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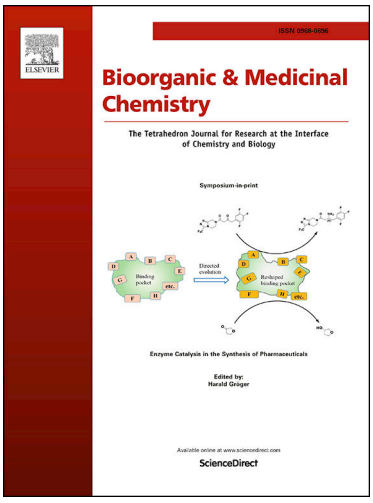

New Thiopyrimidine-Benzenesulfonamide Conjugates as Selective Carbonic Anhydrase II Inhibitors: Synthesis, *in vitro* Biological Evaluation, and Molecular Docking Studies

Heba T. Abdel-Mohsen, Ahmed M. El Kerdawy, Mohamed A. Omar, Emanuela Berrino, Ahmed S. Abdelsamie, Hoda I. El Diwani, Claudiu T. Supuran

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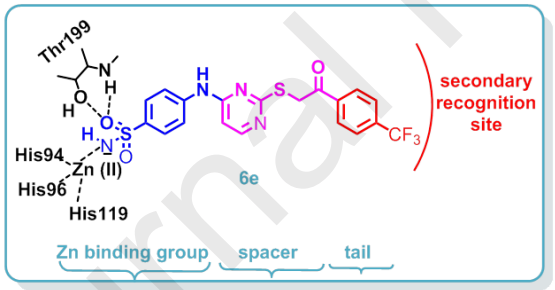
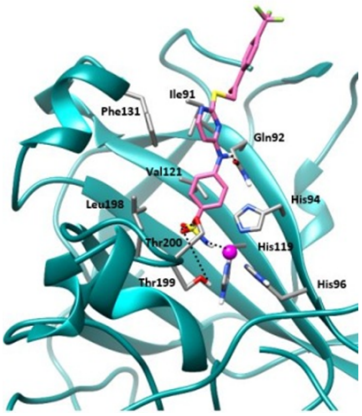


Compd	hCA II K_i (µM)	Selectivity ratio for hCA II		
		I/II	IX/II	XII/II
6e	0.04	21.8	980	22.5



6e in CA II active site

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ABSTRACT

In the present work, a new series of thiopyrimidine-benzenesulfonamide conjugates was designed, synthesized and tested as carbonic anhydrase (CA, EC 4.2.1.1) inhibitors. Our design strategy was based on the molecular hybridization of the benzenesulfonamide moiety as a zinc binding group (ZBG), an alkylated thiopyrimidine moiety as a spacer and (un)substituted phenyl moieties with various electronic and hydrophobic environments as a tail. The designed and synthesized compounds were evaluated against four human (h) CA isoforms hCA I, hCA II, hCA IX and hCA XII. Series **6** showed promising activity and selectivity toward the cytosolic isoforms hCA I and hCA II versus the membrane bound isoforms hCA IX and hCA XII. Compounds **6e** and **6f** showed K_i of 0.04 µM against hCA II with a selectivity of 15.8- to 980-fold towards hCA II over hCA I, hCA IX, hCA XII isoforms. Molecular docking in the hCA II active site attributed the promising inhibitory activity of series **6** to the interaction of their sulfonamide moiety with the active site Zn^{2+} ion as well as its hydrogen bonding with the key amino acids Thr199 and Thr200. Through hydrophobic interaction, the benzenesulfonamide and the thiopyrimidine moieties interact with the hydrophobic side chains of the amino acids Val121/Leu198 and Ile91/Phe131, respectively. These results indicated that the designed and synthesized series is an interesting scaffold that can be further optimized for the development of selective antiglaucoma drugs.

1. Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are a class of metalloenzymes that use zinc as a metal cofactor to catalyze the reversible inter-conversion of carbon dioxide and bicarbonate ion [1,2]. To date more than sixteen human carbonic anhydrase (hCA) isoforms have been discovered [3,4]. They have different molecular features, organs and tissues distribution, expression levels as well as different kinetic properties [5]. For instance, CA I, CA II, CA III and CA XIII isoforms are present in the cytosol; while CA IV, CA IX, CA XII, CA XIV and CA XV are membrane bound. Besides, CA VA and CA VB are present in the mitochondria [3-5]. hCAs are involved in different physiological as well as pathological processes [6-8]. Thus, they are considered a well-established therapeutic target for treatment of a wide range of pathological disorders [9-11]. For instance, CA II contributes in the regulation of the bicarbonate concentration in the eyes; hence, its inhibition is regarded as a successful strategy for the intraocular

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extracellular isoforms CA IX and CA XII are strongly upregulated in different cancers such as breast, renal, colorectal, and non-small cell lung cancer in response to hypoxia. They play an outstanding role in controlling the cellular pH system that protects the tumor cells from the hypoxia-induced acidosis; hence, maintain cell viability and proliferation. For that reason, targeting CA IX and CA XII is proposed to be a promising approach for the development of novel anticancer agents for the treatment of hypoxic tumors [15-19].

Generally, the catalytic active site of mammalian α-CAs is a cone-shaped cavity that is partitioned into two conserved environments, hydrophobic and hydrophilic walls, with a Zn^{2+} ion at the bottom. The Zn^{2+} ion is tetrahedrally coordinated with three histidine residues (His94, His96, and His119) and a solvent molecule; it can bind to hydroxide ion followed by reaction with carbon dioxide to yield bicarbonate [20-22]. In this way, CA inhibitors should contain certain group, zinc binding group (ZBG), that can successfully coordinate with the binding site Zn^{2+} ion. ZBG is coupled with variable chemical moieties which are known as the compound "tail" that facilitate the interactions with specific residues within the active site. Although the carbonic anhydrase binding site is highly conserved among the different isoforms, there is variability in the polarity and hydrophobicity of its peripheral part [20-24].

Over the years, sulfonamides and their bioisosteres, v*iz.*, sulfamates and sulfamides, are considered as the main investigated and clinically applied class of hCA inhibitors [11]. Acetazolamide (AAZ) (**I**), methazolamide (**II**), ethoxzolamide (**III**), dichlorphenamide (**IV**), dorzolamide **(V)** and sulthiame (**VI**), are examples of CA inhibitors that are clinically used for the treatment of glaucoma, epilepsy, and obesity [25-30]. Despite this achieved progress, most of the classical sulfonamide-like CA inhibitors are unselective toward the different hCA isoforms. Hence, their long-term application is associated with off-target side effects [25]. Consequently, the development of isozyme-selective inhibitors is regarded as a major challenge and has been under extensive study in the last few years [31].

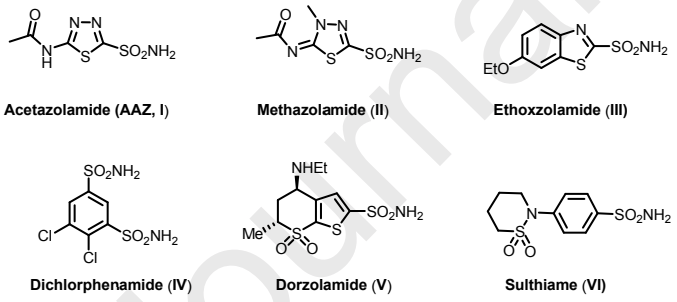


Figure 1. Structures of clinically used CA inhibitors

The knowledge of the CA active site topology presents a prospective strategy for the development of selective inhibitors. The amino acid residues that exist in and around the upper part of the CA active site form several selective binding sub-pockets which can be utilized for designing selective inhibitors. The tail approach, which is based on introducing different moieties to the aromatic sulfonamide ring that selectivity forces the interaction with isoform unique residues in the peripheral part of the active site, is regarded as a successful approach for targeting the CA selectivity [20-24, 32].

Structural hybridization, which covalently links two or more pharmacophores into a single structural framework, is considered a successful approach for the discovery of new scaffolds with

nost common *N*-based heterocyclic systems that play an important role in the different metabolic as well as cellular processes [34]. Therefore, pyrimidines and fused pyrimidines are considered privileged scaffolds for the development of various small molecules with promising therapeutic applications [35-38].

Against this background, some studies reported the structural hybridization of classical CA inhibitors that exhibit a benzenesulfonamide scaffold as ZBG, with the pyrimidine or fused pyrimidine moieties as a suitable tail for the synthesis of different carbonic anhydrase inhibitors of different therapeutic targets [39-41]. Nocentini *et al.* [39] reported the discovery of a new series of antitumor hCA inhibitors by incorporating the uracil or purine moieties to the classical benzenesulfonamide scaffold, for example, compounds **VII** and **VIII** showed K_i = 4.8 and 25.7 nM, respectively, against hCA IX and K_i = 0.85 and 17.7 nM, respectively, against hCA II. In addition, compound **IX** displayed K_i = 8.2 nM against CA II using the stopped flow CO_2 hydration assay [39] (Figure 2). Also, El-Azab *et al.* [40] reported the synthesis and the promising CA inhibitory activity of a series of benzene sulfonamide-quinazoline conjugates. For example, compound **X** revealed K_i = 0.73 nM against CA II (Figure 2). Moreover, Casini *et al.* [41] reported the potent CA inhibitory activity of a series of 4-(2-aminopyrimidin-4-yl-amino)-benzenesulfonamides. For instance, compounds **XI** and **XII** showed K_i = 1 and 4 nM, respectively against CA II and demonstrated a strong topical antiglaucoma properties (Figure 3).

