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Synthesis of benzamide derivatives with thiourea-substituted benzenesulfonamides as carbonic anhydrase inhibitors

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Abstract

The novel compounds with the chemical structure of *N*-({4-[*N*′-(substituted) sulfamoyl]phenyl}carbamothioyl)benzamide (**1a–g**) and 4-fluoro-*N*-({4-[*N*′-(substituted)sulfamoyl]phenyl}carbamothioyl)benzamide (**2a–g**) were synthesized as potent and selective human carbonic anhydrase (hCA) I and hCA II candidate inhibitors. The aryl part was changed to sulfacetamide, sulfaguanidine, sulfanilamide, sulfathiazole, sulfadiazine, sulfamerazine, and sulfametazine. The K_i values of compounds **1a–g** were in the range of 20.73 ± 4.32 to 59.55 ± 13.07 nM (hCA I) and 5.69 ± 0.43 to 44.81 ± 1.08 nM (hCA II), whereas the K_i values of compounds **2a–g** were in the range of 13.98 ± 2.57 to 75.74 ± 13.51 nM (hCA I) and 8.15 ± 1.5 to 49.86 ± 6.18 nM (hCA II). Comparing the K_i values of the final compounds and acetazolamide, compound **1c** with the sulfanilamide moiety (K_i = 5.69 ± 0.43 nM, 8.8 times) and **2f** with the sulfamerazine moiety (K_i = 8.15 ± 1.5 nM, 6.2 times) demonstrated promising and selective inhibitory effects against the hCA II isoenzyme, the main target protein in glaucoma. Furthermore, compounds **1d** (K_i = 20.73 ± 4.32, 4 times) and **2d** (K_i = 13.98 ± 2.57, 5.9 times), which have the sulfathiazole moiety, were found as potent hCA I inhibitors. Compounds **1c** and **2f** can be considered as the lead compounds determined in the present study, which can be investigated further to alleviate glaucoma symptoms.

KEYWORDS

benzamide, carbonic anhydrase, enzyme inhibitors, sulfonamides, thiourea

1 | INTRODUCTION

Carbonic anhydrases (CAs; E.C. 4.2.1.1) are metalloenzymes with important roles in various diseases. They are considered as target proteins in diuretic, epilepsy, glaucoma, cancer, and obesity. The irregular expression of CA isoenzymes leads to pathological problems. Among human CAs (hCA), common hCA I and hCA II isoenzymes in the human body are well-known and most-studied targets. hCA I plays role in edema, whereas hCA II is the main target protein in glaucoma.[1–6]

Several primary sulfonamides and their derivatives are known as an attractive and versatile compound class of CA inhibitors.[7,8] They are mainly employed as antiglaucoma and diuretics agents in clinic applications.[9,10] Well-known sulfonamide-based CA inhibitors include acetazolamide, ethoxzolamide, and methazolamide. Benzenesulfonamides and sulfonamides exhibit inhibition effects through interaction with Zn^{2+} ion at the active site of the enzyme and amino acid residues are the proposed effect mechanism.[7–9,11,12] Although primary sulfonamides are known as the most valuable pharmacophoric group for CA inhibitors, secondary sulfonamides were also reported with their selective and effective CA inhibitory activity against several CAs isoenzymes with a similar effect mechanism as primary sulfonamides.[13–15] Several CA inhibitors with primary or secondary benzenesulfonamide are presented in Figure 1. From this perspective, we designed novel compounds that incorporated both primary and secondary sulfonamide pharmacophores as novel CA I

