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New 1*H*-indole-2,3-dione 3-thiosemicarbazones with 3-sulfamoylphenyl moiety as selective carbonic anhydrase inhibitors

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Abstract

1-Methyl/ethyl/benzyl-5-(un)substituted 1*H*-indole-2,3-diones (**2**, **3**, and **4**) were synthesized by reaction of 5-(un)substituted 1*H*-indole-2,3-diones (**1**) with methyl iodide, ethyl chloride, and benzyl bromide. (3-Sulfamoylphenyl)isothiocyanate (**6**) was obtained by the treatment of 3-aminobenzenesulfonamide (**5**) with thiophosgene. Compound **6** was reacted with hydrazine to yield 4-(3-sulfamoylphenyl) thiosemicarbazide (**7**). Novel 1-(un)substituted/methyl/ethyl/benzyl-5-(un) substituted 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazone] derivatives (**8**–**11**) were prepared by condensation of **7** and **1**–**4**. The structures of the synthesized compounds were confirmed by elemental analysis and spectral data. Inhibition of the widely distributed cytosolic off-targets human carbonic anhydrases (hCAs) I and II, and two tumor-associated membrane-bound isoforms (hCAs IX and XII), by **8**–**11** was investigated. The hCA II inhibitory effects of all tested compounds were in the subnanomolar to low nanomolar levels (K_i = 0.32–83.3 nM), and generally high selectivity for hCA II isoenzyme over hCA I, IX, and XII isoenzymes was observed. The strongest inhibitors of hCA II, 1-benzyl-5-(trifluoromethoxy)-substituted **11c** (K_i = 0.32 nM) and 1-ethyl-5-chloro-substituted **10e** (K_i = 0.35 nM), were docked within the enzyme active site. Molecular modeling studies with the most effective hCA IX and XII inhibitors were also carried out.

KEYWORDS
antitumour activity, inhibitors, substituent effect, synthesis

1 | INTRODUCTION

Carbonic anhydrases (CAs), which catalyze the reversible hydration of carbon dioxide to bicarbonate are metalloenzymes with 16 isoforms identified in humans. Although this reaction can take place without a catalyst, it is fairly slow to meet metabolic needs at physiological pH. CAs carry Zn(II) ion that is coordinated by three histidine residues (His94, His96, and His119) and water at their active sites to provide the nucleophile required for the catalytic reaction.[1–6] They play an essential role in crucial physiological and pathological processes, such as transport of carbon dioxide/bicarbonate, pH homeostasis, respiration, electrolyte secretion in various tissues and organs, gluconeogenesis, lipogenesis, ureagenesis, and tumorigenicity.[6–9] CAs I and II play a role in many important normal physiological processes,

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such as the regulation of acid–base homeostasis. Development of many drugs with different pharmacological activities, such as diuretic,[10] antiglaucoma,[11] antiepileptic,[12] and antiobesity,[13] are aimed at inhibiting these isoforms. Expression of CAs IX and XII is restricted in normal tissues as these isozymes are mainly detected in tumor cells and induced by tumor hypoxia. Therefore, these membrane-bound isozymes have become targets for antitumor activity.[14–18]

The sulfonamide group, which can interact with the zinc ion in the active site of the CA enzyme, is the main pharmacophoric group for CA inhibition studies. Indeed strong inhibitors usually are sulfonamide derivatives carrying aromatic or heteroaromatic rings. Nonselective primary sulfonamides, such as acetazolamide (AAZ), have been used systemically in the treatment of glaucoma for more than 60 years. Second-generation antiglaucoma drugs, such as dorzolamide and brinzolamide, are highly selective CA II inhibitors. They are used topically and side effects are significantly reduced in these agents.[19,20]