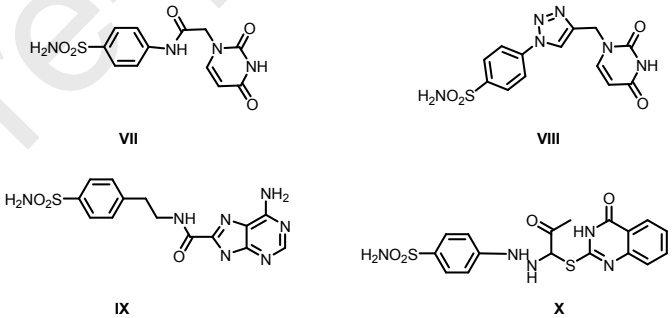


Figure 2. Examples for reported pyrimidine- and fused pyrimidine–sulfonamide conjugates **VII-X** as CA inhibitors

Recently, it was reported that some 2-thioxo-1,2,3,4-tetrahydropyrimidines displayed carbonic anhydrase inhibitory properties [42]. For instance, **XIII** and **XIV** showed K_i of 30.63 and 49.78 nM, respectively, against CA II [42]. Guided by the structures of **XI** and **XII** [41] as well as **XII** and **XIV** [42], isosteric replacement of the 2-pyrimidine benzenesulfonamide moiety of **XI** and **XII** with an alkylated thiopyrimidine moiety was carried out in order to design a hybridized series of pyrimidine-sulfonamide conjugates incorporating primary and secondary benzenesulfonamide scaffold as ZBG, the alkylated thiopyrimidine moiety as a spacer, and 4-(un)substituted phenyl moiety with various electronic and hydrophobic environments as a tail (Figure 3). It was considered for a long time that primary sulfonamides act as the most effective CA inhibitors because of their ability to coordinate the Zn^{2+} ion in the deprotonated form making highly stable enzyme-inhibitor adducts. [26,43]. Recent data confirmed on the other hand the ability of secondary benzenesulfonamides to act as potent and selective hCA inhibitors, in some cases showing comparable activity to that of primary sulfonamides [43]. In CA II, the substitution on the secondary sulfonamide amino group is accommodated in the small hydrophobic pocket defined by Trp209, Val143 and Val121, which represents the CO_2 binding pocket [44-46]. Hence, this

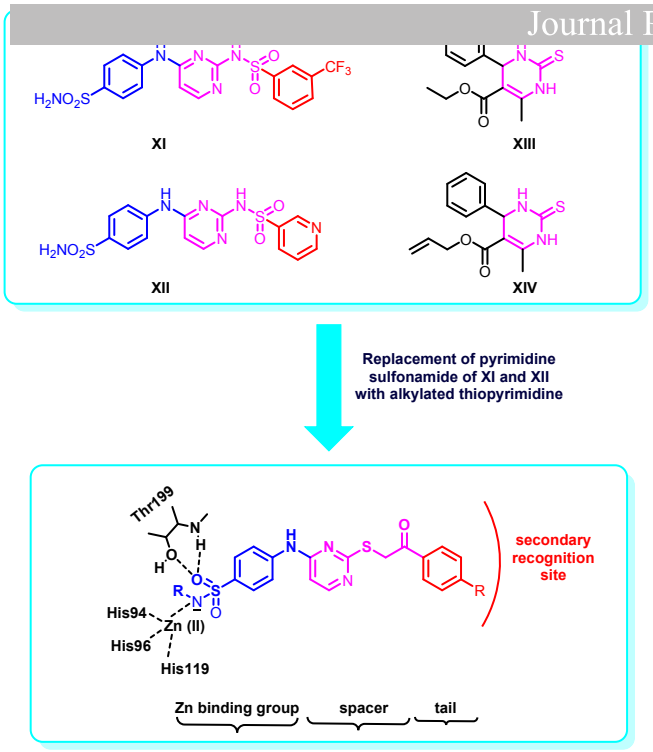


Figure 3. The design strategy for the novel pyrimidine-sulfonamide conjugates as hCA inhibitors

neglected class turned to attract the researchers aiming at the discovery of novel CA inhibitors [43]. Based on these recent facts, we were curious in this study to examine the effect of introducing five different substitutions, namely acetyl, thiazolyl, 5-methylisoxazol-3-yl, pyridyl and pyrimidinyl groups to the corresponding primary sulfonamide. On the other hand, the incorporation of the selected alkylated thiopyrimidine moiety will have a twofold impact on the designed structures a) It affords an additional hydrophobicity to the parent compounds **XI**, **XII** and so stronger binding to the hydrophobic hCA II sub-pockets, and b) It has the required flexibility needed to accommodate the distal 4-(un)substituted phenyl moiety properly in hCA II secondary recognition site. The designed and synthesized conjugates were subsequently evaluated *in vitro* for their inhibitory activity on the cytosolic hCA I and hCA II and on the transmembrane hCA IX and hCA XII isoforms. Moreover, docking simulation studies were carried out to study their binding mode in the hCA II active site and to investigate their structure activity relationship (SAR).

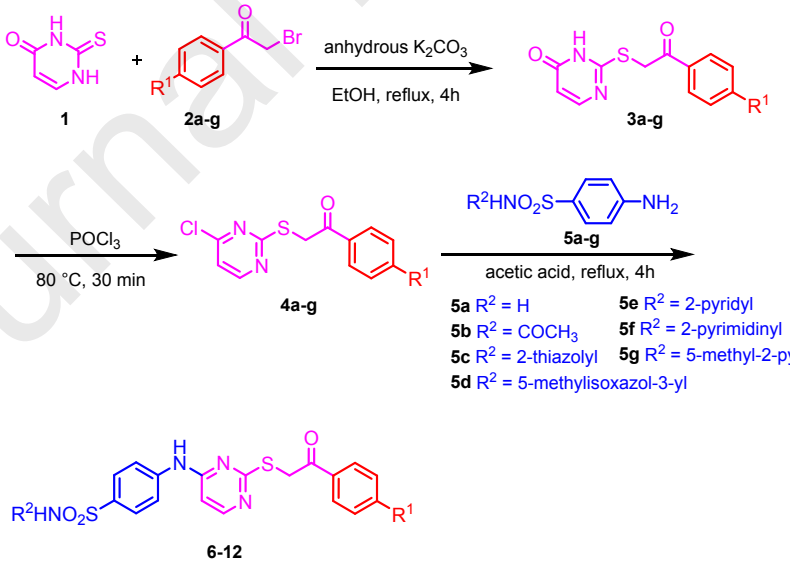
2. Results and discussion

2.1. Chemistry

The synthesis of the target pyrimidine-benzenesulfonamide conjugates **6-12** was carried out through initial reaction of thiouracil (**1**) with different 2-bromoacetophenone derivatives **2a-g** under basic conditions to afford the corresponding intermediates **3** [47,48]. The intermediates **3** were subsequently chlorinated by the reaction with phosphorus oxychloride to give **4** [47]. Reaction of **4** with different benzenesulfonamides in acetic acid under reflux gave the target products **6-12** in good yields.

2.2. Biological evaluation

The synthesized pyrimidine-sulfonamide conjugates **6-12** as well as acetazolamide; AAZ (I), the positive control, were evaluated for their potency to inhibit cytosolic hCA I (associated with edema), cytosolic hCA II (associated with glaucoma), and transmembrane hCA IX and hCA XII (associated with tumors)



5a R^2 = H
5b R^2 = $COCH_3$
5c R^2 = 2-thiazolyl
5d R^2 = 5-methylisoxazol-3-yl
5e R^2 = 2-pyridyl
5f R^2 = 2-pyrimidinyl
5g R^2 = 5-methyl-2-pyrimidinyl

2-4a R^1 = H
2-4b R^1 = F
2-4c R^1 = Cl
2-4d R^1 = Br
2-4e R^1 = CF_3
2-4f R^1 = OCH_3
2-4g R^1 = Ph

6a R^1 = H, R^2 = H
6b R^1 = F, R^2 = H
6c R^1 = Cl, R^2 = H
6d R^1 = Br, R^2 = H
6e R^1 = CF_3, R^2 = H
6f R^1 = OCH_3, R^2 = H
6g R^1 = Ph, R^2 = H
7 R^1 = Cl, R^2 = $COCH_3$
8a R^1 = H, R^2 = 2-thiazolyl
8b R^1 = F, R^2 = 2-thiazolyl
8c R^1 = Cl, R^2 = 2-thiazolyl
8d R^1 = CF_3, R^2 = 2-thiazolyl
8e R^1 = OCH_3, R^2 = 2-thiazolyl

9a R^1 = F, R^2 = 5-methylisoxazol-3-yl
9b R^1 = Cl, R^2 = 5-methylisoxazol-3-yl
9c R^1 = OCH_3, R^2 = 5-methylisoxazol-3-yl
10a R^1 = H, R^2 = 2-pyridyl
10b R^1 = Cl, R^2 = 2-pyridyl
10c R^1 = OCH_3, R^2 = 2-pyridyl
11 R^1 = OCH_3, R^2 = 2-pyrimidinyl
12a R^1 = H, R^2 = 4-methyl-2-pyrimidinyl
12b R^1 = F, R^2 = 4-methyl-2-pyrimidinyl
12c R^1 = Cl, R^2 = 4-methyl-2-pyrimidinyl
12d R^1 = CF_3, R^2 = 4-methyl-2-pyrimidinyl
12e R^1 = OCH_3, R^2 = 4-methyl-2-pyrimidinyl

Scheme 1. Synthesis of pyrimidine-sulfonamide conjugates **6-12**

isof[illegible] An hydration assay [49]. The inhibition results are depicted in table 1.