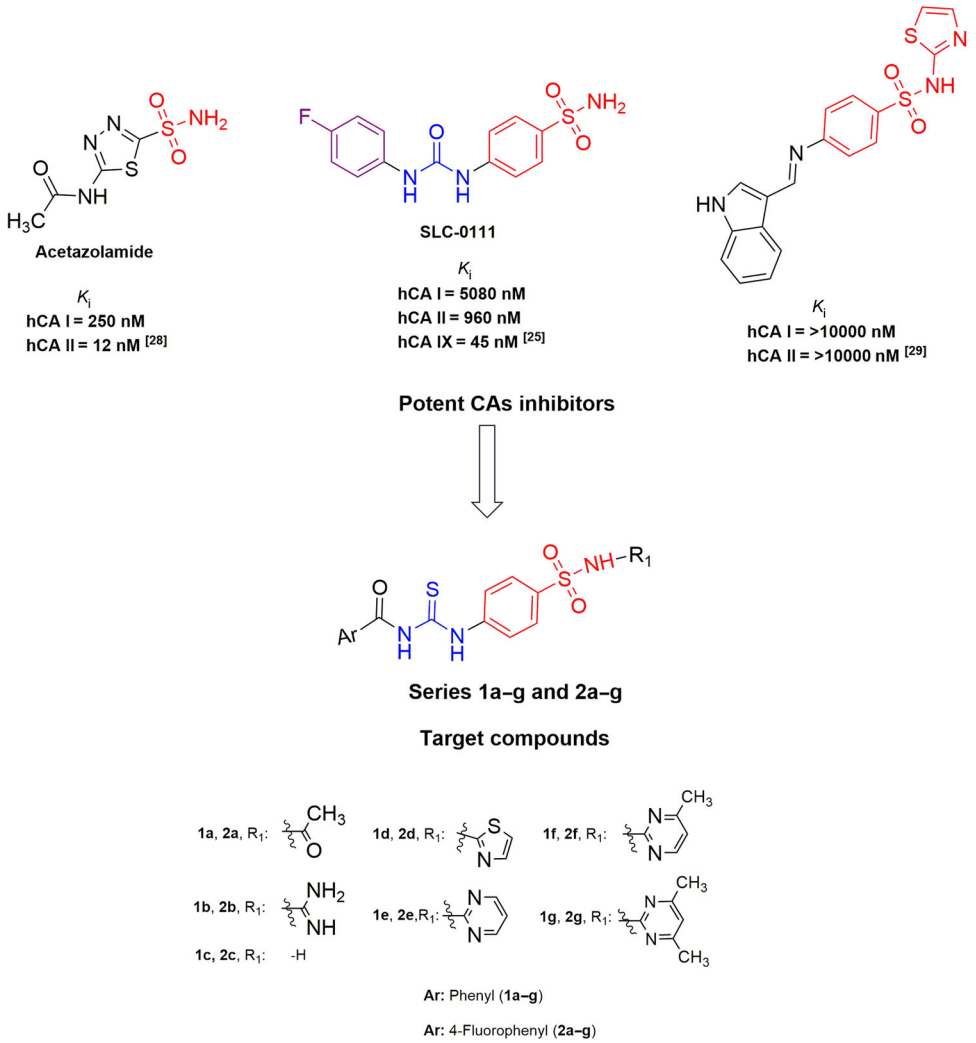


FIGURE 1 Structures of some carbonic anhydrase inhibitors[16,17]

and CA II inhibitors in the present study to observe their effects on bioactivity.

Furthermore, compounds derived from urea and its sulfur analogue thiourea have continuously been used in medicinal chemistry to design new bioactive compounds due to their promising physicochemical and pharmacological features. Also, these compounds have many biological activities including anticancer, antiviral, and antimicrobial activities, and they also acted as 15-lipoxygenase inhibitors, polo-like kinase 1 (Plk1), polo-box domain (PBD) inhibitors, anti-Alzheimer's disease agents, CA inhibitors, and so forth.[18–24]

Primary sulfonamide-based urea derivative SLC-0111, designed by the tail-approach method, exhibited selective inhibition toward CA IX and CA XII isoenzymes associated with cancer against targets CA I and CA II. SLC-0111 (Figure 1) was referred to in clinical trials related with hypoxic tumors.[25–27] It was suggested that the ureido moiety of SLC-0111 may lead to an increase in flexibility of the tail of the compound, which may cause various several conformational changes at the active site of the enzyme. These flexible conformations allow several favorable interactions between inhibitor and CAs isoenzymes.[25–29] Despite the significant SLC-0111 behavior as selective CA IX inhibitor, many compounds were reported to exhibit unselective inhibition toward CA isoenzymes. This unfavorable case leads to several side effects. Thus, a new drug design strategy, that is, the tail approach, was preferred in the design of novel CAs inhibitors in the present study.

Several drug molecules such as encorafenib, dacomitinib, and lorlatinib that include a fluorine atom have been known with their significant clinical pharmacological uses.[30] It was considered that the substitution of fluorine would lead to more potent compounds with increased resistance against drug metabolism, as it regulates the reactivity and stability of the compounds.[30] Furthermore, fluorine atom modulates pK_a, lipophilicity, and hydrophobic interactions that affect the physicochemical properties, which play a role in the pharmacokinetic process of the compound. Due to the significant molecular properties of fluorine, it has been considered in novel drug design strategies.[10,31]

In the present study, we combined primary or secondary benzenesulfonamide and thioureido pharmacophores in a molecule with the

tail approach drug design to investigate promising enzyme inhibitors. *N*-({4-[*N*′-(Substituted)sulfamoyl]phenyl}carbamothioyl)benzamides (**1a–g**) and 4-fluoro-*N*-({4-[*N*′-(substituted)sulfamoyl]phenyl}carbamothioyl) benzamides (**2a–g**) were designed and synthesized, and their enzyme inhibitory activities against hCA I and hCA II were investigated to discover possible promising drug candidate/s in the current study.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

In the present study, we synthesized two series of benzamide derivatives with thiourea-substituted benzenesulfonamides in two steps (Scheme 1). First, aroyl isothiocyanate derivatives were synthesized by the reaction of aroyl chlorides (benzoyl chloride for **1a–g** and 4-fluorobenzoyl chloride for **2a–g** series) and potassium thiocyanate in acetone. The intermediates were not isolated and used without any further purification for the next step. Then, aroyl isothiocyanates were reacted with substituted sulfonamides (sulfacetamide [**1a**, **2a**], sulfaguanidine [**1b**, **2b**], sulfanilamide [**1c**, **2c**], sulfathiazole [**1d**, **2d**], sulfadiazine [**1e**, **2e**], sulfamerazine [**1f**, **2f**], and sulfamethazine [**1g, 2g**]) to obtain final compounds **1a–g** and **2a–g** (Scheme 1). All final compounds **1a–g** and **2a–g** were characterized by spectral methods. Compounds **1a**, **1b**, **2a**, **2b**, and **2f** have been reported for the first time in the present study. Compounds **1c**, **2c**, **1d**, **2d**, **1e**, **2e**, **1f**, **1g**, and **2g** with bioactivities have been reported only in a few studies.[32–35]

^{1}H NMR (nuclear magnetic resonance) spectral data were presented for compound **1b** as follows: Signals of protons attached with thiourea moiety were at δ 12.78 and 12.14 ppm as a singlet, whereas the signal of the proton on the sulfamoyl group was observed at 11.73 as a singlet. Methyl protons on the acetyl group were observed at δ 1.96 ppm as a singlet. Besides, ^{13}C NMR spectra revealed that aryl and aliphatic carbon peaks of the compounds were observed in the expected areas. High-resolution mass spectrometry (HRMS) data also confirmed the molecular weight of the compound **1b** (calculated $[M+Na]^+$: 400.0396 and found $[M+Na]^+$: 400.0405).

