Bioorganic & Medicinal Chemistry 22 (2014) 5133–5140

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Quinazoline–sulfonamides with potent inhibitory activity against the α-carbonic anhydrase from *Vibrio cholerae*

Ahmed M. Alafeefy [a,*], Mariangela Ceruso [b], Abdul-Malek S. Al-Tamimi [a], Sonia Del Prete [c], Clemente Capasso [c], Claudiu T. Supuran [b,d,*]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, Salman Bin Abdulaziz University, PO Box 173, Alkharj 11942, Saudi Arabia
[b] Università degli Studi di Firenze, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Rm 188, Via della Lastruccia 3, I 50019 Sesto Fiorentino (Firenze), Italy
[c] Istituto di Bioscienze e Biorisorse, CNR, Via Pietro Castellino 111, 80131 Napoli, Italy
[d] Università degli Studi di Firenze, Neurofarba Department, Section of Pharmaceutical and Nutriceutical Sciences, Via U. Schiff 6, 50019 Sesto Fiorentino (Florence), Italy

ARTICLE INFO

Article history:
Received 30 June 2014
Revised 8 August 2014
Accepted 13 August 2014
Available online 21 August 2014

Keywords:
Carbonic anhydrase
Vibrio cholera
Sulfonamide
Enzyme inhibitor
Quinazoline

ABSTRACT

Thirteen novel sulfonamide derivatives incorporating the quinazoline scaffold were synthesized by simple, eco-friendly procedures. These compounds were tested for their ability to inhibit the α-carbonic anhydrases (CA, EC 4.2.1.1) from *Vibrio cholerae* (VchCA) as well as the human α-CA isoforms, hCA I and hCA II. Nine compounds were highly effective, nanomolar inhibitors of the pathogenic enzyme VchCA. Three of them were also highly effective sub-nanomolar inhibitors of the cytosolic isoform II. The best VchCA inhibitor had a K_I of 2.7 nM. Many of these developed compounds showed high selectivity for inhibition of the bacterial over the mammalian CA isoforms, with one compound possessing selectivity ratios as high as 97.9 against hCA I and 9.7 against hCA II. Compound **9d** was another highly effective VchCA inhibitor presenting a selectivity ratio of 99.1 and 8.1 against hCA I and hCA II, respectively. These results suggest that sulfonamides with quinazoline backbone could be considered suitable tools to better understand the role of bacterial CAs in pathogenesis.

1. Introduction

Cholera is an infectious human disease of the small intestine and is caused by the gram negative bacterium *Vibrio cholerae*. It is characterized by massive loss of water and electrolytes which leads to severe dehydration and hypovolemic shock followed by death if not well treated.[1] Globally, it was estimated that it affects 3–5 million people and causes 100.000–130.000 deaths annually. In developing countries, cholera spreads among victims mainly through contaminated water sources, and countries without proper sanitation techniques have greater incidence of such horrible disastrous disease.[2]

It was reported that the growth of *Vibrio cholera* during infection of a host initiates a complex regulatory cascade that results in the production of a regulatory protein that directly activates transcription of the genes encoding cholera toxins and other virulence inducer genes.[3] Although the in vivo signals that induce *V. cholerae* virulence gene expression have not yet been determined, *V. cholerae* has been shown to modulate the expression of its virulence genes in vitro which could be explained by the bicarbonate induced alkalinity of the small intestine.[4]

Consequently, recently, it was reported that the intestinal hypoxic conditions enables *V. cholera* to express a number of virulence activators. It has also been shown that *V. cholerae* may sense intestinal anoxic signals by multiple components to activate virulence.[5] Another potential inducer of virulence gene expression is sodium bicarbonate, which is present at a high concentration in the upper small intestine. Consequently, bicarbonate is considered the first positive effector for ToxT, the major direct transcription activator of the virulence genes.[6]

Jeffrey and Basel reported that ethoxzolamide, a potent sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitor, outstandingly controlled the bicarbonate-mediated virulence induction, suggesting that conversion of CO_2 into bicarbonate by carbonic anhydrase plays a major role in virulence induction.[1] Recently our group succeeded to clone an α-CA enzyme from this pathogen, *Vibrio cholerae*, which was named VchCA.[7] This enzyme showed a significant catalytic activity for the hydration of CO_2 to produce HCO_3^-, and was greatly inhibited by sulfonamide and sulfamate containing molecules.[7–11]

Based on the aforementioned results we proposed VchCA as a new target for the antibiotic development program. In the present

* Corresponding authors. Tel.: +966 537600100; fax: +966 011 5886060 (A.M.A.); tel./fax: +39 055 4573005 (C.T.S.).
E-mail addresses: a.alafeefy@sau.edu.sa (A.M. Alafeefy), claudiu.supuran@unifi.it (C.T. Supuran).

http://dx.doi.org/10.1016/j.bmc.2014.08.015
0968-0896/

paper we continued our efforts in developing sulfonamide CAIs as potential antibacterial agents, and report the synthesis of certain quinazoline derivatives crowned with sulfonamide moiety. In addition to the bacterial enzyme VchCA, these novel compounds were investigated for their ability to inhibit the physiologically most important, cytosolic isoforms CA I and II.

2. Results and discussion

2.1. Sequence and phylogenic analysis

An alignment of the amino acid sequences of VchCA with hCAs I and II is shown in Figure 1 in order to identify salient features of this bacterial enzyme. It may be observed that, like the other investigated α-CAs, VchCA has the conserved three His ligands, which coordinate the Zn(II) ion crucial for catalysis (His94, His96, and His119, hCA I numbering system). The proton shuttle residue (His64) is also conserved in all these enzymes. This residue assists the rate-determining step of the catalytic cycle, transferring a proton from the water coordinated to the Zn(II) ion to the environment with formation of zinc hydroxide nucleophilic species of the enzyme. VchCA has also the gate-keeping residues (Glu106 and Thr199), which orientate the substrate for catalysis and are also involved in the binding of inhibitors. The only unique macro feature in the primary structure of the bacterial α-CAs with respect to the mammalian enzymes was the absence of four amino acid loops indicated in Figure 1 by the '- - -' symbols. This feature is typical of all bacterial α-CAs investigated so far. Deletion of these loops makes the bacterial proteins smaller and more compact.

A phylogenetic analysis of VchCA and other α-CAs (such as human and bacterial α-CAs) is shown in Figure 2. It may be observed that VchCA is most closely related to the *Neisseria gonorrhoeae* (NgoCA). The next closest relatives of these enzymes are the pathogenic bacterium, *Helicobacter pylori* and the thermophilic bacterium enzyme SspCA, isolated from the extremophile *Sulfurihydrogenibium yellowstonense*. The human enzymes clustered together in the upper branches of the radial dendrogram shown in Figure 2.

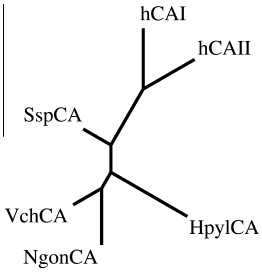


Figure 2. Phylogenetic analyses of VchCA and other α-CAs from human and bacterial sources. The phylogenetic tree was constructed using the program PhyML 3.0. Legend: for VchCA, hCA I and hCA II see Figure 1; NgoCA, *Neisseria gonorrhoeae* (accession no. CAA72038.1); HphyCA, *Helicobacter pylori* (accession no. NP_223829.1); SspCA, *Sulfurihydrogenibium yellowstonense* YO3AOP1 (accession no. ACD66216.1).