Structure -Activity Relationship

The obtained results show that the primary benzenesulfonamide-pyrimidine conjugates **6** strongly inhibited the cytosolic isoform hCA I with K_i ranged from 0.10 μM to 0.87 μM. The only exceptions are **6c** and **6g** which showed moderate (K_i = 1.94 μM) to complete loss (K_i > 100 μM) of inhibitory activity, respectively.

Comparison of the effect of the phenyl group substitution at 4-position on the inhibitory activity showed that the 4-fluorophenyl **6b** and unsubstituted phenyl **6a** derivatives possessed inhibition potential of K_i = 0.10 μM and K_i = 0.27 μM, respectively, compared to AAZ (K_i = 0.25 μM) against the cytosolic isoform h CA I. The *p*-bromophenyl **6d** (K_i = 0.41 μM), *p*-methoxyphenyl **6f** (K_i = 0.63 μM) and *p*-(trifluoromethyl)phenyl **6e** (K_i = 0.87 μM) apparent decrease in the inhibitory potency was observed in case of using *p*-chlorophenyl group **6c** (K_i = 1.94 μM) as a tail, whereas, loss of inhibitory activity was found in case of incorporating a biphenyl group **6g** (K_i > 100 μM) as a tail. In the case of pyrimidine-secondary benzenesulfonamide conjugates **7-12** a marked decrease or loss of the activity was observed in comparison to the equivalent primary sulfonamide derivatives **6**. The only exception was **8c** (K_i = 1.75 μM) which showed a slight increase in the potency in comparison to **6c** (K_i = 1.94 μM).

Nearly, all the pyrimidine-primary benzenesulfonamide conjugates **6** strongly inhibited the cytosolic isoform hCA II with inhibition potential ranging between K_i = 0.04 μM to 0.09 μM in comparison to the reference drug AAZ (K_i = 0.012 μM). Compound **6g** was the only exception which showed a moderate inhibition activity with K_i of 0.73 μM. Compounds **6e** and **6f** with 4-(trifluoromethyl)phenyl and *p*-methoxyphenyl substituents, respectively, are the most potent derivatives with K_i of 0.04 μM.

Table 1: Dissociation constants K_i (μM) for CA isoforms in response to **6-12** and **AAZ** using a stopped flow CO_2 hydrase assay [49] and selectivity ratio for hCA II.

Entry	Compound	Dissociation constants K_i (μM) for CA isoforms				Selectivity ratio for hCA II		
		hCA I	hCA II	hCA IX	hCA XII	I/II	IX/II	XII/II
1	**6a**	0.27	0.05	0.16	0.53	5.4	3.2	10.6
2	**6b**	0.10	0.09	0.82	0.82	1.1	9.1	9.1
3	**6c**	1.94	0.05	>100	0.40	38.8	>2000	8
4	**6d**	0.41	0.07	>100	0.94	5.9	>1428	13.42
5	**6e**	0.87	0.04	39.20	0.90	21.8	980	22.5
6	**6f**	0.63	0.04	5.17	0.71	15.8	129.3	17.8
7	**6g**	>100	0.73	0.23	0.70	>137	0.32	0.96
8	**7**	74.6	0.34	>100	1.35	219	294.1	3.97
9	**8a**	73.6	0.54	>100	0.41	136.3	185.2	0.75
10	**8b**	>100	0.57	>100	>100	>175.4	>175.4	>175.4
11	**8c**	1.75	0.85	0.55	3.18	2.05	0.65	3.74
12	**8d**	84.5	0.83	>100	0.36	101.8	>120.5	0.43
13	**8e**	>100	0.88	>100	>100	>113.6	>113.6	>113.6
14	**9a**	>100	80.52	92.12	>100	>1.24	1.14	>1.24
15	**9b**	>100	70.15	8.94	48.44	>1.42	0.13	0.69
16	**9c**	49.16	6.76	>100	0.54	7.27	>14.79	0.08
17	**10a**	>100	45.41	>100	9.31	>2.20	>2.20	0.21
18	**10b**	>100	55.9	>100	4.0	>1.79	>1.79	0.072
19	**10c**	>100	>100	>100	0.80	nd	nd	nd
20	**11**	>100	>100	2.51	7.09	nd	nd	nd
21	**12a**	>100	74.6	4.48	3.15	>1.34	0.06	0.04
22	**12b**	64.4	3.15	4.87	0.31	20.44	1.54	0.10
23	**12c**	>100	21.79	83.81	6.29	>4.59	3.85	0.29
24	**12d**	>100	59.06	0.49	6.44	>1.69	0.008	0.11
25	**12e**	>100	7.07	0.53	8.67	>14.14	0.07	1.23
26	**AAZ**	0.25	0.012	0.025	0.0057	20.83	2.08	0.48

This phenyl and *p*-chlorophenyl groups, respectively, showing K_i of 0.05 μM. Whereas compounds **6d** and **6b** exhibiting *p*-bromophenyl group and *p*-fluorophenyl moiety as a tail displayed K_i = 0.07 and 0.09 μM, respectively. A decrease in the inhibitory activity to a sub-micromolar range (K_i = 0.34-0.88 μM) was observed by the incorporation of a secondary sulfonamide moiety having acetyl **7** or thiazolyl **8** groups. However, a very weak to complete disappearance of inhibitory activity was observed in using secondary sulfonamides having 5-methylisoxazol-3-yl **9** (K_i = 6.76-80.52 μM), pyridyl **10** (K_i = 45.41 - > 100 μM), 2-pyrimidinyl **11** (K_i > 100 μM) or 4-methyl-2-pyrimidinyl **12** (K_i = 3.15 - 74.6 μM) moieties.

The membrane bound tumor associated isoform hCA IX is highly inhibited by the primary sulfonamides **6a** and **6g** with K_i = 0.16 and 0.23 μM, respectively and they were the most potent compounds among the series. This is followed by compound **6b** which exhibit K_i = 0.82 μM. An increase in the potency of **6c** (K_i > 100 μM) was observed by incorporating a 4-methyl-2-pyrimidinyl in **12c** (K_i = 83.81 μM), 5-methylisoxazol-3-yl in **9b** (K_i = 8.94 μM) and reaches its maximum potency by incorporating 2-thiazolyl group in **8c** (K_i = 0.55 μM). Also, huge increase in the potency of the primary sulfonamides **6e** (K_i = 39.20 μM) and **6f** (K_i = 5.17 μM) was observed by the introduction of 4-methyl-2-pyrimidinyl substituent in **12d** (K_i = 0.49 μM) and **12e** (K_i = 0.53 μM), respectively. These results confirm our assumptions that the incorporation of secondary sulfonamides can be advantageous in some cases.

Weak hCA XII inhibitory potency was observed by all the synthesized compounds. Primary sulfonamides **6** showed activity in the range of K_i = 0.40 to 0.94 μM. A slight increase in the potency was observed by introduction of 2-thiazolyl moiety in **8a** (K_i = 0.41 μM) in comparison to the primary sulfonamide derivative **6a** (K_i = 0.53 μM). However, a decrease in the potency was observed by incorporating a pyridyl group in **10a** (K_i = 9.31 μM) or 4-methyl-2-pyrimidinyl group in **12a** (K_i = 3.15 μM). An increase in the potency was observed by introduction of 4-methyl-2-pyrimidinyl group in **12b** (K_i = 0.31 μM) in comparison to **6b** (K_i = 0.82 μM) while total loss in the potency was observed by including 2-thiazolyl moiety in **8b** (K_i > 100 μM) or 5-methylisoxazol-3-yl group in **9a** (K_i > 100 μM). A decrease in potency was observed by introducing an acetyl group in **7** (K_i = 1.35 μM), 2-thiazolyl group in **8c** (K_i = 3.18 μM), 5-methylisoxazol-3-yl group in **9b** (K_i = 48.44 μM), pyridyl group in **10b** (K_i = 4.0 μM), 4-methyl-2-pyrimidinyl group in **12c** (K_i = 6.29 μM) in comparison to **6c** (K_i = 0.40 μM). Incorporation of thiazolyl moiety in **8d** (K_i = 0.36 μM) resulted in more than two-fold increase in potency in comparison to **6e** (K_i = 0.90 μM), whereas, huge decrease in the potency appeared with compound exhibiting 4-methyl-2-pyrimidinyl moiety **12d** (K_i = 6.44 μM).). A slight increase in the potency appeared in the secondary sulfonamide exhibiting 5-methylisoxazol-3-yl group **9c** (K_i = 0.54 μM) when compared to the primary sulfonamide congener **6f** (K_i = 0.71 μM), whereas, a slight decrease to complete loss of the activity appeared by the introduction of pyridyl group in **10c** (K_i = 0.80 μM), 2-pyrimidinyl moiety in **11** (K_i = 7.09 μM), 4-methyl-2-pyrimidinyl group in **12e** (K_i = 8.67 μM) and thiazolyl group in **8e** (K_i > 100 μM) (Table 1).