2.2 | Inhibitory potency of the compounds toward CAs

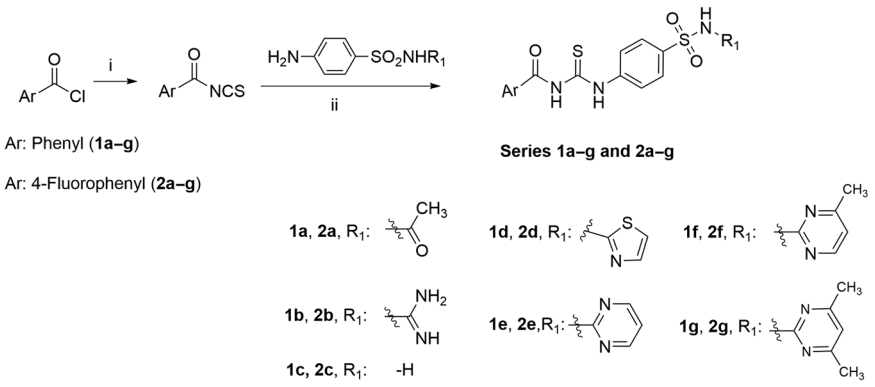
Compounds **1a–g** and **2a–g** were tested against slow cytosolic hCA I and the fast cytosolic hCA II isoenzymes, as the main CA inhibitor classes had a sulfonamide group. The findings concerning the compounds are presented in Table 1.

The CA isoenzymes were effectively inhibited by compounds **1a–g**. IC_{50} values of the compounds were 13.07–31.5 nM against both isoenzymes. IC_{50} values were calculated in the range of 17.77–31.50 nM (hCA I) and 13.07–24.75 nM (hCA II), whereas IC_{50} values of the reference drug AZA were 48.78 nM (hCA I) and 43.76 (hCA II). Compounds **1a–g** were 1.5–2.7 times and 2.1–3.3 times more potent when compared with reference drug AZA against hCA I and hCA II, respectively, based on the IC_{50} values. The compound *N*-({4-[*N*′-(thiazol-2-yl)sulfamoyl]phenyl}carbamothioyl)benzamide (**1d**) was the best inhibitor against hCA I and hCA II isoenzymes, with IC_{50} values of 17.77 and 13.07 nM, respectively.

K_i values of compounds **1a–g** were also in the range of 20.73 ± 4.32 to 59.55 ± 13.07 nM (hCA I) and 5.69 ± 0.43 to 44.81 ± 1.08 nM (hCA II), whereas AZA's K_i values were 82.13 ± 4.56 nM (hCA I) and 50.27 ± 3.75 nM (hCA II). Compound **1d**, *N*-({4-[*N*′-(thiazol-2-yl) sulfamoyl]phenyl}carbamothioyl)benzamide, was the best inhibitor against hCA I, with K_i = 20.73 ± 4.32 nM, whereas compound **1c**, *N*-({4-sulfamoylphenyl}carbamothioyl)benzamide, was found as the best CA inhibitor in the series against hCA II, with a K_i value of 5.69 ± 0.43 nM.

IC_{50} values of the compound **2a–g** were in the range of 23.90–33.0 nM (hCA I) and 21.66–32.48 nM (hCA II), whereas for the reference drug AZA, IC_{50} values were 48.78 nM (hCA I) and 43.76 nM (hCA II). Compounds **2a–g** with 4-fluorophenyl moiety also exhibited significant CA inhibition potential. CA inhibitory effects of the compounds **2a–g** were higher when compared with AZA. The IC_{50} values were 1.5–2.0 times and 1.3–2.0 times more potent against hCA I and hCA II, respectively. Compound **2d**, 4-fluoro-*N*-({4-[*N*′-(thiazol-2-yl)sulfamoyl]phenyl}carbamothioyl)benzamide, can be considered as the potent compound, with IC_{50} = 23.9 and IC_{50} = 21.66 nM against hCA I and hCA II, respectively.