2.2. Chemistry

A large number of aromatic and heteroaromatic were shown to possess significant CA inhibitory activity against many classes of CAs and diverse isoforms.[7,8] The nature of the aromatic/heterocyclic scaffold is crucial for the binding of these compounds to the enzyme, as the sulfonamide moiety (in deprotonated form) coordinates the Zn(II) from the enzyme active site, whereas the organic scaffold participates in interactions (positive or negative ones) with amino acid residues and water molecules from the cavity, which may lead to a stabilization (in case of positive interactions) or destabilization (in case of clashes) of the enzyme-inhibitor complex. As a consequence, exploration of diverse heterocyclic scaffolds incorporating sulfamoyl moieties may lead to interesting compounds, both in terms of efficacy for the inhibition of the various CA isoforms, as well as in terms of selectivity for some isoforms over other ones which may be off targets, such as for example the house-keeping, widespread human isoforms hCA I and II.[7,8] Thus, the rationale for the drug design presented here was to explore compounds incorporating a ring system rarely investigated for inhibition of these enzymes, that is, the quinazolin-4-one.

```
VchCA   MKKTTWVLAMVASMSFGVQASEWGYEGEHAPEHWGKVAPLCAEGKNQSPIDVAQSV---E
hCAI    -----------------MASPDWGYDDKNGPEQWSKLYPI-ANGNNQSPVDIKTSETKHD
hCAII   ------------------MSHHWGYGKHNGPEHWHKDFPI-AKGERQSPVDIDTHTAKYD
                         :  ***   :.**:* *  *: *:*:.***:*:       :

                                64                                  94 96
VchCA   ADLQPFTLNY-QGQVVGLLNNGHTL---------QAIVRGNNPLQIDGKTFQLKQFHFH-
hCAI    TSLKPISVSYNPATAKEIINVGHSFHVNFEDNDNRSVLKG-GPFS---DSYRLFQFHFHW
hCAII   PSLKPLSVSYDQATSLRILNNGHAFNVEFDDSQDKAVLKG-GPLD---GTYRLIQFHFHW
        ..*:*::::.*  .    ::* **::          .::::.* .*:.    ::.* ****

                106           119
VchCA   -----TPSENLLKGKQFPLEAHFVH------------ADEQGNLAVVAVMYQVGSENP-L
hCAI    GSTNEHGSEHTVDGVKYSAELHVAHWNSAKYSSLAEAASKADGLAVIGVLMKVGEANPKL
hCAII   GSLDGQGSEHTVDKKKYAAELHLVHWNT-KYGDFGKAVQQPDGLAVLGIFLKVGSAKPGL
               **: :.  ::. * *..*             ..: ..***:.:: :**. :* *

                                                   199
VchCA   LKVLTA--DMPTKGNSTQLTQGIPLADWIPESKHYYRFNGSLTTPPCSEGVRWIVLKEPA
hCAI    QKVLDALQAIKTKGKRAPFTNFDP-STLLPSSLDFWTYPGSLTHPPLYESVTWIICKESI
hCAII   QKVVDVLDSIKTKGKSADFTNFAA-RGLLPESLDYWTYPGSLTTPPLLECVTWIVLKEPI
         **: .   : ***: : :*:  .    :*.*  :: : **** **  * * **: **.


VchCA   HLSNQQEQQLSAV-----------MGHNNRPVQPHNARLVLQAD-
hCAI    SVSSEQLAQFRSLLSNVEGDNAVPMQHNNRPTQPLKGRTVRASF-
hCAII   SVSSEQVLKFRKLNFNGEGEPEELMVDNWRPAQPLKNRQIKASFK
         :*.:*  ::  :           *  *.**.** : * :  :
```

Figure 1. Alignment of VchCA, hCA I and hCA II amino acid sequences. The proton shuttle residue (His64), the zinc ligands (His 94, 96 and 119) and the gate keeper residues (Glu106 and Thr199) are conserved in the bacterial and mammalian sequences. The hCA I numbering system was used. The asterisk (*) indicates identity at all aligned positions; the symbol (:) relates to conserved substitutions, while (.) means that semi-conserved substitutions are observed. Multialignment was performed with the program Clustal W, version 2.1. Legend: VchCA, *Vibrio cholerae* (accession number: AFC59768.1); hCA I, *Homo sapiens*, isoform I (accession no. NP_001158302.1); hCA II, *Homo sapiens*, isoform II (accession no. AAH11949.1).

The approach to prepare the target potentially bioactive quinazoline derivatives **8a–h** and thioxoquinazolin-4-one analogues **9a–e** was via a generalized route as depicted in Schemes 1–3. The starting materials, thioxoquinazoline derivatives **3a–h** and their 2,4-dithioxo isosteres **4a–e**, were prepared according to reported literature procedure.[12,13] The appropriate anthranilic acid derivatives **1a–e** was reacted with the selected isothiocyanate derivative in boiling ethanol containing catalytic amount of triethylamine. The structure of these intermediates was confirmed by IR, NMR and MS spectra (see Section 4 for details).

It is thought that in certain instances, conversion of the oxoquinazoline derivatives to the thioxo isosteres could contribute to the biological activity.[14] Recently, and after in silico prediction, certain thioxoquinazoline derivatives showed inhibitory potencies at submicromolar levels against the catalytic domain of PDE7A1.[15] Such consideration led us to explore some selected thioxoquinazoline isomers as possible inhibitors of VchCA isoform. Consequently, the 2-thioxoquinazolin-4-thione derivatives **4a–e** were obtained by treatment of compounds **3a–e** with phosphorus penta sulfide in dry pyridine. 2-Chloro-*N*-(4-sulfamoylphenyl)-acetamide was prepared by acylation of sulfanilamide at room temperature under basic condition. Alkylation of both quinazoline derivatives **3a–h** and **4a–e** with 2-chloro-*N*-(4-sulfamoylphenyl)-acetamide was carried out afterwards to afford the target derivatives endowed with the sulfonamide functionality **8a–h**, and **9a–e**, respectively. The structure of the intermediate and target molecules were confirmed by elemental analyses, IR, NMR and MS spectra and were in accordance with the suggested structure.

The I.R. spectra of compounds **8a–h** showed two absorption bands at around 3350 and 3250 cm^{-1} due to NH and NH_2. Two strong carbonyl bands ranging from 1725–1685 cm^{-1} were a common feature of these derivatives. Their ^1H NMR showed two D_2O exchangeable signals of NH and NH_2 groups around the regions δ 11.00 and δ 6.30, respectively. The ^{13}C spectra confirmed the presence of the corresponding carbons at their expected shift values specially the two carbonyl peaks at around 160–168 ppm. Conversion of the thioxoquinazolin-4-one **3a–e** derivatives to their corresponding di-thioxo **4a–e** isosteres was confirmed by the absence of one of the carbonyl absorption band characteristic for compounds **3a–e** in the IR and ^{13}C spectra and the concomitant appearance of the new C=S bands instead. Conversely, compounds **9a–e** formation was evidenced by the same tools, where the final compounds exhibited the C=S peaks at δ values above 175 ppm. However, generally the NH, CH_2 and C=O is a common functionality for *N*-(4-sulfamoylphenyl)-acetamido containing compounds such as **8a–h** and **9a–e** as evidenced by their IR, ^1H and ^{13}C NMR spectra. Their mass spectra revealed, in each case, a peak corresponding to the molecular ion in addition to the base peak.

2.3. Carbonic anhydrase inhibition

The inhibition studies of the new quinazolin-4-one sulfonamide derivatives **8a–h** and their 4-thioxo bioisosteres **9a–e** reported here, against the human (h) CA isoforms hCA I, II, and the bacterial enzyme VchCA, are reported in Table 1. Data for the selectivity ratios of the dominant and physiologically relevant human carbonic anhydrase isoforms (hCA I and II) over the bacterial VchCA enzyme with these compounds are also included in Table 1, as they may be the main off-targets in case they are very efficient VchCA inhibitors. The following structure-activity relationship (SAR) can be observed from data of Table 1, for the inhibition of the VchCA investigated here with the new group of quinazoline–sulfonamide derivatives **8a–h** and **9a–e**:

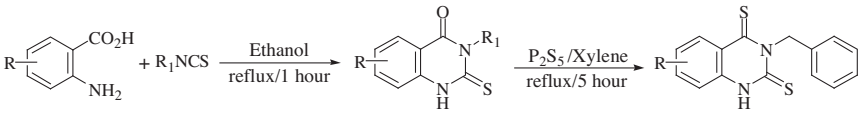
(i) Generally, sulfonamide derivatives with quinazolin-4-one backbone exhibited inhibitory activity towards VchCA comparable to that obtained by those derivatives with quinazolin-4-thione derivatives except for compound **8c**. Compounds **8–e**, **8–f**, **8–h** and **9–e**, were poorly effective as VchCA inhibitors (K_Is >10,000 nM) and noticed no great difference between allyl or benzyl group as 3-substituents.

(ii) Substitution at the 5, 6 and or 8 positions of the quinazoline nucleus led to compounds with varying degrees of activities. The 6-methyl substituted derivatives were more active than the 5-substituted methyl derivatives and the later ones were more active than their 8-methyl substituted quinazoline sulfonamide congeners. Compound **8g** with 6-nitro-3-allyl showed very good inhibitory activity but the 6-nitro-3-benzyl **8h** exhibited moderate activity the same as the its dithioxo congener **9e**.

(iii) In both series the 6-methyl-3-benzyl derivatives (**8–c**, **9–b**) were mostly more active than the standard drug, however the oxoquinazoline series could be considered inferior to the thioxoquinazolines based on the better selectivity ratio of the latter.

(iv) Against the cytosolic slow isoform hCA I, the new quinazoline derivatives and their bioisosteres reported here were generally ineffective inhibitors, with inhibition constants ranging between 674–5432 nM. Only compounds **8a–c** showed medium inhibition activity against this isoform, with K_Is of 86.5–105 nM (Table 1).

