–CH–CH_2–N<), 7.58–7.87 (m, 5H, *Ph*-SO_2-), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 376.99 (377.04), $[M-H]^-$ (calcd) 374.92 (375.03).

4.2.5.10. 2-[Benzenesulfonamido-*N*-(*n*-Lauryl)]-l,3,4-thiadiazole-5-sulfonamide (I′-10). Yield, 41.0%; mp: 92–94 °C; ^{1}H NMR (DMSO-d_6) δ: 0.84–0.87 (t, 3H, *J* = 6.8 Hz; CH_3–$(CH_2)_9$–CH_2–CH_2–N<), 1.13–1.27 (m, 18H, CH_3–C$H_2)_9$–CH_2–CH_2–N<), 1.64–1.67 (m, 2H, *J* = 6.6 Hz; CH_3–$(CH_2)_9$–CH_2–CH_2–N<), 4.10–4.14 (t, 2H, *J* = 6.8 Hz; CH_3–$(CH_2)_9$–CH_2–CH_2–N<), 7.57–7.86 (m, 5H, *Ph*-SO_2–), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: Target $[M+H]^+$ (calcd) 489.12 (489.17), $[M-H]^-$ (calcd) 487.00 (487.15).

4.2.5.11. 2-(Benzenesulfonamido-*N*-benzyl)-l,3,4-thiadiazole-5-sulfonamide (I′-11). Yield, 50.2%; mp: 147–150 °C; ^{1}H NMR (DMSO-d_6) δ: 5.36 (s, 2H, Ph-CH_2–N<), 7.19–7.32 (m, 5H, *Ph*-CH_2–N<), 7.56–7.82 (m, 5H, *Ph*-SO_2–), 8.61 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 411.01 (411.03), $[M-H]^-$ (calcd) 408.92 (409.01).

4.2.5.12. 2-[Benzenesulfonamido-*N*-(4-methoxylbenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-12). Yield, 63.6%; mp: 188–191 °C; ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H, CH_3O-Ph-CH_2–N<), 5.27 (s, 2H, CH_3O-Ph-CH_2–N<), 6.82–6.84 (d, 2H, J = 8.7 Hz; H-3, 5 of CH_3O-*Ph*-CH_2–N<), 7.15–7.18 (d, 2H, J = 8.7 Hz; H-2, 6 of CH_3O-*Ph*-CH_2–N<), 7.57–7.83 (m, 5H, *Ph*-SO_2–), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 440.73 (441.04), $[M-H]^-$ (calcd) 438.93 (439.02).

4.2.5.13. 2-[Benzenesulfonamido-*N*-(*i*-propyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-13). Yield, 67.3%; mp: 163–166 °C; ^{1}H NMR (DMSO-d_6) δ: 1.32–1.34 (d, 6H, J = 6.4 Hz; (CH_3)$_2$–CH–N<), 4.77–4.84 (m, 1H, J = 6.6 Hz; (CH_3)$_2$–C*H*–N<), 7.59–7.88 (m, 5H, *Ph*-SO_2–), 8.60 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 362.98 (363.03), $[M-H]^-$ (calcd) 360.94 (361.01).

4.2.5.14. 2-[Benzenesulfonamido-*N*-(4-bromophenethyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-14). Yield, 71.7%; mp: 193–195 °C; ^{1}H NMR (DMSO-d_6) δ: 2.99–3.03 (t, 2H, J = 6.8 Hz; Br-Ph-CH_2–CH_2–N<), 4.38–4.42 (t, 2H, J = 6.8 Hz; Br-Ph-CH_2–CH_2–N<), 6.99–7.01 (d, 2H, J = 8.1 Hz; H-3, 5 of Br-*Ph*-CH_2–CH_2–N<), 7.29–7.31 (m, 2H, J = 8.4 Hz; H-2, 6 of Br-*Ph*-CH_2–CH_2–N<), 7.58–7.76 (m, 5H, *Ph*-SO_2–), 8.63 (s, 2H, –SO_2–NH_2). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 502.90 (502.95), $[M-H]^-$ (calcd) 500.85 (500.94).

4.2.5.15. 2-[Benzenesulfonamido-*N*-(3,4,5-trimethoxylbenzyl)]-l,3,4-thiadiazole-5-sulfonamide (I′-15). Yield, 69.1%; mp: 84–88 °C; ^{1}H NMR (DMSO-d_6) δ: 3.58 (s, 9H, (CH_3O)$_3$-Ph-CH_2–N<), 5.27 (s, 2H, (CH_3O)$_3$-Ph-CH_2-N<), 6.53 (s, 2H, H-2, 6 of (CH_3O)$_3$-*Ph*-CH_2-N<), 7.55–7.86 (m, 5H, *Ph*-SO_2-), 8.61 (s, 2H, –SO_2-NH_2). ESI-MS, *m/z*: $[M+Na]^+$ (calcd) 522.97 (523.04), $[M-H]^-$ (calcd) 498.94 (499.04).