On the basis of the K_i values, compounds **2a–g** strongly inhibited hCA I isoenzyme, with K_i values of 13.98 ± 2.57 to 75.74 ± 13.51 nM,



SCHEME 1 The synthesis of compounds **1a–g** and **2a–g**. Reaction conditions: (i) KSCN, acetone, reflux, 1.5 hr; (ii) acetone, reflux, 4–8 hr

TABLE 1 Carbonic anhydrase (CA) enzyme inhibition results of compounds **1a–g** and **2a–g**

Compounds	IC_{50}				K_i	
	hCA I (nM)	r^2	hCA II (nM)	r^2	hCA I (nM)	hCA II (nM)
1a	31.50	.9779	19.25	.9918	38.51 ± 7.83	44.81 ± 1.08
1b	22.35	.9919	16.90	.9746	30.91 ± 6.62	24.95 ± 5.90
1c	20.38	.9745	13.59	.9927	40.38 ± 8.15	5.69 ± 0.43
1d	17.77	.9796	13.07	.9727	20.73 ± 4.32	17.93 ± 1.02
1e	23.10	.9798	18.24	.9730	46.55 ± 4.56	38.77 ± 6.77
1 f	24.75	.9832	21.00	.9768	54.26 ± 3.89	16.61 ± 0.87
1 g	26.65	.9975	24.75	.9878	59.55 ± 13.07	12.19 ± 2.24
2a	28.88	.9840	23.90	.9761	64.31 ± 13.16	25.83 ± 1.99
2b	30.88	.9655	32.48	.9738	75.74 ± 13.51	42.99 ± 9.57
2c	24.75	.9718	25.67	.9869	19.88 ± 1.04	49.86 ± 6.18
2d	23.90	.9727	21.66	.9813	13.98 ± 2.57	12.49 ± 1.04
2e	30.13	.9804	31.50	.9729	45.20 ± 1.24	18.92 ± 1.63
2f	33.00	.9834	30.13	.9741	30.17 ± 2.35	8.15 ± 1.50
2g	29.87	.9952	26.65	.9801	55.95 ± 10.72	47.96 ± 7.91
Acetazolamide	48.78	.9878	43.76	.9813	82.13 ± 4.56	50.27 ± 3.75

whereas AZA's K_i value was 82.13 ± 4.56 nM against hCA I isoenzyme. Thus, hCA I isoenzyme inhibition potency of the compounds was 5.9–1.1 times more than AZA. Compound **2d** can be considered as a promising inhibitor against hCA I isoenzyme in the series, with K_i = 13.98 ± 2.57 nM.

Furthermore, for compounds **2a–g**, K_i values were 8.15 ± 1.5 to 49.86 ± 6.18 nM against cytosolic hCA II isoenzyme, whereas AZA's K_i value was 50.27 ± 3.75 nM against hCA II isoenzyme. 4-Fluoro-*N*-({4-[*N*′-(4-methylpyrimidin-2-yl)sulfamoyl]phenyl}carbamothioyl) benzamide (**2f**) was the strongest inhibitor in the series, with a K_i value of 8.15 ± 1.5 nM against hCA II isoenzyme.

Compounds **1c** with phenyl moiety and **2c** with 4-fluorophenyl moiety have been reported for hCA II inhibitory effects in the literature.[33] In previous study findings, compounds **1c** and **2c** have been found to exhibit inhibitory effects against hCA II, with IC_{50} values of 110 nM and 580 nM, respectively, whereas in the present study, these values are 13.59 nM (**1c**) and 25.67 nM (**2c**). However, the differences could be due to the employed techniques. Unsubstituted compound **1c** was more effective when compared with its fluorinated analog **2c**, similar to our findings, against hCA II.

2.3 | Structure–activity relationships (SAR)

The following SAR results could be determined on the basis of compounds' K_i values against hCA I and hCA II. Initially, in series **1a–g** against hCA I, the most potent compound was the thiazole-bearing sulfathiazole derivative **1d**. Compounds **1a**, **1b**, and **1d** bearing secondary sulfonamides were found more effective inhibitors than sulfanilamide moiety-bearing compound **1c**, which has a primary sulfonamide. However, compounds sulfadiazine (**1e**), sulfamerazine (**1f**), and sulfamethazine (**1g**) which have six-membered pyrimidine ring were less effective when compared with **1c**. Among pyrimidine derivatives, it could be suggested that substitution of the additional methyl groups on the ring led to unfavorable results. Thus, methyl groups caused steric hindrance in the compounds, which affected the interaction at the active side of the enzyme. However, the acetyl and guanidine substitution in compounds **1a** and **1b** led to an increased activity when compared with **1c**. The SAR data indicated that a five-membered ring and small groups led to positive effects on hCA I inhibitory potency of the benzamide derivatives with thiourea-substituted benzenesulfonamides. Also, in series **1a–g** against hCA II, the most potent compound was the sulfanilamide derivative **1c** in contrast to the hCA I results. Surprisingly, in this series against hCA II, sulfamerazine-bearing **1f** and sulfamethazine-bearing **1g** derivatives, except sulfadiazine-bearing **1e**, on six-membered pyrimidine significantly inhibited the hCA II, whereas they were not considerably effective against hCA I. Thus, methyl groups on pyrimidine enhanced the inhibitory activity against hCA II as compared with their effects on hCA I. Enzyme selectivity of the drugs or drug candidates is one of the crucial properties of enzyme-targeted diseases. For instance, the compound that targets glaucoma should selectively inhibit the hCA II enzyme. Among **1a–g** series, compound **1c** with primary sulfonamide was approximately eightfold more selective toward hCA II, which the main target of glaucoma. This suggested that **1c** could be considered as a potential candidate compound in this series.

The SAR results of compounds **2a–g** bearing fluorophenyl could be summarized as follows. Compound **2d** with sulfathiazole core was the strongest inhibitor in the series. This situation was similar to hCA I findings. The substitution of the fluorine atom in compound **2d**

increased the activity by 1.5 times when compared with its corresponding phenyl analog **1d**. When primary sulfonamide derivative **1c** converted to its fluorinated analog **2c**, the activity was also increased two times. When pyrimidine ring-bearing compounds **2e**–**g** were considered, fluorination of the compounds slightly increased the activity as compared with their non-fluorinated analogs **1e**–**g**. However, sulfacetamide (**2a**) and sulfaguanidine (**2b**) derivatives exhibited a lower activity against hCA I when compared with their corresponding analogs **1a** and **1b**. The SAR data revealed that fluorination of the compounds generally increased the enzyme inhibitory activity against hCA I.