Most of the newly synthesized derivatives exhibited a preferential selectivity for the glaucoma associated isoform hCA II over hCA I and hCA IX. In series **6**, the derivatives **6a** and **6d** displayed 5-fold higher selectivity toward hCA II over hCA I. Introduction of *p*-chloro, *p*-CF_3, *p*-methoxy, *p*-Ph in **6c**, **6e**, **6f** and **6g**, respectively, resulted in about 15 to more than 137 hCA I/hCA

n 3- and 9-fold higher selectivity toward hCA II over the membrane associated isoform hCA IX. However, an apparent increase in the selectivity was observed by introducing chloro, bromo, trifluoromethyl or methoxy groups at the 4 position of the phenyl moiety in **6c**, **6d**, **6e**, **6f**, respectively, these derivatives demonstrated >2000, >1428, 980, and 129 hCA IX/hCA II selectivity index, respectively. For series **7-12**, most of the compounds showed higher selectivity toward the hCA II over hCA IX, for instance, the derivative **7** showed 294-fold higher selectivity toward hCA II over hCA IX. The secondary sulfonamide derivatives **8c**, **9b**, **12a**, **12d**, and **12e** are the only exceptions with higher selectivity toward hCA IX over hCA II. Studying the selectivity of the synthesized series toward hCA II over hCA XII showed that derivatives having primary sulfonamide groups **6a-f** have 8- to 22-fold higher selectivity toward hCA II over hCA XII. Compound **6g** is the only exception; it displayed higher selectivity toward hCA IX and hCA XII over hCA II. Compound **8b** showed more than 175 higher selectivity toward hCA II over hCA XII. Secondary sulfonamides exhibiting thiazolyl **8a,d**, 5-methylisoxazol-3-yl **9b,c,** pyridyl **10a-c**, pyrimidinyl **11** and 4-methyl-pyrimidinyl substituent **12a-d** showed higher selectivity toward hCA XII over hCA II.

These results indicated that the synthesized 2-thioxopyrimidine-benzensulfonamide conjugates **6** are interesting scaffold with higher selectivity toward hCA II. Hence, it can be further optimized for the development of new drugs that can be applied for the treatment of glaucoma. In addition, the incorporation of secondary sulfonamides results in an increase in the inhibitory activity in some cases against CA IX and CA XII while no apparent increase in the potency was observed against CA I and CA II.

2.4. Molecular docking study

Docking simulations were carried out to study the binding pattern of the newly synthesized sulfonamide conjugate**s** in the active site of the target anhydrase CA II to elucidate their structure activity relationship (SAR). The molecular docking protocol was first validated by running self-docking of the co-crystalized ligand (AAZ) in the active site of CA II (PDB ID: 3HS4) [50]. The self-docking validation reproduced the co-crystallized ligand's experimental binding mode indicating that the docking protocol used is adequate for the intended docking analysis. This is shown by the small RMSD between the experimental co-crystallized ligand pose and the docked pose of 0.867Å; and by the capability of the docking pose to reproduce all the key interactions achieved by the co-crystallized ligands in the active site with Zn^{2+}, Thr199 and Thr200 (Figure 4).

Table 2 shows the predicted docking score of the most potent compounds **6a-g**. Generally, as can be seen in figure 5, the sulfonamide moiety acted as the ZBG interacting agent with the active site Zn^{2+} ion. Moreover, it interacts through hydrogen bonding with the key amino acids Thr199 and Thr200. In addition, the linker NH between the benzenesulfonamide and the pyrimidine moieties establishes a hydrogen bonding interaction with Gln92 backbone carbonyl group. This additional interaction could be responsible for the potent effect of series **6** on CA II and their selectivity toward its inhibition. Through hydrophobic interaction, the benzenesulfonamide and the thiopyrimidine moieties interact with the hydrophobic side chains of the amino acids Val121/Leu198 and Ile91/Phe131, respectively (For further details See SI).

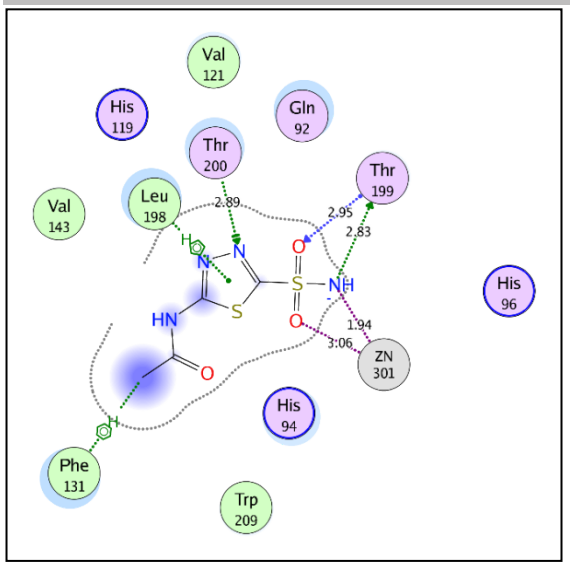
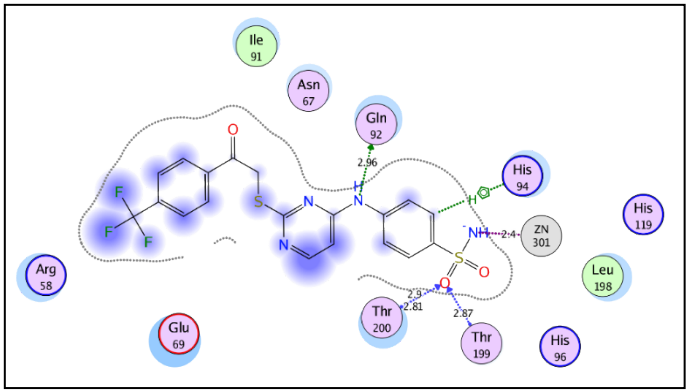


Figure 4. 2-Dimensional interaction diagram showing the AAZ docking pose interactions with the key amino acids in the CA II active site (PDB ID: 3HS4).

Table 2. Docking energy scores (*S*) in kcal/mol for the newly synthesized **6a-g** in CA II active site

Compound	Energy score (S) kcal/mol
6a	−9.44
6b	−9.31
6c	−9.33
6d	−9.93
6e	−10.1
6f	−10.23
6g	−9.95
AAZ	−7.68

Compounds **6e** and **6f**, which showed the most potent inhibitory activity of CA II with K_i of 0.04 µM, are showing the most negative energy score of −10.10 and −10.23 kcal/mol, respectively. Compound **6e** shows this high activity due to the additional polar interaction taking place between its polar fluorine atoms of its CF_3 with the positively charged guanidinium group of Arg58 (Figure 5A). Whereas, the high activity of compound **6f** could be attributed to the additional H-bond interaction performed by the *p*-methoxy group on the distal phenyl group with Asn67 (For further details see SI).

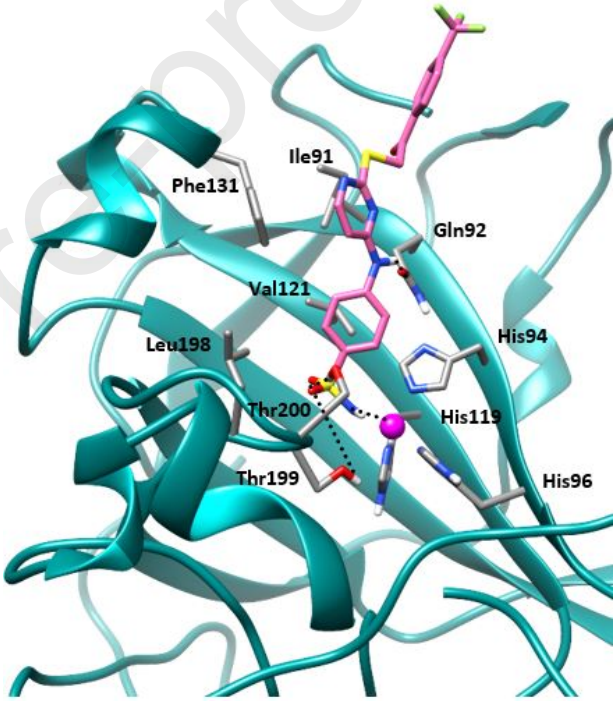


(A)

(B)

Figure 5. 2-Dimensional diagram (**A**) and 3-dimensional representation (**B**) of compound **6e** showing its interaction with the CA II active site.

Although the secondary sulfonamides **7**, **8**, **9**, **10**, **11** and **12** could accomplish the planned hydrophobic interaction by their substituent on the sulfonamide amino group with the hydrophobic side chains of the CO_2 pocket Val121, Leu141, Val143, Leu198, Val207 and Trp209, they could not achieve a high inhibitory activity as appeared in their experimental results. This could be attributed to their inability to achieve the proper coordination



geometry with the Zn^{2+} cation and the twisting of their whole structure within the binding site relative to the common binding pattern of CA inhibitors (For further details see SI)

3. Conclusion

In summary, a molecular hybridization approach of a benzensulfonamide moiety (ZBG), an alkylated thiopyrimidine moiety (spacer) and an (un)substituted phenyl moiety (tail) was implemented for the design of a new series of thiopyrimidine-benzenesulfonamides **6-12**. The designed and synthesized compounds **6-12** were examined for their inhibitory activity on four carbonic anhydrase (CA, EC 4.2.1.1) isoforms hCA I, hCA II, hCA IX and hCA XII using stopped-flow CO_2 hydration assay. Series **6** exerted a promising activity and selectivity toward the cytosolic isoforms hCA I and hCA II versus the membrane bound isoforms hCA IX and hCA XII. The thiopyrimidine-benzenesulfonamides **6e** and **6f** showed K_i of 0.04 uM against hCA II and 15.8- to 980-fold selectivity towards hCA II over the other tested isoforms. Molecular docking simulations of the newly

synt /cm-1 promising hCA II inhibitory activity of series **6** to the ability of their to interact with the key elements in the active site *viz*. the Zn^{2+} ion and the key amino acids Thr199 and Thr200. Through hydrophobic interaction, the benzene sulfonamide and the thiopyrimidine moieties interact with the hydrophobic side chains of the amino acids Val121/Leu198 and Ile91/Phe131, respectively. Moreover, a hydrogen bond interaction is formed between the NH group at the 4-position of the pyrimidine moiety and Gln92 backbone carbonyl group. This additional interaction could be responsible for the potent effect of series **6** on CA II and their selectivity toward its inhibition. Molecular docking studies rationalized the potent inhibitory activity of compound **6e** to the additional polar interaction between the polar fluorine atoms of its CF_3 with the positively charged guanidinium group of Arg58. Whereas, the high activity of compound **6f** can be attributed to the additional H-bond interaction performed by the *p*-methoxy group on the distal phenyl group with Asn67.