4.2.6. Synthesis of 2-[(*p*-toluenesulfonamido)-*N*-methyl]-5-[*N*,*N*-dimethylsulfonamide]-l,3,4-thiadiazole (II-1)

2-(*p*-Toluenesulfonamido)-l,3,4-thiadiazole-5-sulfonamide (**3I**) (0.16 g, 0.5 mmol) was dissolved in 1 mL DMF with stirring at room temperature. Potassium hydroxide (0.10 g, 1.5 mmol) was suspended in DMF (1 mL) then added to the above solution, potassium hydroxide was dissolved slowly. Then a mixture of methyl iodide (1.8 mmol) and DMF (1 mL) was added to the reaction mixture for 1 h at room temperature (monitored by TLC). The solvent was evaporated to dryness in vacuum, the residue was flaxen solid. The compound **II-1** was obtained by chromatographic fractionation. Yield, 74.5%; mp: 172–175 °C; ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3-Ph-SO_2-), 2.90 (s, 6H, (CH_3)$_2$-N-), 3.72 (s, 3H, CH_3-N<), 7.39–7.41 (d, 2H; J = 8.1 Hz; H-3, 5 of CH_3-*Ph*-SO_2-), 7.75–7.77 (d, 2H; J = 8.1 Hz; H-2, 6 of CH_3-*Ph*-SO_2-). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 377.03 (377.04).

4.2.7. Synthesis of 2-(benzenesulfonamido-*N*-benzyl)-5-[*N*,*N*-dibenzylsulfonamido]-l,3,4-thiadiazole (II′-1)

2-Benzenesulfonamido-l,3,4-thiadiazole-5-sulfonamide (**3I′**) (0.16 g, 0.5 mmol) was dissolved into DMF (1 mL) with stirring at room temperature. Potassium hydroxide (0.10 g, 1.5 mmol) was suspended in DMF (1 mL) then added to the above solutions, potassium hydroxide was dissolved slowly. Then a mixture of benzyl bromide (0.31 g, 0.0018 mol) and DMF (1 mL) was added to the reaction mixture and heated to 80 °C in an oil-bath for 4 h (monitored by TLC). The solvent was evaporated to dryness in vacuum, the residue was flaxen solid. The compound **II-1** was obtained by chromatographic fractionation. Yield, 77.4%; mp: 130–132 °C; ^{1}H NMR (DMSO-d_6) δ: 4.49 (s, 4H, (Ph-CH_2)$_2$-N-), 5.31 (s, 2H, Ph-CH_2-N<), 7.17–7.25 (m, 12H; J = 7.5 Hz; 3*Ph*-CH_2-), 7.32–7.35 (m, 3H; J = 8.7 Hz; 3*Ph*-CH_2-), 7.59–7.80 (m, 5H, *Ph*-SO_2-). ESI-MS, *m/z*: $[M+H]^+$ (calcd) 591.04 (591.12).

4.3. Carbonic anhydrase inhibition assay

4.3.1. Isozyme preparation

Human CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pET-28b/hCA II. A practical method for screening hCA II inhibitors was successfully constituted by recombinant hCA II protein expressed in *E. coli* as the source of hCA II enzyme.[17,18]

4.3.2. Evaluation of carbonic anhydrase inhibitory activity

The dosage of AZA was to be a standard. When the inhibition rate of AZA was 50%, the density of AZA was 0.3 μmol L^{-1}, so we use the same density for all compounds to compare their inhibition effects on CA II. Derivatives **I-1**–**I-19**, **I′-1**–**I′-15**, **II-1** and **II′-1** were investigated for their inhibitory effects on hCA II. The hCA II activity was detected under pH 7.6 and 25 °C by its esterase activity which can decompose *p*-nitrophenyl acetate (PNPA) to increase the photoabsorption at 348 nm by UV-240 ultraviolet spectrophotometry (SHIMADZU, Japan).[19,20] 2.2 mL Tris–SO_4^{2-} buffer solution, 0.1 mL compound solution, 0.6 mL substrate solution and 0.1 mL enzyme solution were added to reaction system one by one, the bulk volume of reaction system was 3.0 mL. At first, the absorbance value (A) was corrected to 'zero' by distilled water, then the absorbance value was recorded every 0.5 min within 3.0 min. The curve was made by absorbance value and time, the increase value of absorbance value in 1 min (ΔA/min) was computed by linear part of the curve. The molar extinction coefficient of paranitrophenol was 5.0×10^6 under pH 7.6 and 25 °C. Three formulas were as followed:

(1) ΔA_{348nm}/min = ΔA_{sample}/min − $\Delta A_{compare\ germ}$/min.
(2) Specific activity (U/mg) = (ΔA_{348nm}/min)/(5.0 × mg enzyme/mL solution).
(3) Inhibition rate (%) = [1 − (sample + compound) specific activity/(sample) specific activity] × 100.