The phenyl analog of compound **1c** with sulfanilamide moiety was the most effective inhibitor against hCA II. However, it is interesting to note that the fluorinated derivative **2c** significantly decreased the enzyme activity against hCA II by about 10-fold when compared with **1c**. The most powerful inhibitor against hCA II, **2f**, with sulfamerazine moiety, increased the activity via fluorination when compared with phenyl analog **1f**. Four fluorinated compounds (**2a**, **2d**, **2e**, and **2f**) exhibited an increased activity against hCA II. Briefly, fluorination of the phenyl ring led to either an increase or decrease in the inhibition activity against both isoenzymes. Thus, the impact of fluorine was variable. The overall analysis of the results demonstrated that compounds **1c** with sulfanilamide and **2f** with sulfamerazine could be considered as the most promising and selective hCA II inhibitors, which could be further analyzed in future research.

Druglikeness properties of the oral drugs are identified by Lipinski's rule of five (RO5).[36] Thus, we calculated suitable parameters of the compounds to determine whether they were compatible with the RO5. On the basis of the analysis (Table 2), the following values were determined: logP (lipophilicity, 1.18–2.78), nHBD (number of H-bond donors, 3–4), nHBA (number of H-bond acceptors, 4–6), and MW (molecular weight, 335.4–459.52). All synthesized compounds were found compatible with Lipinski's RO5.

TABLE 2 Druglikeness properties of compounds **1a–g** and **2a–g**, based on Lipinski's RO5

Compounds	Formula	MW (g/mol)	nHBA	nHBD	LogP
1a	$C_{16}H_{15}N_3O_4S_2$	377.44	4	3	1.85
1b	$C_{15}H_{15}N_5O_3S_2$	377.44	4	4	1.18
1c	$C_{14}H_{13}N_3O_3S_2$	335.40	4	3	1.76
1d	$C_{17}H_{14}N_4O_3S_3$	418.51	4	3	2.33
1e	$C_{18}H_{15}N_5O_3S_2$	413.47	5	3	1.87
1f	$C_{19}H_{17}N_5O_3S_2$	427.50	5	3	2.22
1g	$C_{20}H_{19}N_5O_3S_2$	441.53	5	3	2.49
2a	$C_{16}H_{14}FN_3O_4S_2$	395.43	5	3	2.00
2b	$C_{15}H_{14}FN_5O_3S_2$	395.43	5	4	1.52
2c	$C_{14}H_{12}FN_3O_3S_2$	353.39	5	3	1.91
2d	$C_{17}H_{13}FN_4O_3S_3$	436.50	5	3	2.75
2e	$C_{18}H_{14}FN_5O_3S_2$	431.46	6	3	2.31
2f	$C_{19}H_{16}FN_5O_3S_2$	445.49	6	3	2.58
2g	$C_{20}H_{18}FN_5O_3S_2$	459.52	6	3	2.78

Note: Lipinski filter: MW ≤ 500, MlogP ≤ 4.15, N or O ≤ 10, NH or OH ≤ 5.[36]

Abbreviations: MW, molecular weight; nHBA, number of H-bond acceptors; nHBD, number of H-bond donors; RO5, rule of five.

3 | CONCLUSION

In the present study, two series of benzamide derivatives with thiourea-substituted benzenesulfonamides (**1a–g** and **2a–g**) were designed and synthesized as potent and selective CA inhibitors. Spectral techniques confirmed the proposed chemical structure of the compounds. K_i values of compounds **1a–g** were in the range of 20.73 ± 4.32 to 59.55 ± 13.07 nM (hCA I) and 5.69 ± 0.43 to 44.81 ± 1.08 nM (hCA II), whereas K_i values of compounds **2a–g** with 4-fluorobenzoyl chloride were in the range of 13.98 ± 2.57 to 75.74 ± 13.51 nM (hCA I) and 8.15 ± 1.5 to 49.86 ± 6.18 nM (hCA II). Compounds **1c** (K_i = 5.69 ± 0.43 nM, 8.8 times) with sulfanilamide moiety and **2f** (K_i = 8.15 ± 1.5 nM, 6.2 times) with sulfamerazine moiety exhibited promising and selective hCA II isoenzyme inhibition potential, which is the main target protein in glaucoma. Furthermore, compounds **1d** (K_i = 20.73 ± 4.32, 4 times) and **2d** (K_i = 13.98 ± 2.57, 5.9 times) with sulfathiazole moiety were found as potent hCA I inhibitors. However, sulfathiazole derivatives **1d** and **2d** were more effective inhibitors when compared with **1e** and **2e** with sulfamerazine moiety. It appears that the sulfathiazole compounds (**1d** and **2d**), with a five-membered ring, have a significant inhibitory effect on both isoenzymes in terms of K_i values. The SAR data also indicated that fluorination of the phenyl ring led to variable effects against both isoenzymes. All synthesized compounds were also found compatible with Lipinski's RO5.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