Therefore, CA II is an important target in the treatment of glaucoma. CA inhibitors (CAIs) with high selectivity of CA II and possessing strong inhibitory activity are a focus of interest in the development of new antiglaucoma agents. 4-Sulfamoylphenylthiourea derivatives (**I** and **II**) carrying amines, amino acids, and oligopeptides have been reported to have hCA I and IV isoenzymes inhibitory properties. In particular, water-soluble derivatives incorporating hydroxy and mercapto amino acids, hydrophobic amino acids, aromatic amino acids, and dicarboxylic amino acids, displayed selective inhibition in the low nanomolar range. The compounds with balanced hydrophilic and lipophilic properties also showed *in vivo* interesting pharmacological properties.[21,22] These findings suggest that the 4-sulfamoylphenyl derivative selective hCA II inhibitors may be candidates for developing new antiglaucoma agents devoid of ocular side effects. Cyanoguanidine moiety has been used as a bioisosteric group to reduce side effects and improve pharmacological and pharmacokinetic properties, and *N*,*N*′-diarylcyanoguanidines (**III**) were synthesized from sulfanilamide by the replacement of cyanoguanidine with thiourea. The cyanoguanidine derivatives incorporating a 4-sulfamoylphenyl moiety showed potent inhibition against hCA II isoenzyme in the low nanomolar range, as well as high selectivity for hCA II isozyme over hCA I, IV, and IX isoenzymes.[23] 1,3-Diaryltriazene-sulfonamide derivatives (**IV**) have been noted as potent and selective hCA II inhibitors at the subnanomolar to low nanomolar levels.[24]

Meanwhile, the role of some CAs in cancer development and the potential of their sulfonamide inhibitors in cancer treatment have been investigated. Many potent CAIs bearing benzene-sulfonamide moiety have shown efficacy in previous studies and are used clinically for other purposes; they inhibit the growth of various tumor cell lines. These drugs are selective inhibitors of hCAs IX and XII. This has led to the publication of several studies in which benzene-sulfonamide derivatives have been synthesized and assayed as inhibitors of hCAs IX and XII.[25–27]

1*H*-Indole-2,3-dione (isatin) has been considered a versatile intermediate for the design of pharmacological agents. Benzenesulfonamide derivatives bearing an isatin moiety revealed promising activity and different selectivity toward CAs. Recently, many 3-imino/hydrazono-2-indolinone derivatives of benzene sulfonamides have shown different and highly selective inhibitory effects against CAs.[28–35] 3-Imino-2-indolinone-based benzene sulfonamides (**V**) were selective inhibitors of hCA IX (K_i = 1.0–25.0 nM) and XII (K_i = 2.8–53.8 nM) isoenzymes at low nanomolar levels.[31] 3-Imino-2-indolinone derivatives (**VI**) carrying amido-/ureido-substituted benzenesulfonamide groups have been found to inhibit the hCA XII isoenzyme with K_i values in the range of 0.47–2.83 nM.[32] The inhibitory effects of 1-alkyl/benzyl-3-imino-2-indolinone derivatives incorporating an ureido-benzenesulfonamide moiety, which were intended for stronger hydrophobic interactions with the hCA IX active region, were investigated against hCA IX. It was observed that their inhibitory effects for hCA IX (K_i = 4.7–86.1 nM) increased significantly compared to *N*-unsubstituted derivatives (K_i = 192–239 nM). These derivatives were determined to bind by van der Waals interactions inside a large hydrophobic pocket created by T73, P75, P76, L91, L123, and A128 in the hCA IX active region.[33] Some 3-imino/hydrazono-2-indolinone derivatives and their 1-alkyl/benzyl-substituted derivatives (**VII**) showed selective inhibition toward hCA II isoenzyme (K_i = 3.0–332.5 nM).[34] It was noted that hCA IX and XII isoenzymes were inhibited by 3-hydrazono-2-indolinone-based benzenesulfonamide derivatives (**VIII**) in the dose range of 8.9–264.5 and 9.0–78.1 nM, respectively. In this study, the 3-(4-sulfamoylbenzoylhydrazono)-1-ethyl-1*H*-2-indolinone derivative was found to show low nanomolar inhibitory effects against hCAs IX and XII (K_i = 8.9 and 9.2 nM, respectively).[35] 1*H*-Indole-2,3-dione 3-thiosemicarbazone derivatives (**IX**) containing 4-benzene-sulfonamide moiety have been noted as potential hCA IX and XII inhibitors as well as having a high selectivity for hCAs IX and XII compared to hCAs I and II. The compounds displayed K_i values in low nanomolar range against hCAs XII (K_i = 1.2–87.3 nM) and IX (K_i = 13.3–66.3 nM)[36] (Figure 1).