4. Experimental

4.1. Chemistry

4.1.1. General remarks

Chemicals used for synthesis and biological experiments were purchased from commercial suppliers. Precoated silica gel 60 F_{245}aluminium plates (Merck) were used for analytical thin layer chromatography (TLC). Melting points were measured using open capillary tubes on a Stuart SMP30 melting point apparatus and they were uncorrected. Spectral data and elemental analysis of the synthesized compounds were measured in the Micro analytical labs, National Research Centre, Cairo, Egypt. IR spectra (4000–400 cm^{-1}) were recorded applying KBr pellets in a Jasco FT/IR 6100 Fourier transform infrared spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were measured at 500 (125) MHz and 400 (100) MHz on Bruker instruments, using DMSO-d_6 as a solvent.

General procedure I for the synthesis of 3a-g

As previously reported [47], a mixture of 2-thiouracil (**1**), 2-bromoacetophenone derivatives **2a-g**, anhydrous K_2CO_3 and ethanol was heated under reflux for 4h. The reaction mixture was cooled, poured on ice / water and neutralized with 2*N* HCl. The formed precipitate was filtered and dried to yield **3a-g**. Analytically pure products of **3a-g** were obtained by crystallization form methanol.

General procedure II for the synthesis of 4a-g

As previously reported [47], a round bottom flask was charged with **3a-g** and $POCl_3$. The mixture was stirred at 80 °C for 30 min. Subsequently, the reaction was cooled down and poured portion wise on ice. The formed precipitate was collected by filtration followed by drying to obtain **4a-g.**

General procedure III for the synthesis 6-12

A mixture of **4a-g** (0.50 mmol), benzenesulfonamide derivatives **5a-g** (0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Then, the reaction was left to cool followed by pouring on ice-water. The formed precipitate was collected by filtration and left to dry. Analytically pure compounds **6-12** were afforded by crystallization from methanol.

4-((2-((2-Oxo-2-phenylethyl)thio)pyrimidin-4-yl)amino)benzenesulfonamide (6a)

Following the general procedure III, a mixture of **4a** (132.36 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6a** as a pale ^{1} 3391, 2973, 2924, 1640, 1573 and 1456; δ_H (500 MHz; DMSO-d_6) 4.98 (2H, s, CH_2), 6.78 (1H, d, ^3J = 5.0 Hz), 7.23 (2H, br.), 7.51 (2H, d, ^3J = 8.0 Hz), 7.57 (2H, t like, ^3J = 7.0 Hz), 7.68-7.72 (3H, m), 8.03 (2H, d, ^3J = 7.0 Hz), 8.19 (1H, d, ^3J = 5.0 Hz), 11.01 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 39.00, 104.06, 119.97, 126.56, 128.28, 128.94, 133.96, 135.36, 138.58, 141.13, 150.87, 159.39, 167.48, 192.82 ppm; Anal. Calcd for $C_{18}H_{16}N_4O_3S_2$: C, 53.99; H, 4.03; N, 13.99. Found: C, 53.58; H, 4.33; N, 14.15.

4-((2-((2-(4-Fluorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)benzenesulfonamide (6b)

Following the general procedure III, a mixture of **4b** (141.36 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6b** as a pale brown powder (175.11 mg, 84%); mp 216-218 °C; *ύ* max (atr)/cm^{-1} 3345, 3263, 3066, 3013, 2959, 1686, 1636, 1597, 1574, 1562, 1505; δ_H (500 MHz; DMSO-d_6) 4.82 (2H, s, CH_2), 6.55 (1H, d, ^3J = 5.5 Hz), 7.19 (2H, br.), 7.38 (2H, t, ^3J = 7.5 Hz), 7.54 (2H, d, ^3J = 7.5 Hz), 7.69 (2H, d, ^3J = 7.5 Hz), 8.12-8.13 (3H, m), 10.00 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 38.40, 103.93, 115.94 (d, J = 21.8 Hz), 118.96, 126.60, 131.29 (d, J = 9.5 Hz), 132.48 (d, J = 2.6 Hz), 137.32, 142.26, 155.76, 159.29, 165.27 (d, J = 251.4 Hz), 169.18, 192.20 ppm; Anal. Calcd for $C_{18}H_{15}FN_4O_3S_2$: C, 51.66; H, 3.61; N, 13.39. Found: C, 51.36; H, 3.85; N, 13.62.

4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)benzenesulfonamide (6c)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6c** as a pale brown powder (136 mg, 63%), mp 229-231 °C; *ύ* max (atr)/cm^{-1} 3378, 3290, 2923, 1689, 1631, 1568 and 1498; δ_H (400 MHz; DMSO-d_6) 4.81 (2H, s, CH_2), 6.55 (1H, d, ^3J = 5.6 Hz), 7.19 (2H, s), 7.59-7.63 (4H, m), 7.71 (2H, d, ^3J = 8.8 Hz), 8.05 (2H, d, ^3J = 8.8 Hz), 8.11 (1H, d, ^3J = 5.6 Hz), 9.99 ppm (s, 1H); Anal. Calcd for $C_{18}H_{15}ClN_4O_3S_2$: C, 49.71; H, 3.48; N, 12.88. Found: C, 49.34; H, 3.79; N, 12.61.

4-((2-((2-(4-Bromophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)benzenesulfonamide (6d)

Following the general procedure III, a mixture of **4d** (171.81 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6d** as a pale brown powder (170 mg, 71%); mp 232-234 °C; *ύ* max (atr)/cm^{-1} 3383, 3290, 3153, 3077, 2958, 1688, 1632, 1568, 1499 and 1464; δ_H (400 MHz; DMSO-d_6) 4.80 (2H, s, CH_2), 6.55 (1H, d, ^3J = 6.0 Hz), 7.19 (2H, br.), 7.62 (2H, d, ^3J = 8.8 Hz), 7.72 (2H, d, ^3J = 8.4 Hz), 7.77 (2H, d, ^3J = 8.4 Hz), 7.97 (2H, d, ^3J = 8.4 Hz), 8.11 (1H, d, ^3J = 6.0 Hz), 10.00 ppm (1H, s); δ_C (100 MHz; DMSO-d_6) 38.37, 103.98, 119.07, 126.68, 127.85, 130.27, 131.96, 134.79, 137.41, 142.33, 155.81, 159.32, 169.09, 193.11 ppm; Anal. Calcd for $C_{18}H_{15}BrN_4O_3S_2$: C, 45.10; H, 3.15; N, 11.69. Found: C, 45.35; H, 3.44; N, 11.45.

4-((2-((2-Oxo-2-(4-(trifluoromethyl)phenyl)ethyl)thio)pyrimidin-4-yl)amino)benzene sulfonamide (6e)

Following the general procedure III, a mixture of **4e** (166.36 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6e** as a pale brown powder (177 mg, 76 %), mp 105-107 °C; *ύ* max (atr)/cm^{-1} 3448, 3317, 3082, 2909, 1701, 1647 and 1504; δ_H (500 MHz; DMSO-d_6) 4.86 (2H, s, CH_2), 6.55 (1H, d, ^3J = 5.5 Hz), 7.19 (2H, br.), 7.60 (2H, d, ^3J = 8.0 Hz), 7.72 (2H, d, ^3J = 7.5 Hz), 7.93 (2H, d, ^3J = 7.5 Hz), 8.11 (1H, d, ^3J = 5.5 Hz), 8.22 (2H, d, ^3J = 8.0 Hz), 10.01 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 38.62, 103.99,

119.
129.06, 132.88 (q, ^3J = 31.9 Hz), 137.41, 139.02, 142.27, 155.78, 159.31, 168.96, 193.39 ppm; Anal. Calcd for $C_{19}H_{15}F_3N_4O_3S_2$: C, 48.71; H, 3.23; N, 11.96. Found: C, 48.42; H, 3.52; N, 11.74.

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)benzenesulfonamide (6f)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6f** as a pale brown powder (100 mg, 47%); mp 220-222 °C; *ύ* max (atr)/cm^{-1} 3379, 3271, 3093, 3043, 2916, 1674, 1605, 1574 and 1508; δ_H (400 MHz; DMSO-d_6) 3.87 (3H, s, OCH_3), 4.81 (2H, s, CH_2), 6.57 (1H, d, ^3J = 5.6 Hz), 7.08 (2H, d, ^3J = 8.4 Hz), 7.18 (2H, br), 7.53 (2H, d, ^3J = 8.4 Hz), 7.69 (2H, d, ^3J = 8.4 Hz), 8.02 (2H, d, ^3J = 8.8 Hz), 8.14 (1H, d, ^3J = 5.6 Hz), 10.14 ppm (1H, s); δ_C (100 MHz; DMSO-d_6) 38.43, 55.58, 103.94, 114.17, 119.32, 126.63, 128.45, 130.67, 137.70, 141.94, 154.24, 159.35, 163.61, 168.84, 191.57 ppm; Anal. Calcd for $C_{19}H_{18}N_4O_4S_2$: C, 53.01; H, 4.21; N, 13.01. Found: C, 53.28; H, 4.51; N, 13.33.