The chemical structures of the final compounds were confirmed by the NMR spectra: ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz; Varian Mercury Plus spectrometer; Varian Inc., Palo Alto, CA), and HRMS (Shimadzu, Kyoto, Japan). The original spectra are provided as Supporting Information. Chemical shifts (δ) are reported in ppm and coupling constants (J) are expressed in hertz (Hz). Mass spectra (HRMS) for the compounds were taken using a liquid chromatography ion trap time-of-flight tandem mass spectrometer (Shimadzu) equipped with an electrospray ionization (ESI) source, operating in both positive and negative ionization modes. Shimadzu's LCMS Solution software was used for data analysis. Melting points were

determined using an Electrothermal 9100/IA9100 instrument (Bibby Scientific Limited, Staffordshire, UK) and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 HF254 (Merck KGaA). Chloroform/methanol (4.8:0.2) solvent mixture was used as the TLC solvent system. Dimethyl sulfoxide (DMSO)-d_6 (Merck) was used as an NMR solvent.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of compounds 1a–g (Scheme 1)

A solution of benzoyl chloride (1 mmol) in acetone (20 ml) was added dropwise to a suspension of potassium thiocyanate (1 mmol) in acetone (30 ml), and the reaction mixture was refluxed for 1.5 hr to afford isothiocyanates. The reaction mixture was checked with TLC and benzoyl isothiocyanate derivative was formed in a reaction medium. After completion of the reaction, the substituted sulfonamide derivative (1 mmol) was added and the mixture was stirred under reflux for 4–8 hr. Upon completion of the reaction (checked with TLC), the resulting precipitate was collected by filtration and recrystallized from dimethylformamide/ethanol/H_2O (4.2:0.6:0.2) to obtain the pure products **1a–g**.[23]

N-*({4-[N′-(Acetyl)sulfamoyl]phenyl}carbamothioyl)benzamide (**1a**)*

White color solid, mp: 235–237°C, yield 80%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.78 (s, 1H, NH), 12.14 (s, 1H, NH), 11.73 (s, 1H, NH), 8.03–7.94 (m, 6H, Ar-H), 7.68–7.66 (m, 1H, Ar-H), 7.57–7.54 (m, 2H, Ar-H), and 1.96 (s, 3H, –CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.8 (C═S), 169.3 (C═O), 168.6, 143.0, 136.5, 133.7, 132.5, 129.2, 128.9, 128.8, 124.5, and 23.7; HRMS (ESI-MS) $C_{16}H_{15}N_3O_4S_2$, Calculated $[M+Na]^+$: 400.0396; Found $[M+Na]^+$: 400.0405.

N-*({4-[N′-(Diaminomethylene)sulfamoyl]phenyl}carbamothioyl)-benzamide (**1b**)*

White color solid, mp: 242–243°C, yield 85%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.70 (s, 1H, NH), 11.68 (s, 1H, NH), 8.00–7.95 (m, 2H, Ar-H), 7.86–7.84 (m, 2H, Ar-H), 7.80–7.78 (m, 2H, Ar-H), 7.69–7.65 (m, 1H, Ar-H), 7.57–7.53 (m, 2H, Ar-H), and 6.75 (bs, 4H, NH_2); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.8 (C═S), 162.8 (C═O), 158.6, 142.3, 140.8, 133.7, 132.6, 129.2, 128.9, 126.6, and 124.6; HRMS (ESI-MS) $C_{15}H_{15}N_5O_3S_2$; calculated $[M+H]^+$: 378.0689; found $[M+H]^+$: 378.0693.

N-*({4-Sulfamoylphenyl}carbamothioyl)benzamide (**1c**)*

White color solid, mp: 234–236°C, yield 86%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.72 (s, 1H, NH), 11.70 (s, 1H, NH), 8.00–7.98 (m, 2H, Ar-H), 7.93–7.85 (m, 4H, Ar-H), 7.70–7.66 (m, 1H, Ar-H), 7.57–7.53 (m, 2H, Ar-H), and 7.41 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.9 (C═S), 168.7 (C═O), 141.8, 141.4, 133.7, 132.5, 129.2, 128.9, 126.7, and 124.8; HRMS (ESI-MS) $C_{14}H_{13}N_3O_3S_2$; calculated $[M-H]^-$: 334.0326; found $[M-H]^-$: 334.0330.

N-*({4-[N′-(Thiazol-2-yl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**1d**)*

Cream color solid, mp: 245–246°C, yield 84%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.79 (s, 1H, NH), 12.71 (s, 1H, NH), 11.69 (s, 1H, NH), 7.99–7.97 (m, 2H, Ar-H), 7.91–7.82 (m, 4H, Ar-H), 7.69–7.65 (m, 1H, Ar-H), 7.56–7.52 (m, 2H, Ar-H), 7.29–7.27 (m, 1H, Ar-H), and 6.86–6.85 (m, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.7 (C═S), 169.4 (C═O), 168.6, 141.6, 139.9, 133.7, 132.5, 129.2, 128.9, 126.9, 124.9, 124.7, and 108.8; HRMS (ESI-MS) $C_{17}H_{14}N_4O_3S_3$; calculated $[M+Na]^+$: 441.0120; found $[M+Na]^+$: 441.0129.