(v) The physiologically dominant human isoform hCA II was high effectively inhibited by **8a–c**, which showed K_Is in the range of 1.3–1.7 nM, with better inhibition activity than the clinically used acetazolamide **AAZ** (K_I of 12 nM). Derivative **8g** showed high inhibition potency against this cytosolic isoform with K_I of the same order of magnitude



1a-c **2a, b** **3a-h** **4a-e**

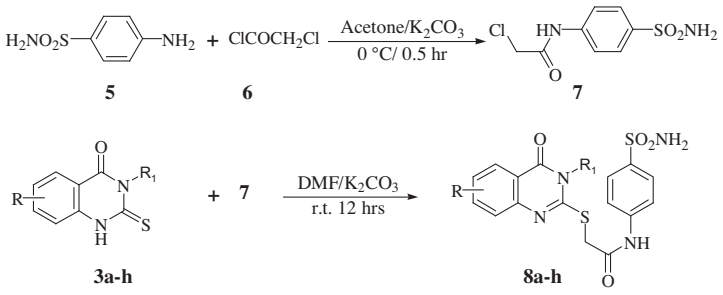
1-	R
a	6-CH_3.
b	5-CH_3.
c	3-CH_3.
d	3-OCH_3.
e	5-NO_2.

2-	R_1
a	$CH_2CH{=}CH_2$.
b	$CH_2C_6H_5$.

3-	R	R_1
a	5-CH_3.	$CH_2CH{=}CH_2$.
b	6-CH_3.	$CH_2CH{=}CH_2$.
c	6-CH_3.	$CH_2C_6H_5$.
d	8-CH_3	$CH_2C_6H_5$.
e	6-OCH_3.	$CH_2C_6H_5$.
f	8-OCH_3.	$CH_2C_6H_5$.
g	6-NO_2.	$CH_2CH{=}CH_2$.
h	6-NO_2.	$CH_2C_6H_5$.

4-	R
a	5-CH_3.
b	6-CH_3.
c	8-CH_3.
d	8-OCH_3.
e	6-NO_2.

Scheme 1. Synthesis of 3,5,6 and/or 8 substituted-2-thio-4-oxoquinazoline and 2-thio-4-thioxoquinazoline derivatives **3a–h** and **4a–e**.



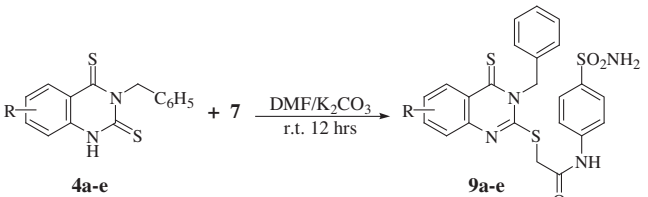
5 6 7

3a-h 8a-h

3-	R	R_1
a	5-CH_3	$CH_2CH{=}CH_2$
b	6-CH_3	$CH_2CH{=}CH_2$
c	6-CH_3	$CH_2C_6H_5$
d	8-CH_3	$CH_2C_6H_5$
e	6-OCH_3	$CH_2C_6H_5$
f	8-OCH_3	$CH_2C_6H_5$
g	6-NO_2	$CH_2CH{=}CH_2$
h	6-NO_2	$CH_2C_6H_5$

8-	R	R_1
a	5-CH_3	$CH_2CH{=}CH_2$
b	6-CH_3	$CH_2CH{=}CH_2$
c	6-CH_3	$CH_2C_6H_5$
d	8-CH_3	$CH_2C_6H_5$
e	6-OCH_3	$CH_2C_6H_5$
f	8-OCH_3	$CH_2C_6H_5$
g	6-NO_2	$CH_2CH{=}CH_2$
h	6-NO_2	$CH_2C_6H_5$

Scheme 2. Synthese of substituted sulfa derivative **7** and 2,3,5,6 and/or 8-substituted-4-oxoquinazoline derivatives **8a–h**.



4a-e 9a-e

4-	R
a	5-CH_3
b	6-CH_3
c	8-CH_3
d	8-OCH_3
e	6-NO_2

9-	R
a	5-CH_3
b	6-CH_3
c	8-CH_3
d	8-OCH_3
e	6-NO_2

Scheme 3. Synthesis of 2,3,5,6 and/or 8-substituted-4-thioxoquinazoline derivatives **9a–e**.

Table 1
Inhibition data against human (h) isoforms hCA I, II (cytosolic), and bacterial enzyme VchCA of derivates **8a–h**, **9a–e** and acetazolamide **AAZ** (as standard inhibitor) by a stopped-flow CO_2 hydrase assay[17]

Compound	K_I* (nM)			Selectivity ratios	
	hCA I	hCA II	VchCA	VchCA/hCA I	VchCA/hCA II
8-a	105	1.3	8.1	13.0	0.2
8-b	119	1.8	6.0	14.7	0.2
8-c	86.5	1.7	2.7	10.7	0.2
8-d	2078	208.4	8.5	256.6	25.7
8-e	4761	233.8	>10,000	–	–
8-f	5432	208.9	>10,000	–	–
8-g	674	12.1	6.6	83.27	1.5
8-h	3371	122.9	>10,000	–	–
9-a	4168	67.5	7.7	514.5	8.3
9-b	793	78.26	5.5	97.9	9.7
9-c	2162	114.2	8.7	266.9	14.1
9-d	803	65.3	7.9	99.1	8.1
9-e	4549	96.0	>10,000	–	–
AAZ	250	12.0	6.8	36.7	1.76

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

as acetazolamide. Some of the remaining derivatives, such as **9a–b**, **9d** and **9e**, were medium potency hCA II inhibitors (K_Is in the range of 44.0–91.9 nM). The other compounds reported here **8d–8f**, **8h** and **9c** showed ineffective hCA II inhibitory activity, with K_Is in the range of 114.2–233.8 nM (Table 1).

(vi) The bacterial enzyme VchCA was generally highly inhibited by the quinazolines reported here, with K_Is in the range of 2.7–8.5 nM. Several low nanomolar VchCA inhibitors were detected here. However, the best VchCA inhibitor was derivative **8c** (K_I of 2.7 nM), which was also a very strong inhibitor of hCA II with K_I of 1.7 nM and a medium potency inhibitor of the cytosolic isoform hCA I with K_I of 86.5 nM. The least effective VchCA inhibitors were **8e–f**, **8g** and **9e**, which showed K_Is higher than 10,000 nM. Although most of the compounds reported here inhibited efficiently VchCA, no significant change of K_I values has been observed. Therefore, SAR is almost impossible to delineate as all substitution patterns lead to highly effective inhibitors of this bacterial CA, VchCA.

(vii) Some of the potent VchCA inhibitors reported here also showed a high selective inhibition of the bacterial CA over the human isoforms. Indeed, compounds **8d**, **9b** and **9c** possessing selectivity ratios ranging from 97.9 to 266.9 against hCA I and 9.7 to 25.7 against hCA II, represents the most interesting CAIs reported here (Table 1). Furthermore, another highly effective VchCA inhibitor presenting similar selectivity ratios to the previous compound as high as 99.1 against hCA I and 8.1 against hCA II was observed for compound **9d**.

Overall the compounds presented better selectivity inhibition ratios against hCA I than hCA II and some of them were low selective inhibitor for the bacterial isoform over the human ones such as **8a–c**. It is also interesting to notice that acetazolamide **AAZ** possesses lower selectivity ratios for inhibiting VchCA against hCA I and II (36.7 and 1.76, respectively) than the derivatives **8d–8h** reported here, although it acts as an efficient VchCA inhibitor.

3. Conclusion

A series of quinazolin-4-one and quinazolin-4-thione derivatives endowed with the biologically interesting benzenesulfonamide moiety were designed and synthesized as potential CAIs. The CA inhibitory activity of these compounds was assessed against the human pathogenic bacterium *Vibrio cholera* VchCA and the human cytosolic isoforms hCA I and II. Most of the novel tested sulfonamide derivatives showed interesting activities against VchCA enzyme and three of them potently inhibited hCA II at a subnanomolar concentration and were more potent than

acetazolamide **AAZ** (as standard inhibitor). According to the results obtained in the current study, many of the highly effective VchCA inhibitors reported here also possessed excellent selectivity ratios for inhibiting the bacterial over the human enzymes.

4. Experimental

4.1. Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Southborough, UK) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60G F254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infra red spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on Bruker AC-400 Ultra Shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using TMS as internal standard and peak multiplicities are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Electron Impact Mass Spectra were recorded on a Shimadzu GC-MS-QP 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were performed, on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), at the Micro-analytical Unit, Faculty of Science, Cairo University, Cairo, Egypt, and the found results were within ±0.4% of the theoretical values.