4-((2-((2-([1,1'-Biphenyl]-4-yl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)benzenesulfonamide (6g)

Following the general procedure III, a mixture of **4g** (170.41 mg, 0.50 mmol), sulfanilamide (**5a**) (86.10 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **6g** as a pale brown powder (125 mg, 53%); mp 263-265 °C; *ύ* max (atr)/cm^{-1} 3413, 3290, 3189, 3051, 2924, 1635, 1594, 1550 and 1495; δ_H (400 MHz; DMSO-d_6) 5.02 (2H, s, CH_2), 6.82 (1H, d, ^3J = 6.0 Hz), 7.26 (2H, br.), 7.44 (2H, d, ^3J = 7.0 Hz), 7.51 (2H, like, ^3J = 7.2 Hz), 7.63 (2H, d, ^3J = 8.0 Hz), 7.77-7.79 (3H, m), 7.87 (2H, d, ^3J = 7.6 Hz), 8.11 (2H, d, ^3J = 7.6 Hz), 8.21 (1H, d, ^3J = 6.0 Hz) and 11.12 ppm (1H, s); δ_C (100 MHz; DMSO-d_6) 38.87, 104.07, 120.36, 126.67, 126.84, 127.09, 127.23, 128.51, 129.09, 134.12, 138.83, 138.96, 141.12, 145.42, 150.49, 159.46, 167.35, 192.44 ppm; Anal. Calcd for $C_{24}H_{20}N_4O_3S_2$: C, 60.49; H, 4.23; N, 11.76. Found: C, 60.25; H, 4.54; N, 11.61.

N-((4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)phenyl)sulfonyl) acetamide (7)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfacetamide (**5b**) (107.12 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **7** as a white powder (120 mg, 50%); mp 247-249 °C; *ύ* max (atr)/cm^{-1} 3439, 2926, 1684, 1635 and 1564; δ_H (400 MHz; DMSO-d_6) 1.89 (3H, s, CH_3), 4.83 (2H, s, CH_2), 6.56-6.58 (1H, m), 7.74-7.75 (2H, m), 7.61-7.69 (4H, m), 8.04-8.06 (2H, m), 8.13-8.14 (1H, m), 10.12 (1H, s), 11.68 ppm (1H, br.); Anal. Calcd for $C_{20}H_{17}ClN_4O_4S_2$: C, 50.37; H, 3.59; N, 11.75. Found: C, 50.65; H, 3.28; N, 11.91.

4-((2-((2-Oxo-2-phenylethyl)thio)pyrimidin-4-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (8a)

Following the general procedure III, a mixture of **4a** (132.36 mg, 0.50 mmol), sulfathiazole (**5c**) (127.66 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **8**a as a pale brown powder (185.21 mg, 77%); mp 181-183 °C; *ύ* max (atr)/cm^{-1} 3444, 3348, 3101, 2812, 1678, 1566 and 1539; δ_H (400 MHz; DMSO-d_6) 4.84 (2H, s, CH_2), 6.53 (1H, d, ^3J = 5.6 Hz), 6.82 (1H, d, ^3J = 4.8 Hz), 7.24 (1H, d, ^3J = 4.4 Hz), 7.45 (2H, d, ^3J = 8.8 Hz), 7.54 (2H, t like, ^3J = 7.6 Hz), 7.64-7.68 (3H, m), 8.05 (2H, d, ^3J = 8.0 Hz), 8.12 (1H, d, ^3J = 6.0 Hz), 9.97 (1H, s) and 12.60 ppm (1H, br.); Anal. Calcd for $C_{21}H_{17}N_5O_3S_3$: C, 52.16; H, 3.54; N, 14.48. Found: C, 52.31; H, 3.37; N, 14.20.

yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (8b)

Following the general procedure III, a mixture of **4b** (141.36 mg, 0.50 mmol), sulfathiazole (**5c**) (127.66 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **8b** as a pale brown powder (140.29 mg, 56%); mp 187-189 °C; *ύ* max (atr)/cm^{-1} 3433, 3089, 2925, 1632 and 1460; δ_H (400 MHz; DMSO-d_6) 4.82 (2H, s, CH_2), 6.53 (1H, d, ^3J = 5.0 Hz), 6.79 (1H, d, ^3J = 5.5 Hz), 7.21-7.22 (1H, m), 7.35-7.39 (2H, m), 7.45 (2H, d, ^3J = 7.6 Hz), 7.63 (2H, d, ^3J = 7.2 Hz), 8.12-8.14 (3H, m), 9.97 (1H, s) and 11.77 ppm (1H, br.); Anal. Calcd for $C_{21}H_{16}FN_5O_3S_3$: C, 50.29; H, 3.22; N, 13.96. Found: C, 50.11; H, 3.41; N, 13.65.

4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(thiazol-2-yl)benzene-sulfonamide (8c)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfathiazole (**5c**) (127.66 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **8c** as a white powder (180.00 mg, 70%), mp 143-145 °C; *ύ* max (atr)/cm^{-1} 3436, 2924, 1679, 1634, 1570 and 1535; δ_H (500 MHz; DMSO-d_6) 4.81 (2H, s, CH_2), 6.53 (1H, d, ^3J = 5.5 Hz), 6.79 (1H, d, ^3J = 3.5 Hz), 7.21 (1H, d, ^3J = 4.0 Hz), 7.52 (2H, d, ^3J = 8.0 Hz), 7.63 (2H, d like, ^3J = 8.0 Hz), 7.66 (2H, d like, ^3J = 8.0 Hz), 8.06 (2H, d, ^3J = 8.0 Hz), 8.10 (1H, d, ^3J = 5.5 Hz) and 9.99 ppm (1H, s); δ_C (125 MHz; DMSO-d_6) 38.49, 103.97, 107.93, 118.82, 125.15, 126.86, 129.00, 130.18, 134.41, 135.60, 138.72, 142.47, 155.77, 159.28, 168.63, 169.16 and 192.67 ppm; Anal. Calcd for $C_{21}H_{16}ClN_5O_3S_3$: C, 48.69; H, 3.11; N, 13.52. Found: C, 48.41; H, 3.42; N, 13.32.

4-((2-((2-Oxo-2-(4-(trifluoromethyl)phenyl)ethyl)thio)pyrimidin-4-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (8d)

Following the general procedure III, a mixture of **4e** (166.36 mg, 0.50 mmol), sulfathiazole (**5c**) (127.66 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **8d** as a pale brown powder (187.34 mg, 68%); mp 222-224 °C; *ύ* max (atr)/cm^{-1} 3483, 3421, 3294, 3089, 3043, 1686, 1620 and 1562; δ_H (500 MHz; DMSO-d_6) 4.86 (2H, s, CH_2), 6.54 (1H, d, ^3J = 5.0 Hz), 6.80 (1H, br.), 7.22 (1H, br.), 7.54 (2H, d, ^3J = 8.0 Hz), 7.68 (2H, d, ^3J = 8.0 Hz), 7.94 (2H, d, ^3J = 7.5 Hz), 8.10 (1H, d, ^3J = 5.0 Hz), 8.24 (2H, d, ^3J = 7.5 Hz), 10.00 (1H, s) and 12.61 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 38.65, 104.02, 107.94, 118.90, 123.73 (q, J = 271.3 Hz), 124.33, 125.84 (q, J = 3.6 Hz), 126.85, 129.08, 132.92 (q, J = 31.9 Hz), 135.38, 138.98, 142.55, 155.78, 159.27, 168.57, 169.00 and 193.29 ppm; Anal. Calcd for $C_{22}H_{16}F_3N_5O_3S_3$: C, 47.91; H, 2.92; N, 12.70. Found: C, 47.75; H, 2.77; N, 12.93.

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (8e)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfathiazole (**5c**) (127.66 mg, 0.50 mmol), acetic acid (15 mL) was refluxed for 4h. Work up afforded **8e** as a white powder (160.00 mg, 62%), mp 139-141 °C; *ύ* max (atr)/cm^{-1} 3483, 3421, 3090, 2900, 1670, 1636 and 1597; δ_H (400 MHz; DMSO-d_6) 3.89 (3H, s, OCH_3), 4.83 (2H, s, CH_2), 6.60 (1H, br.), 6.81 (1H, d, ^3J = 4.8 Hz), 7.10 (2H, d, ^3J = 8.8 Hz), 7.22 (1H, d, ^3J = 4.4 Hz), 7.44 (2H, d, ^3J = 8.8 Hz), 7.65 (2H, d, ^3J = 8.8 Hz), 8.04 (2H, d, ^3J = 8.8 Hz), 8.14 (1H, d, ^3J = 5.6 Hz), 10.31 (1H, br.) and 12.62 ppm (1H, br.); Anal. Calcd for $C_{22}H_{19}N_5O_4S_3$: C, 51.45; H, 3.73; N, 13.64. Found: C, 51.21; H, 3.45; N, 13.91.