N-*({4-[N′-(Pyrimidin-2-yl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**1e**)*

Light cream color solid, mp: 250–252°C, yield 88%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.75 (s, 1H, NH), 11.70 (s, 1H, NH), 8.54–8.52 (m, 2H, Ar-H), 8.03–7.97 (m, 7H, Ar-H, NH), 7.69–7.65 (m, 1H, Ar-H), 7.56–7.52 (m, 2H, Ar-H), and 7.08–7.05 (m, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.7 (C═S), 168.6 (C═O), 158.9, 157.3, 142.3, 137.8, 133.7, 132.5, 129.2, 128.9, 128.7, 124.3, and 116.3; HRMS (ESI-MS) $C_{18}H_{15}N_5O_3S_2$; calculated $[M+Na]^+$: 436.0509; found $[M+Na]^+$: 436.0502.

N-*({4-[N′-(4-Methylpyrimidin-2-yl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**1f**)*

Light white color solid, mp: 231–232°C, yield 80%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.75 (s, 1H, NH), 11.69 (s, 1H, NH), 8.35–8.33 (m, 1H, Ar-H), 8.03–7.94 (m, 7H, Ar-H, NH), 7.68–7.65 (m, 1H, Ar-H), 7.56–7.53 (m, 2H, Ar-H), 6.92–6.91 (m, 1H, Ar-H), and 2.33 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.7 (C═S), 168.6 (C═O), 158.7, 157.2, 156.9, 142.1, 138.0, 133.7, 132.5, 129.2, 128.9, 124.1, and 23.7; HRMS (ESI-MS) $C_{19}H_{17}N_5O_3S_2$; calculated $[M+H]^+$: 428.0846; found $[M+H]^+$: 428.0834.

N-*({4-[N′-(4,6-Dimethylpyrimidin-2-yl)sulfamoyl]phenyl}-carbamothioyl)benzamide (**1g**)*

White color solid, mp: 216–218°C, yield 84%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.74 (s, 1H, NH), 11.69 (s, 1H, NH), 8.03–7.92 (m, 7H, Ar-H), 7.68–7.64 (m, 1H, Ar-H), 7.56–7.52 (m, 2H, Ar-H), 6.76 (s, 1H, NH), and 2.26 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.2 (C═S), 168.1 (C═O), 158.9, 157.2, 156.0, 142.3, 141.4, 133.2, 132.0, 128.69, 128.66, 128.4, 123.4, and 22.7; HRMS (ESI-MS) $C_{20}H_{19}N_5O_3S_2$; calculated $[M+H]^+$: 442.1002; found $[M+H]^+$: 442.1006.

4.1.3 | General procedure for the synthesis of compounds 2a–g (Scheme 1)

A solution of fluorobenzoyl chloride (1 mmol) in acetone (20 ml) was added dropwise to a suspension of potassium thiocyanate

(1 mmol) in acetone (30 ml), and the reaction mixture was refluxed for 1.5 hr to afford isothiocyanates. The reaction mixture was checked with TLC and benzoyl isothiocyanate derivative was formed in a reaction medium. After completion of the reaction, the substituted sulfonamide derivative (1 mmol) was added and the mixture was stirred under reflux for 3–6 hr. Upon completion of the reaction (checked with TLC), the resulting precipitate was collected by filtration and recrystallized from dimethylformamide/ethanol/H_2O (4.2:0.6:0.2) to obtain the pure products **2a–g**.[23]

4-Fluoro-N-*({4-[N′-(acetyl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**2a**)*

Light white color solid, mp: 247–248°C, yield 90%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.72 (s, 1H, NH), 12.14 (s, 1H, NH), 11.78 (s, 1H, NH), 8.09–8.06 (m, 2H, Ar-H), 8.02–7.94 (m, 4H, Ar-H), 7.41–7.36 (m, 2H, Ar-H), and 1.95 (s, 3H, –CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.3 (C═S), 168.8 (C═O), 166.9, 142.5, 136.0, 131.8, 131.7, 128.3, 123.9, 115.6, 115.4, and 23.2; HRMS (ESI-MS) $C_{16}H_{14}N_3O_4FS_2$; calculated $[M+Na]^+$: 418.0302; found $[M+Na]^+$: 418.0284.

4-Fluoro-N-*({4-[N′-(diaminomethylene)sulfamoyl]phenyl}-carbamothioyl)benzamide (**2b**)*

Light cream color solid, mp: 240–241°C, yield 82%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.64 (s, 1H, NH), 11.73 (s, 1H, NH), 8.09–8.06 (m, 2H, Ar-H), 7.85–7.78 (m, 4H, Ar-H), 7.41–7.37 (m, 2H, Ar-H), and 6.75 (bs, 4H, NH_2); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.2 (C═S), 167.1 (C═O), 163.7, 158.1, 141.8, 140.3, 131.8, 128.6, 126.1, 124.1, and 115.6; HRMS (ESI-MS) $C_{15}H_{14}N_5O_3FS_2$; calculated $[M+H]^+$: 396.0595; found $[M+H]^+$: 396.0569.

4-Fluoro-N-*({4-sulfamoylphenyl}carbamothioyl)benzamide (**2c**)*

White color solid, mp: 215–216°C, yield 80%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.67 (s, 1H, NH), 11.76 (s, 1H, NH), 8.09–8.06 (m, 2H, Ar-H), 7.92–7.85 (m, 3H, Ar-H), and 7.42–7.37 (m, 5H, Ar-H, NH); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.9 (C═S), 167.6 (C═O), 166.7, 164.2, 141.8, 132.3, 129.1, 126.8, 124.8, and 116.1; HRMS (ESI-MS) $C_{14}H_{12}N_3O_3FS_2$; calculated $[M-H]^-$: 352.0231; found $[M-H]^-$: 352.0228.