4.1.1. General procedure for preparation of 2-thioxo-3-substituted-5, 6 or 8-substituted-3*H*-quinazolin-4-ones 3a–h[12,13]

A mixture of the appropriate anthranilic acid derivative, **1a–e** (0.01 mol), the appropriate isothiocyanate derivative (0.012 mol), and TEA (2 mL) in absolute ethanol (50 mL) was heated under reflux for 1 h. The reaction mixture was then cooled and the solvent was evaporated under vacuum. The obtained residue was washed with water, filtered, dried and crystallized from absolute ethanol to give the title compounds.

4.1.1.1. 3-Allyl-2,3-dihydro-5-methyl-2-thioxoquinazolin-4(1*H*)-one 3a[12,13]. Yield (90%); mp 172–174 °C; IR (KBr) *ν*: 3268 (NH), 1685 (C=O), 1271 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.44 (s, 3H, CH_3), 4.42–4.41 (d, 2H, J = 4.1 Hz, NCH_2), 5.12–5.11 (d, 2H, J = 4.5 Hz, Allyl-CH_2), 5.91–5.90 (m, 1H, Allyl-C*H*), 6.72–6.71 (d, J = 7.0 Hz, 1H, ArH), 6.98–6.99 (d, J = 7.1 Hz, 1H, ArH), 7.25–7.26 (t, J = 7.2 Hz, 1H, ArH), 11.75 (s, 1H, NH), ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.9 (*C*H$_3$), 40.6 (NCH$_2$), 116.9, 125.1, 125.2, 125.9, 127.9, 127.7, 128.8, 137.4, 138.6 (Ar-*C*), 160.2 (C=O), 176.5 (C=S). MS (*m*/*z*): 232 [M$^+$]. Anal. ($C_{12}H_{12}N_2OS$, 232.30) C, 62.04 (62.21); H, 5.21 (4.88); N, 12.06 (12.19); S, 13.80 (14.03).

4.1.1.2. 3-Allyl-2,3-dihydro-6-methyl-2-thioxoquinazolin-4(1*H*)-one 3b[12,13]. Yield (92%); mp 183–185 °C; IR (KBr) *ν*: 3271 (NH), 1690 (C=O), 1258 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 2.39 (s, 3H, CH_3), 4.48–4.47 (d, 2H, J = 3.2 Hz, NCH_2), 5.13–5.12 (d, 2H, J = 4.6 Hz, Allyl-CH_2), 5.90 (m, 1H, Allyl-C*H*), 7.62–7.61 (d, J = 7.2, 1H, Ar-*H*), 7.85–7.84 (d, J = 6.7, 1H, Ar-*H*), 8.11 (s, 1H, Ar-*H*), 11.55 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 16.2 (*C*H$_3$), 41.1 (NCH$_2$), 117.3, 120.5, 122.1, 123.0, 127.4, 129.5, 132.7, 134.9, 136.4, 137.8, 141.6, 144.7, 154.3 (Ar-C), 160.7 (C=O), 178.1 (C=S). MS (*m*/*z*): 232 [M$^+$]. Anal. ($C_{12}H_{12}N_2OS$, 232.30) C, 62.04 (61.87); H, 5.21 (5.37); N, 12.06 (11.79); S, 13.80 (13.89).

4.1.1.3. 3-Benzyl-2,3-dihydro-6-methyl-2-thioxoquinazolin-4(1*H*)-one 3c[12,13]. Yield (86%); mp 168–166 °C; IR (KBr) *ν*: 3284 (NH), 1686 (C=O), 1263 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 2.35 (s, 3H, CH_3), 4.45–4.44 (d, 2H, J = 3.4 Hz, NCH_2), 7.63–7.62 (m, 3H, Ar-*H*), 7.67–7.64 (d, J = 7.6, 2H, Ar-*H*), 7.88–7.86 (d, J = 8.6, 2H, Ar-*H*), 8.15 (s, 1H, Ar-*H*), 11.54 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 15.9 (*C*H$_3$), 45.3 (NCH$_2$), 121.2, 125.7, 126.4, 127.2, 128.8, 129.1, 133.5, 136.7, 137.1, 152.0 (Ar-C), 161.6 (C=O), 178.3 (C=S). MS (*m*/*z*): 282 [M$^+$]. Anal. ($C_{16}H_{14}N_2OS$, 282.36) C, 68.06 (68.19); H, 5.00 (4.79); N, 9.92 (10.16); S, 11.36 (11.17).

4.1.1.4. 3-Benzyl-2,3-dihydro-8-methyl-2-thioxoquinazolin-4(1*H*)-one 3d[12,13]. Yield (92%); mp 167–169 °C; IR (KBr) *ν*: 3287 (NH), 1683 (C=O), 1266 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 2.32 (s, 3H, CH_3), 4.60–4.59 (d, 2H, J = 3.6 Hz, NCH_2), 7.67–7.65 (m, 3H, Ar-*H*), 7.68–7.65 (d, J = 7.5, 2H, Ar-*H*), 8.02–8.01 (m,3H, Ar-*H*), 11.14 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 16.3 (*C*H$_3$), 41.5 (NCH$_2$), 121.4, 125.1, 126.8, 127.7, 128.2, 129.1, 133.0, 136.5, 138.7, 152.7 (Ar-C), 160.7, (C=O), 179.3 (C=S). MS (*m*/*z*): 282 [M$^+$]. Anal. ($C_{16}H_{14}N_2OS$, 282.36) C, 68.06 (68.23); H, 5.00 (4.81); N, 9.92 (10.25); S, 11.36 (11.14).

4.1.1.5. 3-Benzyl-2,3-dihydro-6-methoxy-2-thioxoquinazolin-4(1*H*)-one 3e[12,13]. Yield (86%); mp 165–167 °C; IR (KBr) *ν*: 3287 (NH), 1681 (C=O), 1269 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 3.73 (s, 3H, OCH_3), 4.26–4.25 (d, J = 7.0 Hz, 2H, NCH_2), 7.65–7.63 (m, 3H, Ar-*H*), 7.66–7.65 (d, J = 7.5 Hz, 2H, Ar-*H*), 7.83–7.82 (d, J = 7.3 Hz, 2H, Ar-*H*), 8.11 (s, 1H, Ar-*H*), 10.99 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 43.0 (NCH$_2$), 55.6 (OCH$_3$), 121.7, 125.2, 127.0, 127.4, 128.4, 129.1, 133.4, 135.7, 151.5, (Ar-C), 162.5 (C=O), 184 (C=S). MS (*m*/*z*): 298 [M$^+$]. Anal. ($C_{16}H_{14}N_2O_2S$, 298.35) C, 64.41 (64.72); H, 4.73 (4.96); N, 9.39 (9.11); S, 10.75 (10.59).

4.1.1.6. 3-Benzyl-2,3-dihydro-8-methoxy-2-thioxoquinazolin-4(1*H*)-one 3f[12,13]. Yield (89%); mp 163–165 °C; IR (KBr) *ν*: 3289 (NH), 1681 (C=O), 1264 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 3.77 (s, 3H, OCH_3), 4.27–4.26 (d, J = 6.5 Hz, 2H, NCH_2), 7.67–7.64 (m, 4H, Ar-*H*), 7.67–7.66 (d, J = 7.6, 2H, Ar-*H*), 7.88–7.86 (d, J = 8.7, 2H, Ar-*H*), 11.14 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 43.1 (NCH$_2$), 55.5 (OCH$_3$), 121.3, 125.1, 126.6, 127.3, 128.7, 129.2, 133.7, 135.1, 151.5 (Ar-C), 161.1, (C=O), 184.3 (C=S). MS (*m*/*z*): 298 [M$^+$]. Anal. ($C_{16}H_{14}N_2O_2S$, 298.35) C, 64.41 (64.62); H, 4.73 (4.89); N, 9.39 (9.17); S, 10.75 (10.94).

4.1.1.7. 3-Allyl-2,3-dihydro-6-nitro-2-thioxoquinazolin-4(1*H*)-one 3g[12,13]. Yield (82%); mp 197–199 °C; IR (KBr) *ν*: 3277 (NH), 1680 (C=O), 1258 cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 3.82–3.81 (d, 2H, J = 3.6 Hz, NCH_2), 5.15–5.14 (d, 2H, J = 4.9 Hz, Allyl-CH_2), 5.88–5.87 (m, 1H, Allyl-C*H*), 7.62–7.61 (dd, J_1 = 7.0 Hz, J_2 = 3.8 Hz, 2H, Ar-*H*), 7.98 (s, 1H, Ar-*H*), 10.87 (s, D$_2$O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 39.0 (NCH$_2$), 122.5, 123.7, 127.1, 129.5, 133.6, 134.5, 136.3, 137.2, 154.9 (Ar-C), 159.8, (C=O), 185.7 (C=S). MS (*m*/*z*): 263 [M$^+$]. Anal. ($C_{11}H_9N_3O_3S$, 263.27) C, 50.18 (49.98); H, 3.45 (3.69); N, 15.96 (16.17); S, 12.18 (11.89).