4-((2-((2-(4-Fluorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (9a)

Following the general procedure III, a mixture of **4b** (141.36 mg, 0.50 mmol), sulfamethoxazole (**5d**) (126.64 mg, 0.50 mmol)

and .71; as a pale brown powder (111.00 mg, 44%); mp 217-219 °C; ύ max (atr)/cm^{-1} 3437, 2924, 1635, 1570 and 1500; δ_H (400 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 4.82 (2H, s, CH_2), 6.06 (1H, br.), 6.55 (1H, d, ^3J = 4.8 Hz), 7.27 (2H, t, ^3J = 8.0 Hz), 7.37 (2H, t, ^3J = 8.0 Hz), 7.53 (2H, d, ^3J = 8.0 Hz), 7.72 (2H, d, ^3J = 8.0 Hz), 8.13 (1H, br.), 10.08 (1H, s) and 11.26 ppm (1H, s); Anal. Calcd for $C_{22}H_{18}FN_5O_4S_2$: C, 52.90; H, 3.63; N, 14.02. Found: C, 52.65; H, 3.89; N, 14.42.

4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (9b)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfamethoxazole (**5d**) (126.64 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **9b** as a white powder (189 mg, 73%), mp 135-137 °C; ύ max (atr)/cm^{-1} 3309, 3163, 3086, 1678, 1620, 1566 and 1500; δ_H (400 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 4.86 (2H, s, CH_2), 6.08 (1H, s), 6.65 (1H, d, ^3J = 6.0 Hz), 7.57 (2H, d, ^3J = 8.4 Hz), 7.62 (2H, d, ^3J = 8.0 Hz), 7.75 (2H, d, ^3J = 8.4 Hz), 8.05 (2H, d, ^3J = 8.0 Hz), 8.15 (1H, d, ^3J = 5.6 Hz), 10.46 (1H, s) and 11.29 ppm (1H, s); Anal. Calcd for $C_{22}H_{18}ClN_5O_4S_2$: C, 51.21; H, 3.52; N, 13.57. Found: C, 51.54; H, 3.28; N, 13.32.

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (9c)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfamethoxazole (**5d**) (126.64 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **9c** as a white powder (135.60 mg, 53%), mp 128-130 °C; ύ max (atr)/cm^{-1} 3422, 3086, 2972, 1673, 1603, 1567 and 1501; δ_H (500 MHz; DMSO-d_6) 2.30 (3H, s, CH_3), 3.88 (3H, s, OCH_3), 4.79 (2H, s, CH_2), 6.05 (1H, s), 6.55 (1H, d, ^3J = 5.0 Hz), 7.08 (2H, d, ^3J = 8.0 Hz), 7.49 (2H, d, ^3J = 8.0 Hz), 7.72 (2H, d, ^3J = 8.5 Hz), 8.04 (2H, d, ^3J = 8.0 Hz), 8.15 (1H, d, ^3J = 5.0 Hz), 10.08 (1H, s) and 11.26 ppm (1H, s); δ_C (125 MHz; DMSO-d_6) 12.08, 38.29, 55.55, 95.25, 104.12, 114.08, 118.87, 127.90, 128.56, 130.66, 131.83, 143.76, 155.96, 157.59, 159.23, 163.53, 169.50, 170.21 and 191.62 ppm; Anal. Calcd for $C_{23}H_{21}N_5O_5S_2$: C, 54.00; H, 4.14; N, 13.69. Found: C, 54.35; H, 4.36; N, 13.25.

4-((2-((2-Oxo-2-phenylethyl)thio)pyrimidin-4-yl)amino)-N-(pyridin-2-yl)benzene sulfonamide (10a)

Following the general procedure III, a mixture of **4a** (132.36 mg, 0.50 mmol), sulfapyridine (**5e**) (124.65 mg, 0.5 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **10a** as a pale brown powder (128.17 mg, 54%); mp 198-200 °C; ύ max (atr)/cm^{-1} 3437, 2927, 1673, 1633, 1569 and 1499; δ_H (400 MHz; DMSO-d_6) 4.84 (2H, s, CH_2), 6.53 (1H, d, ^3J = 5.2 Hz), 6.84-6.86 (1H, m), 7.07 (1H, d, ^3J = 8.4 Hz), 7.55 (4H, d like, ^3J = 6.8 Hz), 7.67 (4H, d, ^3J = 7.2 Hz), 7.99-8.01 (1H, m), 8.05 (2H, d, ^3J = 6.8 Hz), 8.11 (1H, d, ^3J = 5.2 Hz), 10.00 (1H, s) and 11.71 ppm (1H, br.); Anal. Calcd for $C_{23}H_{19}N_5O_3S_2$: C, 57.85; H, 4.01; N, 14.67. Found: C, 57.62; H, 4.31; N, 14.43.

4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(pyridin-2-yl)benzenesulfonamide (10b)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfapyridine (**5e**) (124.65 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **10b** as a white powder (130.25 mg, 51%), mp 213-215 °C; ύ max (atr)/cm^{-1} 3432, 2925, 1674, 1636 and 1569; δ_H (400 MHz; DMSO-d_6) 4.81 (2H, s, CH_2), 6.53-6.54 (1H, m), 6.85-6.86 (1H, m), 7.09-7.11 (1H, m), 7.50-7.52 (2H, m), 7.63-7.68 (3H, m), 7.75-7.76 (2H, m), 8.00-8.10 (4H, m), 10.00 (1H, s) and 11.74 ppm (1H, br.); Anal. Calcd H, 3.81; N, 13.95.

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)-N-(pyridin-2-yl)benzenesulfonamide (10c)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfapyridine (**5e**) (124.65 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **10c** as a white powder (145.23 mg, 57%), mp 141-143 °C; ύ max (atr)/cm^{-1} 3431, 2973, 2930, 1670, 1635, 1600, 1501 and 1464; δ_H (400 MHz; DMSO-d_6) 3.87 (3H, s, OCH_3), 4.79 (2H, s, CH_2), 6.53 (1H, d, ^3J = 5.6 Hz), 6.85 (1H, t, ^3J = 6.4 Hz), 7.05-7.10 (3H, m), 7.53 (2H, d, ^3J = 8.8 Hz), 7.66 (2H, d like, ^3J = 8.8 Hz), 7.69-7.75 (1H, m), 7.99-8.00 (1H, m), 8.05 (2H, d, ^3J = 8.4 Hz), 8.12 (1H, d, ^3J = 5.6 Hz), 10.01 (1H, s) and 11.63 ppm (1H, br.); δ_C (100 MHz; DMSO-d_6) 38.29, 55.54, 103.94, 113.69, 114.09, 118.78, 127.70, 127.82, 128.21, 128.53, 130.65, 142,71, 142.75, 155.50, 155.52, 159.25, 161.13, 163.55, 169.32 and 191.60 ppm; Anal. Calcd for $C_{24}H_{21}N_5O_4S_2$: C, 56.79; H, 4.17; N, 13.80. Found: C, 56.41; H, 4.35; N, 13.72

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(pyrimidin-2-yl)benzenesulfonamide (11)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfadiazine (**5f**) (125.14 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **11** as a pale brown powder (180.34 mg, 71%), mp 165-167 °C; ύ max (atr)/cm^{-1} 3437, 3092, 2930, 1635, 1577 and 1498; δ_H (500 MHz; DMSO-d_6) 3.86 (3H, s, OCH_3), 4.86 (2H, s, CH_2), 6.65 (1H, d, ^3J = 5.0 Hz), 7.02 (1H, br.), 7.08 (2H, d, ^3J = 8.0 Hz), 7.69-7.74 (4H, m), 8.03 (2H, d, ^3J = 8.0 Hz), 8.16 (1H, d, ^3J = 5.0 Hz), 8.46-8.47 (2H, m) and 10.52 ppm (1H, s); δ_C (125 MHz; DMSO-d_6) 38.61, 55.57, 104.12, 114.14, 115.75, 119.06, 128.39, 128.76, 130.66, 133.52, 142.85, 153.68, 156.93, 158.32, 159.31, 163.63, 168.63, 172.04 and 191.44 ppm; Anal. Calcd for $C_{23}H_{20}N_6O_4S_2$: C, 54.32; H, 3.96; N, 16.53. Found: C, 54.11; H, 3.69; N, 16.86

N-(4-Methylpyrimidin-2-yl)-4-((2-((2-oxo-2-phenylethyl)thio) pyrimidin-4-yl)amino)benzenesulfonamide (12a)

Following the general procedure III, a mixture of **4a** (132.36 mg, 0.50 mmol), sulfamerazine (**5g**) (132.15 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **12a** as a pale brown powder (162.22 mg, 66%); mp 238-240 °C; ύ max (atr)/cm^{-1}, 3438, 3351, 3099, 3044, 2924, 1679, 1611 and 1566; δ_H (500 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 4.85 (2H, s, CH_2), 6.55 (1H, d, ^3J = 5.5 Hz), 6.88 (1H, d, ^3J = 3.5 Hz), 7.53 (2H, t, ^3J = 7.5 Hz), 7.66-7.69 (5H, m), 8.03 (2H, d, ^3J = 7.5 Hz), 8.13 (1H, d, ^3J = 5.5 Hz), 8.28 (1H, d, ^3J = 3.5 Hz), 10.06 (1H, br.) and 11.60 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 23.27, 38.56, 104.08, 114.82, 118.39, 126.32, 128.25, 128.46, 128.81, 128.99, 133.75, 135.68, 143.15, 155.66, 156.58, 159.25, 169.21, 172.03 and 193.49 ppm; Anal. Calcd for $C_{23}H_{20}N_6O_3S_2$: C, 56.08; H, 4.09; N, 17.06. Found: C, 56.35; H, 4.27; N, 17.35.