4-Fluoro-N-*({4-[N′-(thiazol-2-yl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**2d**)*

Light cream color solid, mp: 239–240°C, yield 88%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.80 (s, 1H, NH), 12.66 (s, 1H, NH), 11.74 (s, 1H, NH), 8.07–8.05 (m, 2H, Ar-H), 7.90–7.82 (m, 4H, Ar-H), 7.38 (t, 2H, Ar-H, J = 7.4 Hz), 7.28 (s, 1H, Ar-H), and 6.86 (s, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.7 (C═S), 169.4 (C═O), 167.5, 164.2, 166.8, 141.6, 139.9, 132.3, 129.1, 126.9, 124.7, 116.1, and 108.8; HRMS (ESI-MS) $C_{17}H_{13}N_4O_3F\ S_3$; calculated $[M+H]^+$: 437.0207; found $[M+H]^+$: 437.0187.

4-Fluoro-N-*({4-[N′-(pyrimidin-2-yl)sulfamoyl]phenyl}carbamothioyl)-benzamide (**2e**)*

White color solid, mp: 240–241°C, yield 86%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.69 (s, 1H, NH), 11.75 (s, 1H, NH), 8.54–8.48 (m, 2H, Ar-H), 8.08–7.95 (m, 7H, Ar-H, NH), 7.40–7.36 (m, 2H, Ar-H), and 7.08–7.06 (m, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.2 (C═S), 166.9 (C═O), 166.2, 163.7, 158.3, 156.8, 141.8, 137.3, 131.8, 128.2, 123.8, 115.6, and 115.4; HRMS (ESI-MS) $C_{18}H_{14}N_5O_3FS_2$; calculated $[M+H]^+$: 432.0595; found $[M+H]^+$: 432.0603.

4-Fluoro-N-*({4-[N′-(4-methylpyrimidin-2-yl)sulfamoyl]phenyl}-carbamothioyl)benzamide (**2f**)*

Light cream color solid, mp: 235–236°C, yield 86%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.69 (s, 1H, NH), 11.75 (s, 1H, NH), 8.32 (dd, 1H, Ar-H, J = 9.8, 4.5 Hz), 8.08–7.93 (m, 7H, Ar-H, NH), 7.40–7.36 (m, 2H, Ar-H), 6.92–6.91 (m, 1H, Ar-H), and 2.33 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.2 (Carddp═S), 166.9 (C═O), 166.2, 163.7, 156.4, 141.6, 137.5, 131.8, 131.7, 128.4, 123.6, 115.6, 115.4, and 23.2; HRMS (ESI-MS) $C_{19}H_{16}N_5O_3FS_2$; calculated $[M+H]^+$: 446.0751; found $[M+H]^+$: 446.0747.

4-Fluoro-N-*({4-[N′-(4,6-dimethylpyrimidin-2-yl)sulfamoyl]phenyl}-carbamothioyl)benzamide (**2g**)*

White color solid, mp: 210–211°C, yield 84%. ^{1}H NMR (DMSO-d_6, ppm, 400 MHz), δ 12.69 (s, 1H, NH), 11.74 (s, 1H, NH), 8.08–8.01 (m, 5H, Ar-H), 7.92 (d, 2H, Ar-H, J = 7.2 Hz), 7.40–7.35 (m, 2H, Ar-H), 6.76 (s, 1H, NH), and 2.26 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6, ppm, 100 MHz) δ 179.1 (C═S), 167.0 (C═O), 166.2, 163.7, 155.9, 141.4, 137.9, 131.8, 131.7, 128.7, 123.4, 115.6, 115.4, and 22.7; HRMS (ESI-MS) $C_{20}H_{18}N_5O_3FS_2$; calculated $[M+H]^+$: 460.0908; found $[M+H]^+$: 460.0915.

4.2 | Pharmacological/biological assays

4.2.1 | Carbonic anhydrase enzyme assay

The purification of cytosolic CA isoenzymes (CA I and CA II) was previously described with a simple one-step method by Sepharose-4BL-tyrosine-sulfanilamide affinity chromatography.[37] The protein quantity in the column effluents was determined spectrophotometrically at 280 nm. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was applied with a BioRad Mini Gel system, Mini-PROTEAN system, Bio-Rad Laboratories, Inc., after purification of both CA isoenzymes. Briefly, it was performed in acrylamide for the running (10%) and the stacking gel (3%) contained SDS (0.1%), respectively. The increase in absorbance of the reaction medium was spectrophotometrically recorded at 348 nm. Activities of CA isoenzymes were determined according to a method by Verpoorte et al.[38] Also, the quantity of protein was determined at 595 nm according to the Bradford method.[39] Bovine serum albumin was used as standard protein. The experimental procedure was based on the procedures reported in the literature.[4–6,10,40,41]

4.2.2 | Calculation of IC_{50} and K_i values

An activity (%)–compound graph was drawn to calculate the CA inhibition potential of the compounds. The IC_{50} values were obtained from activity (%) versus compound plots. Three different concentrations were used to calculate K_i values. The Lineweaver–Burk plots[42] were drawn and calculations were performed as described in detail before.[4–6,10,40,41]

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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