4.1.1.8. 3-Benzyl-2,3-dihydro-6-nitro-2-thioxoquinazolin-4(1*H*)-one 3h[12,13]. Yield (89%); mp 185–183 °C; IR (KBr) *ν*: 3295 (NH), 1684 (C=O), 1252 (C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 4.34 (s, 2H, NCH_2), 7.60–7.59 (m, 3H, Ar-*H*),

7.67–7.65 (d, J = 7.0, 2H, Ar-H), 7.98–7.97 (d, J = 7.7, 2H, Ar-H), 8.11 (s, 1H, Ar-H), 10.98 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 44.3 (NCH_2), 121.5, 125.7, 126.4, 127.2, 128.5, 129.1, 133.7, 136.2, 138.3, 152.5 (Ar-C), 161.8 (C=O), 184.5. MS (m/z): 313 [M$^+$]. Anal. ($C_{15}H_{11}N_3O_3S$, 313.33) C, 57.50 (57.36); H, 3.54 (3.77); N, 13.41 (3.28); S, 10.23 (10.14).

4.1.2. General procedure for preparation of 2-thioxo-3-substituted-5, 6 or 8-substituted-3*H*-quinazolin-4-thiones 4a–e[12,13]

A mixture of 2-thioxo-3-benzyl-3*H*-quinazolin-4-one **3a–e** derivative, **1a–e** (0.015 mol) and phosphorus penta-sulfide (4.0 g, 0.018 mol) in xylene (50 ml) was heated under reflux for 5 h. The reaction mixture was filtered while hot, the filtrate was cooled and the separated solid was washed with water, filtered, dried and crystallized from dimethylformamide to give the title compounds.

4.1.2.1. 3-Allyl-2,3-dihydro-5-methyl-2-thioxoquinazolin-4(1*H*)-thione 4a[12,13]. Yield (54%); mp 207–209 °C; IR (KBr) ν: 3261 (NH), 1285, 1235 (2C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 4.41–4.40 (d, 2H, J = 4.5 Hz, NCH_2), 5.13–5.12 (d, 2H, J = 4.8 Hz, Allyl-CH_2), 5.93–5.92 (m, 1H, Allyl-CH), 6.75–6.74 (d, J = 7.2 Hz, 1H, ArH), 6.96–6.97 (d, J = 7.5 Hz, 1H, ArH), 7.21–7.22 (t, J = 7.0 Hz, 1H, ArH), 11.92 (s, 1H, NH), ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.1 (CH_3), 41.5 (NCH_2), 118.0, 125.5, 125.6, 126.2, 127.1, 127.7, 128.0, 138.5, 139.5 (Ar-C), 176.5, 179.5 (2C=S). MS (m/z): 248 [M$^+$]. Anal. ($C_{12}H_{12}N_2S_2$, 248.30) C, 58.03 (57.81); H, 4.87 (4.65); N, 11.28 (10.99); S, 25.82 (26.05).

4.1.2.2. 3-Allyl-2,3-dihydro-6-methyl-2-thioxoquinazolin-4(1*H*)-thione 4b[12,13]. Yield (42%); mp 211–213–185 °C; IR (KBr) ν: 3260 (NH), 1282, 1240 (2C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 4.41–4.40 (d, 2H, J = 4.8 Hz, NCH_2), 5.13–5.11 (d, 2H, J = 4.3 Hz, Allyl-CH_2), 5.93–5.92 (m, 1H, Allyl-CH), 6.77–6.75 (d, J = 10.1 Hz, 1H, ArH), 6.96–6.97 (d, J = 7.4 Hz, 1H, ArH), 7.21–7.23 (t, J = 7.7 Hz, 1H, ArH), 11.95 (s, 1H, NH), ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.0 (CH_3), 41.3 (NCH_2), 118.2, 125.1, 125.6, 126.0, 127.0, 127.7, 128.4, 138.4, 139.5 (Ar-C), 174.5, 179.5 (2C=S). MS (m/z): 248 [M$^+$]. Anal. ($C_{12}H_{12}N_2S_2$, 248.30) C, 58.03 (57.85); H, 4.87 (4.69); N, 11.28 (11.19); S, 25.82 (26.02).

4.1.2.3. 3-Benzyl-2,3-dihydro-6-methyl-2-thioxoquinazolin-4(1*H*)-thione 4c[12,13]. Yield (46%); mp 162–164 °C; IR (KBr) ν: 3284 (NH), 1286, 1253 (2C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.37 (s, 3H, CH_3), 4.45–4.43 (d, 2H, J = 3.8 Hz, NCH_2), 7.64–7.63 (m, 3H, Ar-H), 7.67–7.66 (d, J = 4.6, 2H, Ar-H), 7.88–7.87 (d, J = 8.6, 2H, Ar-H), 8.05 (s, 1H, Ar-H), 11.52 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 17.1 (CH_3), 44.5 (NCH_2), 120.8, 125.1, 126.6, 127.8, 128.5, 129.4, 133.1, 136.9, 137.5, 151.8 (Ar-C), 176.3, 178.7 (2C=S). MS (m/z): 298 [M$^+$]. Anal. ($C_{16}H_{14}N_2S_2$, 282.36) C, 64.39 (64.22); H, 4.73 (4.52); N, 9.39 (9.24); S, 21.49 (21.65).

4.1.2.4. 3-Benzyl-2,3-dihydro-8-methyl-2-thioxoquinazolin-4(1*H*)-thione 4d[12,13]. Yield (45%); mp 160–162 °C; IR (KBr) ν: 3271 (NH), 1277, 1251 (2C=S) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.36 (s, 3H, CH_3), 4.46–4.44 (d, 2H, J = 3.1 Hz, NCH_2), 7.74–7.72 (m, 3H, Ar-H), 7.87–7.85 (d, J = 5.5, 2H, Ar-H), 7.93–7.92 (d, J = 8.2, 2H, Ar-H), 8.15 (s, 1H, Ar-H), 11.83 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 17.1 (CH_3), 44.4 (NCH_2), 120.2, 125.0, 126.5, 127.1, 128.6, 129.3, 133.4, 136.8, 137.4, 152.1 (Ar-C), 177.3, 179.7 (2C=S). MS (m/z): 298 [M$^+$]. Anal. ($C_{16}H_{14}N_2S_2$, 282.36) C, 64.39 (64.25); H, 4.73 (4.57); N, 9.39 (9.26); S, 21.49 (21.61).

4.1.2.5. 3-Benzyl-2,3-dihydro-6-methoxy-2-thioxoquinazolin-4(1*H*)-thione 4e[12,13]. Yield (46%); mp 160–162 °C; IR (KBr) ν: 3274 (NH), 1275, 1222 (2C=S) cm^{-1}; ^{1}H NMR (400 MHz, DSMO-d_6) δ 3.73 (s, 3H, OCH_3), 4.22–4.23 (d, J = 7.0 Hz, 2H, NCH_2), 7.63–7.61 (m, 3H, Ar-H), 7.68–7.69 (d, J = 7.1 Hz, 2H, Ar-H), 7.84–7.82 (d, J = 9.3 Hz, 2H, Ar-H), 8.15 (s, 1H, Ar-H), 11.35 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 44.6 (NCH_2), 55.8 (OCH_3), 121.3, 125.4, 127.1, 127.4, 128.1, 129.4, 133.5, 135.6, 151.1, (Ar-C), 172.9, 180.2 (2C=S). MS (m/z): 314 [M$^+$]. Anal. ($C_{16}H_{14}N_2OS_2$, 298.35) C, 61.12 (60.91); H, 4.49 (4.31); N, 8.91 (9.20); S, 20.40 (20.67).

4.1.3. Synthesis of 2-chloro-*N*-(4-sulfamoylphenyl)-acetamide 7[16]

To a solution of sulfanilamide **5** (1.72 gm, 0.01 mol) and K_2CO_3 (2.76 g, 0.02 mol) in acetone (20 mL), 2-chloroacetyl chloride **6** (1.354 g, 0.012 mol) was added drop-wise. The reaction mixture was stirred at 0 °C for 0.5 h then left to warm to room temperature. The progress of the reaction was monitored by TLC till completion. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to afford the crude product, which was washed with water and crystallized from ethanol to afford analytically pure product of compound **7**.