4.3.3. Evaluation of some compounds' antihypoxic effects in mice

AZA was a positive control drug, the antihypoxic effects of some compounds in mice were evaluated.[21] Kunming mice were randomly divided into blank control group, positive control group and target compound group. In dose of 80 mg/kg, target compounds, AZA and physiological saline were administered to the target compound group, positive control group and blank control group for five days, respectively. The mice were fasted for 12 h before experiment, and were dealed with intragastric administration after 1 h experimentized. After putting the mice into sealed bottles (250 mL) with 10 g of Soda Lime, the survival time of the mice was recorded.

4.4. Docking studies

The molecular modeling calculations and docking studies were performed using Dock6. The program operated under 'Linux' operating system installed on the Xeon with a 2.6 MHz processor and 1G RAM.

The X-ray crystallographic structure of hCA II complexed with brinzolamide (1A42) was obtained from the Protein Data Bank. The enzyme was prepared for docking studies where: (i) The ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) Virtual screen was used to discover the inhibitor of hCA II.

The ligand were minimize by MMFF94 force field.[22]

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.061. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Gao, W.; Zhang, S. F.; Zhao, Q. L.; Liu, Y.; Luo, X. H.; Liu, H. P.; Li, N. B. *J. Fourth Mil. Med. Univ.* **2008**, *29*, 151.
2. Hackett, P. H.; Roach, R. C. *N. Eng. J. Med.* **2001**, *345*, 107.
3. Chu, X. Q.; Ge, R. L.; Ma, Y. C. *Chin. J. Crit. Care Med.* **2003**, *11*, 797.
4. Reyes, E.; Izquierdo, N. J.; Blasini, M. *Bol. Asoc. Med. P. R.* **1997**, *89*, 51.
5. Lindskog, S. *Pharmacol. Ther.* **1997**, *74*, 1.
6. Swenson, E. R.; Teppema, L. J. *J. Appl. Physiol.* **2007**, *102*, 1305.
7. Leaf, D. E.; Goldfarb, D. S. *J. Appl. Physiol.* **2007**, *102*, 1313.
8. Clapp, J. W.; Darien, C.; Roblin, R. O. Patents, US2554816. 1951-05-29.
9. Arslan, O.; Küfrevioğlu, O. I.; Nalbantoğlu, B. *Bioorg. Med. Chem.* **1997**, *5*, 515.
10. James, R. V.; Darien, C.; Joyce, A. E. Patents, US2721204. 1955-10-18.
11. Richard, W. Y.; Kathryn, H. D.; Joyce, A. E. *J. Am. Chem. Soc.* **1956**, *78*, 4649.
12. Moustakas, D. T.; Lang, P. T.; Pegg, S.; Pettersen, E.; Kuntz, I. D.; Brooijmans, N.; Rizzo, R. C. *J. Comput. Aided Mol. Des.* **2006**, *20*, 601.
13. Powers, R. A.; Morandi, F.; Shoichet, B. K. *Structure* **2002**, *10*, 1013.
14. Stams, T.; Chen, Y.; Boriack-Sjodin, P. A.; Hurt, J. D.; Liao, J.; May, J. A.; Dean, T.; Laipis, P.; Silverman, D. N.; Christianson, D. W. *Protein Sci.* **1998**, *7*, 556.
15. Grünberg, S.; Stubbs, M. T.; Klebe, G. *J. Med. Chem.* **2002**, *45*, 3588.
16. Di Fiore, A.; Scozzafava, A.; Winum, J. Y.; Montero, J. L.; Pedone, C.; Supuran, C. T.; De Simone, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1726.
17. Winum, J. Y.; Innocenti, A.; Vullo, D.; Montero, J. L.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5082.
18. Ilies, M.; Supuran, C. T.; Scozzafava, A.; Casini, A.; Mincione, F.; Menabuoni, L.; Caproiu, M. T.; Maganu, M.; Banciu, M. D. *Bioorg. Med. Chem.* **2000**, *8*, 2145.
19. Armstrong, J. M.; Myers, D. V.; Verpoorte, J. A.; Edsall, J. T. *J. Biol. Chem.* **1966**, *241*, 5137.
20. Arslan, O.; Küfreviolu, Ö. I.; Nalbantolu, B. *Bioorg. Med. Chem.* **1997**, *5*, 515.
21. He, T.; Ming, L.; Wang, S. B.; Wu, Q.; Yin, Y. Y.; Li, W. P. *Chin. Pharm. Bull.* **2004**, *20*, 576.
22. Thomas, A. H. *J. Comput. Chem.* **1996**, *17*, 490.