4-((2-((2-(4-Fluorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (12b)

Following the general procedure III, a mixture of **4b** (141.36 mg, 0.50 mmol), sulfamerazine (**5g**) (132.15 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **12b** as a pale brown powder (150.36 mg, 59%); mp 187-189 °C; ύ max (atr)/cm^{-1} 3436, 3099, 2925, 1676, 1600 and 1503; δ_H (500 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 4.82 (2H, s, CH_2), 6.54 (1H, d, ^3J = 5.0 Hz), 6.88 (1H, br.), 7.36 (2H, t like, ^3J = 8.5 Hz), 7.69-7.74 (4H, m), 8.11-8.12 (3H, m), 8.29 (1H, d, ^3J = 4.0 Hz), 10.04 (1H, s) and 11.54 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 23.25, 38.43, 104.07, 114.88, 115.90 (d, J = 21.88 Hz), 118.47, 128.98, 131.28

(d, ... 157.59, 159.23, 165.27 (d, J = 251.3 Hz), 168.27, 169.19, 172.03 and 192.24 ppm; Anal. Calcd for $C_{23}H_{19}FN_6O_3S_2$: C, 54.11; H, 3.75; N, 16.46. Found: C, 54.42; H, 3.47; N, 16.19.

4-((2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)pyrimidin-4-yl)-amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (12c)

Following the general procedure III, a mixture of **4c** (149.59 mg, 0.50 mmol), sulfamerazine (**5g**) (132.15 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **12c** as a pale brown powder (180.46 mg, 69%), mp 211-213 °C; *ύ* max (atr)/cm^{-1} 3428, 3054, 2970, 2926, 1626, 1566 and 1499; δ_H (500 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 4.81 (2H, s, CH_2), 6.55 (1H, d, ^3J = 5.5 Hz), 6.87 (1H, d, ^3J = 4.0 Hz), 7.61 (2H, d, ^3J = 8.0 Hz), 7.73 (2H, d, ^3J = 8.0 Hz), 7.80 (2H, d, ^3J = 8.0 Hz), 8.04 (2H, d, ^3J = 8.0 Hz), 8.11 (1H, d, ^3J = 5.5 Hz), 8.28 (1H, d, ^3J = 4.0 Hz), 10.06 (1H, s) and 11.70 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 23.27, 38.43, 104.12, 114.81, 118.55, 128.19, 128.54, 128.95, 129.03, 130.15, 134.42, 138.64, 142.34, 155.00, 155.79, 156.61, 159.23, 169.06 and 192.84 ppm; Anal. Calcd for $C_{23}H_{19}ClN_6O_3S_2$: C, 52.42; H, 3.63; N, 15.95. Found: C, 52.74; H, 3.81; N, 15.68.

N-(4-Methylpyrimidin-2-yl)-4-((2-((2-oxo-2-(4-(trifluorometh-yl)phenyl)ethyl)thio)pyrimidin-4-yl)amino)benzenesulfonamide (12d)

Following the general procedure III, a mixture of **4e** (166.36 mg, 0.50 mmol), sulfamerazine (**5g**) (132.15 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **12d** as a pale brown powder (184.65 mg, 66%); mp 210-212 °C; *ύ* max (atr)/cm^{-1} 3440, 2926, 2857, 1675, 1629 and 1572; δ_H (400 MHz; DMSO-d_6) 2.28 (3H, s, CH_3), 4.86 (2H, s, CH_2), 6.55 (1H, br.), 6.87 (1H, br.), 7.69-7.92 (8H, m), 8.11-8.28 (2H, m), 10.04 (1H, s) and 11.82 ppm (1H, br.); Anal. Calcd for $C_{24}H_{19}F_3N_6O_3S_2$: C, 51.42; H, 3.42; N, 14.99. Found: C, 51.78; H, 3.15; N, 14.65.

4-((2-((2-(4-Methoxyphenyl)-2-oxoethyl)thio)pyrimidin-4-yl)amino)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide (12e)

Following the general procedure III, a mixture of **4f** (147.38 mg, 0.50 mmol), sulfamerazine (**5g**) (132.15 mg, 0.50 mmol) and acetic acid (15 mL) was refluxed for 4h. Work up afforded **12e** as a white powder (190.44 mg, 73%), mp 230-232 °C; *ύ* max (atr)/cm^{-1} 3434, 3087, 2926, 2855, 1678, 1605 and 1565; δ_H (400 MHz; DMSO-d_6) 2.29 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 4.79 (2H, s, CH_2), 6.54 (1H, d, ^3J = 5.6 Hz), 6.87 (1H, d, ^3J = 5.0 Hz), 7.07 (2H, d, ^3J = 8.8 Hz), 7.69-7.74 (4H, m), 8.02 (2H, d, ^3J = 8.8 Hz), 8.13 (1H, d, ^3J = 5.6 Hz), 8.28 (1H, d, ^3J = 5.2 Hz), 10.04 (1H, s) and 11.52 ppm (1H, br.); δ_C (125 MHz; DMSO-d_6) 23.27, 38.23, 55.59, 104.03, 106.88, 114.11, 118.52, 121.77, 128.55, 129.02, 130.65, 143.21, 155.80, 159.26, 160.43, 163.57, 169.37, 171.16 and 191.88 ppm; Anal. Calcd for $C_{24}H_{22}N_6O_4S_2$: C, 55.16; H, 4.24; N, 16.08. Found: C, 55.44; H, 4.51; N, 16.38.

5.2. Biological evaluation

5.2.1. Carbonic anhydrase inhibition

Photophysics stopped-flow instrument was utilized for evaluating the CA catalyzed CO_2 hydration activity [49]. Phenol red (0.2 mM) was employed as an indicator. The assay was carried out at absorbance maximum of 557 nm, using 20 mM Hepes (pH 7.5) as a buffer, and 20 mM Na_2SO_4 for keeping the ionic strength of the used buffer constant. Initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100s were applied. Subsequently, CO_2 concentrations ranging from 1.7 to 17 mM were applied for the calculating of the kinetic parameters and inhibition constants. Minimum six traces of the initial 5 to 10% of the reaction have been utilized for evaluating the initial velocity ...vere similarly determined and subtracted from the total observed rates. A Stock solution of (0.1 mM) of the synthesized compounds or AAZ in distilled-deionized water was prepared. Further dilutions up to 0.01 nM were carried out using distilled-deionized water. Before starting the assay, the tested inhibitors and enzyme solutions were incubated together for 15 min at room temperature, in order to allow the generation of the E-I complex. The inhibition constants were calculated by the application of non-linear least-squares methods using the Cheng-Prusoff equation. The results were presented as the mean from at least three different experiments.

5.3. Molecular Docking

All studies of molecular modeling were carried out using the software Molecular Operating Environment (MOE, 2010.10). All minimizations were performed with MOE until an RMSD gradient of 0.1 kcal·mol^{-1}Å^{-1} with MMFF94x force field and the partial charges were automatically calculated. The X-ray crystallographic structure of CAII co-crystallized with acetazolamide AAZ as inhibitor (PDB ID: 3HS4) [50] were downloaded from the protein data bank [https://www.rcsb.org/]. First, removal of water molecules and ligands not engaged in binding in the active site were carried out. The enzyme structure was then prepared with default alternatives for the simulation of molecular docking using Protonate 3D protocol in MOE. The enzyme structure was then prepared for the molecular docking simulation using *Protonate 3D* protocol in MOE with default options. The co-crystalized AAZ was used to locate the binding site for molecular docking. Triangle Matcher placement method and London dG scoring function were used for docking using Zn^{2+} metal ion chelation as a constrain for molecular docking. Docking protocol was first validated by self-docking of the co-crystallized ligand AAZ in the enzyme active site giving a docking pose with an energy score (S) = −7.68 kcal/mol and an RMSD of 0.867 Å from the co-crystalized ligand pose (Figure 4). The validated molecular docking protocol was then used to study the ligand-enzyme interactions of the newly synthesized sulphonamide conjugates in the enzyme active site to predict their binding pattern and to elucidate their SAR (Figure 5) (For further details see SI) .

Conflict of interest

The authors have no conflict of interest to declare.

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Supplementary Material

Ne... Benzenesulfonamide Conjugates as Selective Carbonic Anhydrase II Inhibitors: Synthesis, *in vitro* Biological Evaluation, and Molecular Docking Studies

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Conflict of interest

The authors have no conflict of interest to declare.

Highlights μM

against hCA II.

New Thiopyrimidine-Benzenesulfonamide Conjugates as Selective Carbonic Anhydrase II Inhibitors: Synthesis, *in vitro* Biological Evaluation, and Molecular Docking Studies

Heba T. Abdel-Mohsen*[a], Ahmed M. El Kerdawy[b,c], Mohamed A. Omar[a], Emanuela Berrino[d], Ahmed S. Abdelsamie[a,e], Hoda I. El Diwani[a] and Claudiu T. Supuran*[d]

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- A new series of thiopyrimidine-benzenesulfonamide conjugates was synthesized and evaluated for their CA inhibitory activity.
- Compounds **6a-g** displayed promising inhibitory activity against cytosolic isoforms hCA I and hCA II.
- Compounds **6e** and **6f** demonstrated selectivity of 15.8- to 980-fold towards hCA II over hCA I, hCA IX, hCA XII isoforms.
- Molecular docking simulations of **6a-g** with hCA II revealed key H-bonding interactions with the sulfonamide group and hydrophobic interactions with the benzenesulfonamide and thiopyrimidine moieties.