Compound **7**: Yield (95%); mp 236–238 °C; IR ν 3347 (NH), 1682 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): 4.33 (s, 2H, CH_2), 7.31 (s, 2H, SO_2NH_2), 8.01–8.03 (J = 9.0, 2H, Ar-H), 8.07–8.09 (J = 8.5, 2H, Ar-H), 10.66 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 15.8 (CH_3), 27.6 (SCH_2), 44.6 (NCH_2), 120.1, 127.8, 140.1, 142.4 (Ar-C), 166.3 (C=O); MS m/z (Rel. Int.) 248 (M$^+$). Anal. ($C_8H_9ClN_2O_3S$, 248.68) C, 38.64 (38.35); H, 3.65 (3.81); N, 11.26 (11.05); S, 12.89 (13.06).

4.1.4. General procedure for preparation of 2-substitiuted thioxo-3-substituted-5, 6 or 8-substituted-3*H*-quinazolin-4-ones 8a–h and 9a–e

To a solution of compound **7** (2.48 g, 0.01 mol) in DMF (10 mL), the appropriate thioxoquinazolin-4-one (0.01 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) were added and the mixture was stirred at room temperature for 12 h. Water (15 mL) was added and the mixture was stirred for further 30 min. The separated solid was filtered, washed with cold water, dried and crystallized from absolute ethanol.

4.1.4.1. *N*-4-Aminosulphonylphenyl-2-(3-allyl-4-oxo-6-methyl-quinazolin-2ylthio)-acetamide 8-a. Yield (72%); mp 287–289 °C; IR ν 3343–3297 (NH, NH_2), 1714, 1688 (2C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.23 (s, 3H, CH_3), 3.72 (s, 2H, SCH_2), 3.83–3.82 (d, 2H, J = 4.3 Hz, NCH_2), 5.14–5.13 (d, 2H, J = 4.7 Hz, Allyl-CH_2), 5.92–5.90 (m, 1H, Allyl-CH), 6.28 (s, D_2O exchangeable, 1H, NH_2), 7.64–7.62 (m, 3H, Ar-H), 7.94–7.93 (d, J = 8.7, 2H, Ar-H), 8.13–8.12 (d, J = 7.0, 2H, Ar-H), 10.72 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 15.8 (CH_3), 27.6 (SCH_2), 42.5 (NCH_2), 117.7, 120.3, 122.4, 123.9, 127.5, 129.8, 132.4, 134.4, 136.7, 137.4, 141.0, 144.5, 154.7 (Ar-C), 161.6, 165.8 (2C=O); MS m/z (Rel. Int.) 444 (M$^+$). Anal. ($C_{20}H_{20}N_4O_4S_2$, 444.52) C, 54.04 (54.15); H, 4.53 (4.28); N, 12.60 (12.73); S, 14.43 (14.26).

4.1.4.2. *N*-4-Aminosulphonylphenyl-2-(3-allyl-4-oxo-8-methyl-quinazolin-2yl-thio)-acetamide 8b. Yield (89%); mp 283–285 °C; IR ν 3345–3291 (NH, NH_2), 1725, 1690 (2C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.22 (s, 3H, CH_3), 3.73 (s, 2H, SCH_2), 3.88 (d, 2H, J = 3.2 Hz, NCH_2), 5.16 (d, 2H, J = 4.8 Hz,Allyl-CH_2), 5.92 (m, 1H, Allyl-CH), 6.27 (s, D_2O exchangeable, 1H, NH_2), 7.65–7.62 (d, J = 7.5, 2H, Ar-H), 7.95–7.94 (d, J = 8.6, 2H, Ar-H), 8.13–8.11 (d, J = 8.4, 2H, Ar-H), 8.26 (s, 1H, Ar-H), 10.56 (s, D_2O exchangeable, 1H, NH); ^{13}C NMR (DSMO-d_6) δ 15.5 (CH_3), 26.9 (SCH_2), 40.8

(NCH_2), 117.3, 120.5, 122.1, 123.0, 127.4, 129.5, 132.7, 134.9, 136.4, 137.8, 141.6, 144.7, 154.3 (Ar-C), 160.7, 166.8 (2C=O); MS *m/z* (Rel. Int.) 444 (M^+, 100). Anal. ($C_{20}H_{20}N_4O_4S_2$, 444.52) C, 54.04 (53.88); H, 4.53 (4.78); N, 12.60 (12.49); S, 14.43 (14.57).

4.1.4.3. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-oxo-6-methyl-quinazolin-2yl-thio)-acetamide 8c. Yield (86%); mp 258–260 °C; IR *ν* 3346–3295 (NH, NH_2), 1728, 1685 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 2.26 (s, 3H, CH_3), 3.83 (s, 2H, SCH_2), 4.47 (s, 2H, NCH_2), 6.27 (s, D_2O exchangeable, 2H, NH_2), 7.67–7.64 (m, 5H, Ar-*H*), 7.67–7.64 (d, *J* = 7.6, 2H, Ar-*H*), 7.98–7.97 (d, *J* = 8.6, 2H, Ar-*H*), 8.12–8.10 (d, *J* = 8.5, 2H, Ar-*H*), 8.25 (s, 1H, Ar-*H*), 10.77 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 15.6 (CH_3), 29.1 (SCH_2), 46.8 (NCH_2), 119.3, 120.5, 121.3, 125.4, 126.7, 127.5, 128.7, 129.0, 133.4, 136.0, 138.1, 142.5, 144.8, 154.3 (Ar-C), 161.6, 168.5 (2C=O); MS *m/z* (Rel. Int.) 494 (M^+, 71). Anal. ($C_{24}H_{22}N_4O_4S_2$, 494.58) C, 58.28 (58.35); H, 4.48 (4.27); N, 11.33 (11.40); S, 12.97 (13.22).

4.1.4.4. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-oxo-8-methyl-quinazolin-2yl-thio)-acetamide 8d. Yield (81%); mp 252–254 °C; IR *ν* 3345–3298 (NH, NH_2), 1723, 1689 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 2.22 (s, 3H, CH_3), 3.89 (s, 2H, SCH_2), 4.67 (s, 2H, NCH_2), 6.24 (s, D_2O exchangeable, 1H, NH_2), 7.67–7.65 (m, 3H, Ar-*H*), 7.68–7.65 (d, *J* = 7.5, 2H, Ar-*H*), 7.87–7.85 (d, *J* = 9.0, 2H, Ar-*H*), 8.12–8.10 (d, *J* = 8.5, 2H, Ar-*H*), 8.25–8.23 (m,3H, Ar-*H*), 10.94 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 16.3 (CH_3), 28.5 (SCH_2), 42.9 (NCH_2), 119.7, 120.2, 121.4, 125.1, 126.8, 127.7, 128.2, 129.1, 133.0, 136.5, 138.7, 142.3, 143.9, 152.7 (Ar-C), 160.7, 165.9 (2C=O); MS *m/z* (Rel. Int.) 494 (M^+, 71). Anal. ($C_{24}H_{22}N_4O_4S_2$, 494.58) C, 58.28 (58.31); H, 4.48 (4.59); N, 11.33 (11.14); S, 12.97 (12.71).

4.1.4.5. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-oxo-6-methoxy-quinazolin-2yl-thio)-acetamide 8e. Yield (86%); mp 259–261 °C; IR *ν* 3346–3296 (NH, NH_2), 1727, 1685 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 3.70 (s, 3H, OCH_3), 3.82 (s, 2H, SCH_2), 4.27 (s, 2H, NCH_2), 6.27 (s, D_2O exchangeable, 1H, NH_2), 7.66–7.63 (m, 5H, Ar-*H*), 7.66–7.65 (d, *J* = 7.5, 2H, Ar-*H*), 7.86–7.85 (d, *J* = 7.3, 2H, Ar-*H*), 8.11–8.10 (d, *J* = 7.4, 2H, Ar-*H*), 8.25 (s, 1H, Ar-*H*), 10.92 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 28.8 (SCH_2), 43.6 (NCH_2), 55.9 (OCH_3), 119.3, 121.0, 121.7, 126.0, 127.1, 127.6, 128.4, 129.5, 133.8, 135.1, 141.8, 151.3, 155.2 (Ar-C), 161.4, 166.2 (2C=O); MS *m/z* (Rel. Int.) 510 (M^+, 75). Anal. ($C_{24}H_{22}N_4O_5S_2$, 510.58) C, 56.46 (56.30); H, 4.34 (4.57); N, 10.97 (10.85); S, 12.56 (12.24).