Based on these data, in the present study, we synthesized 1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives to investigate the contribution of the sulfamoyl residue at the 3-position of the phenyl ring to their the hCA I, II, IX, and XII inhibitory activities and to determine the structure–activity relationships. A ligand databank was prepared for the compounds and molecular modeling studies were carried out for hCAs II, IX, and XII.

2 | RESULTS

2.1 | Chemistry

5-(Un)substituted 1*H*-indole-2,3-diones (**1**) were reacted with methyl iodide, ethyl chloride, or benzyl bromide to obtain 1-methyl/ethyl/benzyl-5-(un)substituted 1*H*-indole-2,3-diones (**2**–**4**). (3-Sulfamoylphenyl)isothiocyanate (**6**) was obtained by reacting

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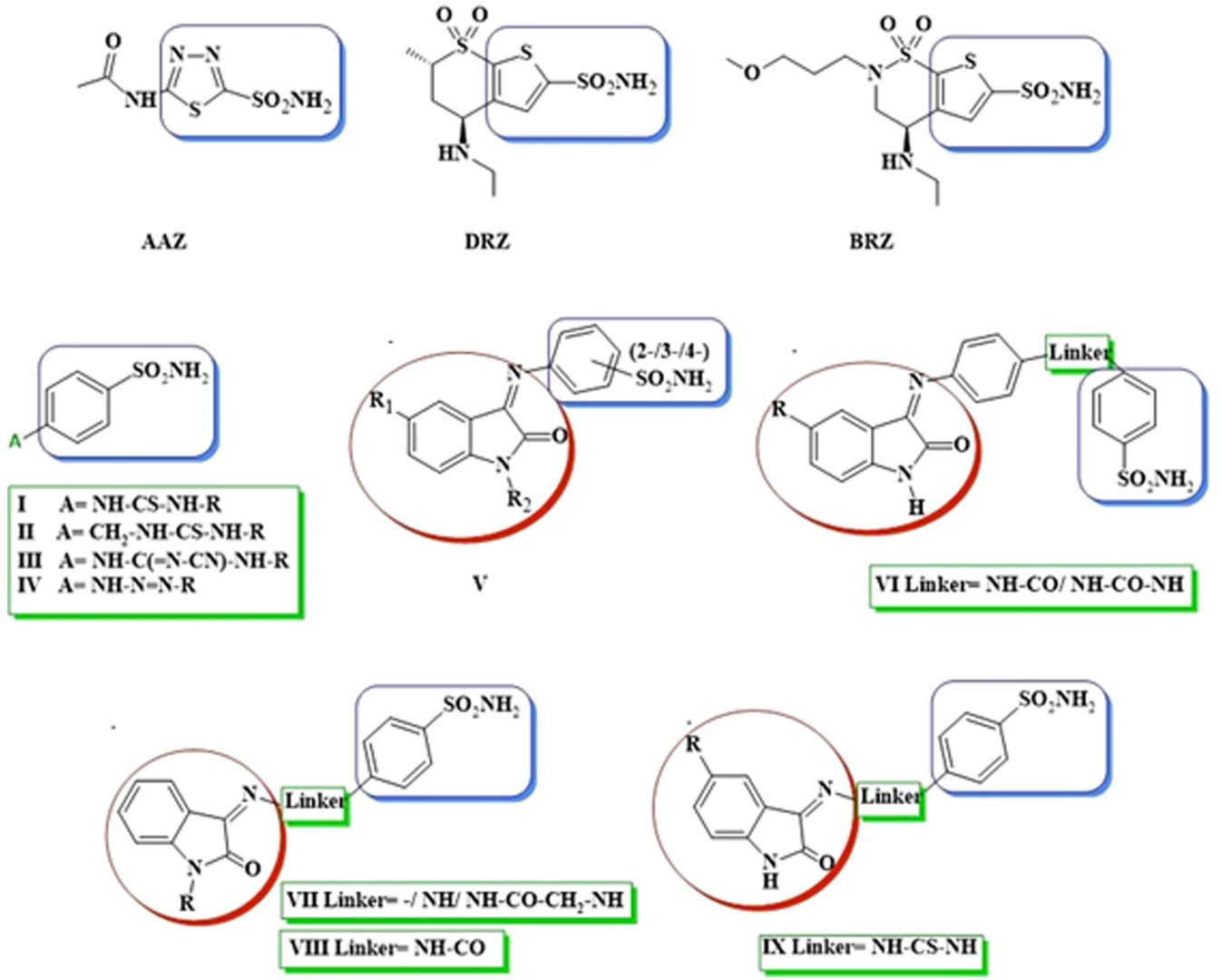