4.1.4.6. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-oxo-8-methoxy-quinazolin-2yl-thio)-acetamide 8f. Yield (86%); mp 263–265 °C; IR *ν* 3339–3299 (NH, NH_2), 1724, 1685 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 3.79 (s, 3H, OCH_3), 3.83 (s, 2H, SCH_2), 4.29 (s, 2H, NCH_2), 6.27 (s, D_2O exchangeable, 1H, NH_2), 7.67–7.64 (m, 3H, Ar-*H*), 7.67–7.66 (d, *J* = 7.6, 2H, Ar-*H*), 7.88–7.86 (d, *J* = 8.7, 2H, Ar-*H*), 8.12–8.11 (d, *J* = 8.6, 2H, Ar-*H*), 8.24–8.22 (m, 3H, Ar-*H*), 10.95 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 28.9 (SCH_2), 43.2 (NCH_2), 56.3 (OCH_3), 119.1, 121.0, 121.6, 125.9, 127.0, 127.5, 128.3, 129.7, 133.9, 135.7, 142.4, 150.3, 153.2 (Ar-C), 161.1, 166.7 (2C=O); MS *m/z* (Rel. Int.) 510 (M^+, 71). Anal. ($C_{24}H_{22}N_4O_5S_2$, 510.58) C, 56.46 (56.53); H, 4.34 (4.47); N, 10.97 (11.20); S, 12.56 (12.74).

4.1.4.7. *N*-4-Aminosulphonylphenyl-2-(3-allyl-4-oxo-6-nitro-quinazolin-2yl-thio)-acetamide 8g. Yield (82%); mp 297–299 °C; IR *ν* 3347–3295 (NH, NH_2), 1727, 1687 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 3.72 (s, 2H, SCH_2), 3.85 (d, 2H, *J* = 3.8 Hz, NCH_2), 5.15 (d, 2H, *J* = 4.9 Hz, Allyl-CH_2), 5.88 (m, 1H, Allyl-C*H*), 6.29 (s, D_2O exchangeable, 1H, NH_2), 7.65–7.63 (d, *J* = 7.3, 2H, Ar-*H*), 7.96–7.95 (d, *J* = 8.6, 2H, Ar-*H*), 8.13–8.12 (d, *J* = 8.0, 2H, Ar-*H*), 8.26 (s, 1H, Ar-*H*), 10.56 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 28.0 (SCH_2), 38.9 (NCH_2), 118.1, 121.0, 122.3, 123.5, 127.3, 129.6, 133.1, 134.2, 136.7, 137.2, 141.0, 144.3, 154.9 (Ar-C), 159.8, 166.1 (2C=O); MS *m/z* (Rel. Int.) 475 (M^+, 82). Anal. ($C_{19}H_{17}N_5O_6S_2$, 475.49) C, 47.99 (48.16); H, 3.60 (3.82); N, 14.73 (14.96); S, 13.49 (13.37).

4.1.4.8. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-oxo-6-nitro-quinazolin-2yl-thio)-acetamide 8h. Yield (69%); mp 221–223 °C; IR *ν* 3346–3295 (NH, NH_2), 1728, 1685 (2C=O) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 3.81 (s, 2H, SCH_2), 4.36 (s, 2H, NCH_2), 6.25 (s, D_2O exchangeable, 1H, NH_2), 7.66–7.64 (m, 5H, Ar-*H*), 7.67–7.65 (d, *J* = 7.0, 2H, Ar-*H*), 7.98–7.97 (d, *J* = 7.7, 2H, Ar-*H*), 8.12–8.10 (d, *J* = 6.6, 2H, Ar-*H*), 8.24 (s, 1H, Ar-*H*), 10.77 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 28.2 (SCH_2), 44.9 (NCH_2), 119.4, 120.5, 121.3, 125.4, 126.7, 127.5, 128.7, 129.0, 133.4, 136.0, 138.1, 142.5, 144.8, 154.3 (Ar-C), 161.6, 168.5 (2C=O); MS *m/z* (Rel. Int.) 525 (M^+, 82). Anal. ($C_{23}H_{19}N_5O_6S_2$, 525.56) C, 52.56 (52.67); H, 3.64 (3.85); N, 13.33 (13.52); S, 12.20 (11.95).

4.1.4.9. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-thioxo-5-methyl-quinazolin-2yl-thio)-acetamide 9a. Yield (70%); mp 255–57 °C; IR *ν* 3344–3288 (NH, NH_2), 1681 (C=O), 1273 (C=S), cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 2.29 (s, 3H, CH_3), 3.83 (s, 2H, SCH_2), 4.33 (s, 2H, NCH_2), 6.23 (s, D_2O exchangeable, 1H, NH_2), 7.62–7.61 (m, 3H, Ar-*H*), 7.66–7.65 (d, *J* = 8.1, 2H, Ar-*H*), 7.95–7.94 (d, *J* = 7.0, 2H, Ar-*H*), 8.11–8.09 (d, *J* = 7.1, 2H, Ar-*H*), 8.25–8.23 (m, 3H, Ar-*H*), 10.77 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 16.2 (CH_3), 27.3 (SCH_2), 45.5 (NCH_2), 118.7, 119.6, 120.8, 126.7, 127.0, 127.6, 128.6, 129.1, 138.6, 141.8, 146.9, 151.2, 159.5 (Ar-C), 160.0, 166.2 (2C=O); MS *m/z* (Rel. Int.) 510 (M^+, 68). Anal. ($C_{24}H_{22}N_4O_3S_3$, 510.65) C, 56.45 (56.59); H, 4.34 (4.51); N, 10.97 (11.18); S, 18.84 (18.97).

4.1.4.10. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-thioxo-6-methyl-quinazolin-2yl-thio)-acetamide 9b. Yield (73%); mp 242–244 °C; IR *ν* 3348–3298 (NH, NH_2), 1688 (C=O), 1275 (C=S) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 2.36 (s, 3H,CH_3), 3.77 (s, 2H, SCH_2), 3.85 (s, 2H, NCH_2), 7.65–7.63 (m, 5H, Ar-*H*), 7.66–7.64 (d, *J* = 7.6, 2H, Ar-*H*), 7.95–7.93 (d, *J* = 8.0, 2H, Ar-*H*), 8.12–8.11 (d, *J* = 6.5, 2H, Ar-*H*), 8.24 (s, 1H, Ar-*H*), 10.79 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 16.1 (CH_3), 27.3 (SCH_2), 47.5 (NCH_2), 118.7, 119.6, 120.8, 126.7, 127.0, 127.6, 128.6, 129.1, 138.6, 141.8, 146.9, 151.2, 159.5 (Ar-C), 160.0 (C=O), 186.5 (C=S); MS *m/z* (Rel. Int.) 510 (M^+, 100). Anal. ($C_{24}H_{22}N_4O_3S_3$, 510.65) C, 56.45 (56.60); H, 4.34 (4.15); N, 10.97 (11.13); S, 18.84 (18.99).

4.1.4.11. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-thioxo-8-methyl-quinazolin-2yl-thio)-acetamide 9c. Yield (76%); mp 265–67 °C; IR *ν* 3344–3295 (NH, NH_2), 1686 (C=O), 1279 (C=S) cm^{-1}; ^1H NMR (DSMO-d_6) *δ* 2.25 (m, 3H, CH_3), 3.75 (s, 2H, SCH_2), 3.86 (s, 2H, NCH_2), 7.64–7.60 (m, 3H, Ar-*H*), 7.65–7.64 (d, *J* = 7.0, 2H, Ar-*H*), 7.95–7.94 (d, *J* = 7.1, 2H, Ar-*H*), 8.12–8.10 (d, *J* = 7.3, 2H, Ar-*H*), 8.27–8.25 (m, 3H, Ar-*H*), 10.83 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) *δ* 16.5 (CH_3), 27.2S(CH_2), 46.1 (NCH_2), 119.2, 119.9, 121.4, 126.1, 127.1, 127.7, 128.5, 129.2, 138.9, 142.0, 147.3, 151.2, 159.4 (Ar-C), 163.4 (C=O), 184.9 (C=S); MS *m/z* (Rel. Int.) 510 (M^+, 100). Anal. ($C_{24}H_{22}N_4O_3S_3$, 510.65) C, 56.45 (56.54); H, 4.34 (4.44); N, 10.97 (10.81); S, 18.84 (19.05).

4.1.4.12. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-thioxo-8-methoxy-quinazolin-2yl-thio)-acetamide 9d. Yield (76%); mp 245–47 °C; IR ν 3344–3298 (NH, NH_2), 1689 (C=O), 1276 (C=S) cm^{-1}; ^1H NMR (DSMO-d_6) δ 3.76 (s, 3H, OCH_3), 3.81 (s, 2H, SCH_2), 4.31 (s, 2H, NCH_2), 6.25 (s, D_2O exchangeable, 1H, NH_2), 7.65–7.63 (m, 3H, Ar-*H*), 7.66–7.65 (d, J = 7.5, 2H, Ar-*H*), 7.88–7.87 (d, J = 8.1, 2H, Ar-*H*), 8.12–8.11 (d, J = 8.3, 2H, Ar-*H*), 8.26–8.25 (m, 3H, Ar-*H*), 10.91 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 28.1 (SCH_2), 43.0 (NCH_2), 55.4 (OCH_3), 120.6, 121.1, 121.9, 125.5, 127.1, 127.7, 128.3, 129.6, 133.2, 135.7, 142.6, 151.0, 154.6 (Ar-C), 160.7 (C=O), 188.5 (C=S); MS m/z (Rel. Int.) 526 (M^+, 74). Anal. ($C_{24}H_{22}N_4O_4S_3$, 526.65) C, 54.73 (54.50); H, 4.21 (4.40); N, 10.64 (10.45); S, 18.27 (17.98).

4.1.4.13. *N*-4-Aminosulphonylphenyl-2-(3-benzyl-4-thioxo-6-nitro-quinazolin-2yl-thio)-acetamide 9e. Yield (76%); mp 239–241 °C; IR ν 3341–3296 (NH, NH_2), 1687 (C=O),1277 (C=S), cm^{-1}; ^1H NMR (DSMO-d_6) δ 3.49 (s, 2H, SCH_2),4.67 (s, 2H, NCH_2), 6.24 (s, D_2O exchangeable, 1H, NH_2), 7.67–7.65 (m, 5H, Ar-*H*), 7.68–7.65 (d, J = 7.5, 2H, Ar-*H*), 7.87–7.85 (d, J = 9.0, 2H, Ar-*H*), 8.12–8.10 (d, J = 8.5, 2H, Ar-*H*), 8.26 (s, 1H, Ar-*H*), 10.94 (s, D_2O exchangeable, 1H, N*H*); ^{13}C NMR (DSMO-d_6) δ 28.5 (SCH_2), 42.9 (NCH_2), 119.7, 120.2, 121.4, 125.1, 126.8, 127.7, 128.2, 129.1, 133.0, 136.5, 138.7, 142.3, 143.9, 152.7 (Ar-C), 160.7 (C=O), 187.2 (C=S); MS m/z (Rel. Int.) 541 (M^+, 68). Anal. ($C_{23}H_{19}N_5O_5S_3$, 541.65) C, 51.00 (51.24); H, 3.54 (3.48); N, 12.93 (13.10); S, 17.76 (17.51).

4.2. CA inhibition studies

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[17] 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) and 20 mM $NaBF_4$ (for maintaining constant the ionic strength), 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 inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (10 mM) were prepared in DMSO and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at RT 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 PRISM 3, and the Cheng–Prusoff equation, as reported earlier,[18–20] and represent the mean from at least three different determinations. All CAs were recombinant proteins obtained as reported earlier by these groups.[18–20]

Acknowledgments

This research was financed by the National Plan of Science, Technology and Innovation (Grant No. 11-MED1874-02), King Saud University, Riyadh (to AF).

References and notes

1. Basel, H. A.; Jeffrey, H. W. *Infect. Immun.* **2009**, 4111.
2. Zuckerman, J. N.; Rombo, L.; Fisch, A. *Lancet Infect. Dis.* **2007**, *7*, 521.
3. Matson, J. S.; Withey, J. H.; DiRita, V. J. *Infect. Immun.* **2007**, *75*, 5542.
4. Chatterjee, A.; Dutta, P. K.; Chowdhury, R. *Infect. Immun.* **1946**, *2007*, 75.
5. Fan, F.; Liu, Z.; Jabeen, N.; Birdwell, L. D.; Zhu, J.; Kan, B. *Infect. Immun.* **2014**, *82*, 1676.
6. Yu, E. S.; Krukonis, R. R.; Dirita, V. J. *Mol. Microbiol.* **2000**, *38*, 67.
7. (a) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168; (b) De Simone, G.; Alterio, V.; Supuran, C. T. *Expert Opin. Drug Discov.* **2013**, *8*, 793; (c) Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3467; (d) Harju, A. K.; Bootorabi, F.; Kuuslahti, M.; Supuran, C. T.; Parkkila, S. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 231.
8. (a) Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 759; (b) Alterio, V.; Di Fiore, A.; D'Ambrosio, K.; Supuran, C. T.; De Simone, G. *Chem. Rev.* **2012**, *112*, 4421; (c) Supuran, C. T. *Expert Opin. Ther. Pat.* **2013**, *23*, 677; (d) Pinard, M. A.; Boone, C. D.; Rife, B. D.; Supuran, C. T.; McKenna, R. *Bioorg. Med. Chem.* **2013**, *21*, 7210; (e) Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 229; (f) Capasso, C.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 379.
9. (a) Del Prete, S.; Isik, S.; Vullo, D.; De Luca, V.; Carginale, V.; Scozzafava, A.; Supuran, C. T.; Capasso, C. *J. Med. Chem.* **2012**, *55*, 10742; (b) De Del Prete, S.; Luca, V.; Scozzafava, A.; Carginale, V.; Supuran, C. T.; Capasso, C. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 23.
10. (a) Çavdar, H.; Ekinci, D.; Talaz, O.; Saraçoğlu, N.; Şentürk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 148; (b) Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 259; (c) Fabrizi, F.; Mincione, F.; Somma, T.; Scozzafava, G.; Galassi, F.; Masini, E.; Impagnatiello, F.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 138; (d) Ekinci, D.; Karagoz, L.; Ekinci, D.; Senturk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 283; (e) Carta, F.; Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2012**, *22*, 79; (f) Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2013**, *23*, 705.
11. (a) Vullo, D.; Isik, S.; Del Prete, S.; De Luca, V.; Carginale, V.; Scozzafava, A.; Supuran, C. T.; Capasso, C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1636; (b) Alp, C.; Maresca, A.; Alp, N. A.; Gültekin, M. S.; Ekinci, D.; Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 294; (c) Carta, F.; Scozzafava, A.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2012**, *22*, 747; (d) Supuran, C. T. *Expert Opin. Ther. Pat.* **2013**, *23*, 677; (e) Supuran, C. T.; Maresca, A.; Gregáň, F.; Remko, M. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 289; (f) Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 261.
12. Al-Omar, M. A.; Abdel-Hamide, S. G.; Hamad, A. A.; El-Subbagh, I. E. *Saudi Pharm. J.* **2004**, *12*, 63.
13. Del Giudice, M. R.; Borioni, A.; Mustazza, C.; Gatta, F. *J. Heterocycl. Chem.* **1994**, *31*, 1503.
14. Castaño, T.; Wang, H.; Campillo, N. E.; Ballester, S.; González-García, C.; Hernández, J.; Pérez, C.; Cuenca, J.; Pérez-Castillo, A.; Martínez, A.; Huertas, O.; Gelpí, J. L.; Luque, F. J.; Ke, H.; Gil, C. *ChemMedChem* **2009**, *4*, 866.
15. Sánchez, A. I.; Martínez-Barrasa, V.; Burgos, C.; Vaquero, J. J.; Alvarez-Builla, J.; Terricabras, E.; Segarra, V. *Bioorg. Med. Chem.* **2013**, *21*, 2370.
16. Gong-Bao, W.; Lin-Fa, W.; Chao-Zhang, L.; Jing, S.; Guang-Ming, Z.; Da-Cheng, Y. *Res. Chem. Intermed.* **2012**, *38*, 77.
17. Khalifah, R. G. *J. Biol. Chem.* **1971**, *246*, 2561.
18. (a) Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. *Eur. J. Med. Chem.* **1998**, *33*, 247; (b) Carta, F.; Supuran, C. T. *Expert Opin. Ther. Pat.* **2013**, *23*, 681; (c) Scozzafava, A.; Supuran, C. T.; Carta, F. *Expert Opin. Ther. Pat.* **2013**, *23*, 725; (d) Gieling, R. G.; Parker, C. A.; De Costa, L. A.; Robertson, N.; Harris, A. L.; Stratford, I. J.; Williams, K. J. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 360.
19. (a) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S. A. *J. Med. Chem.* **2007**, *50*, 1651; (b) Bonneau, A.; Maresca, A.; Winum, J. Y.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 397; (c) Maresca, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 388; (d) Alp, C.; Özsoy, S.; Alp, N. A.; Erdem, D.; Gültekin, M. S.; Küfrevioğlu, Ö. İ.; Şentürk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 818.
20. (a) Supuran, C. T. *Curr. Med. Chem.* **2012**, *19*, 44; (b) Kazancıoğlu, E. A.; Güney, M.; Şentürk, M.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 880; (c) Liu, F.; Martin-Mingot, A.; Lecornué, F.; Maresca, A.; Thibaudeau, S.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2012**, *27*, 886; (d) Maresca, A.; Scozzafava, A.; Vullo, D.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 384; (e) Koz, O.; Ekinci, D.; Perrone, A.; Piacente, S.; Alankus-Caliskan, O.; Bedir, E.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 412.