FIGURE 1 Structures of acetazolamide (AAZ), dorzolamide (DRZ), brinzolamide (BRZ), and benzenesulfonamide derivatives (**I–IX**) reported as carbonic anhydrase inhibitors.

3-aminobenzenesulfonamide (**5**) with thiophosgene. 4-(3-Sulfamoylphenyl)thiosemicarbazide (**7**) was synthesized by a reaction of **6** and hydrazine. 1*H*-Indole-2,3-dione 3-[4-(3-sulfamoylphenyl) thiosemicarbazones] (**8–11**) were prepared by the condensation of **7** and **1–4**. Structures of the synthesized new compounds were confirmed through elemental analysis and spectral (UV, infrared [IR], ^{1}H-NMR [nuclear magnetic resonance], ^{13}C-NMR, two-dimensional heteronuclear single quantum coherence [HSQC-2D], two-dimensional heteronuclear multiple bond correlation [HMBC-2D], and liquid chromatography–mass spectrometry–electrospray ionization [LCMS-ESI]) data (Scheme 1).

The UV spectra of 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl) thiosemicarbazones] (**8–11**) displayed three absorption maxima at 249.0–256.5, 280.5–287.5, and 363.0–369.0 nm regions or four absorption maxima at 236.5–244.0, 250.5–258.7, 281.5–331.4, and 364.0–369.0 nm regions in an ethanolic solution containing trace amounts of DMSO. Absorptions at the 236.5–244.0 nm region are the K bands that characterize the $\pi \rightarrow \pi^*$ transition of the aromatic rings. The absorptions observed at 249.0–258.7 and 280.5–331.4 nm regions were defined as B bands resulting from the $\pi \rightarrow \pi^*$ and R bands due to $n \rightarrow \pi^*$ transitions, respectively. Because the chromophoric azomethine group increased conjugation, the K bands due to the transition of $\pi \rightarrow \pi^*$ were seen at 363.0–369.0 nm. IR spectra of **8–11** exhibited bands in the 3105–3417 cm^{-1} region for NH stretchings of the thioamide, lactam, and sulfonamide functions. The absorption bands due to the lactam C=O and thioamide C=S appeared at 1670–1707 and 1176–1220 cm^{-1}, respectively, whereas the S=O stretchings of the sulfonamide groups showed two separate absorption bands at 1325–1348 and 1143–1178 cm^{-1} regions.

^{1}H NMR spectra of **8–11** showed a singlet at δ 7.43–7.71 ppm region due to sulfonamide NH protons and two separate singlets at δ 10.98–11.49 and 12.56–13.07 ppm regions attributed to the NH

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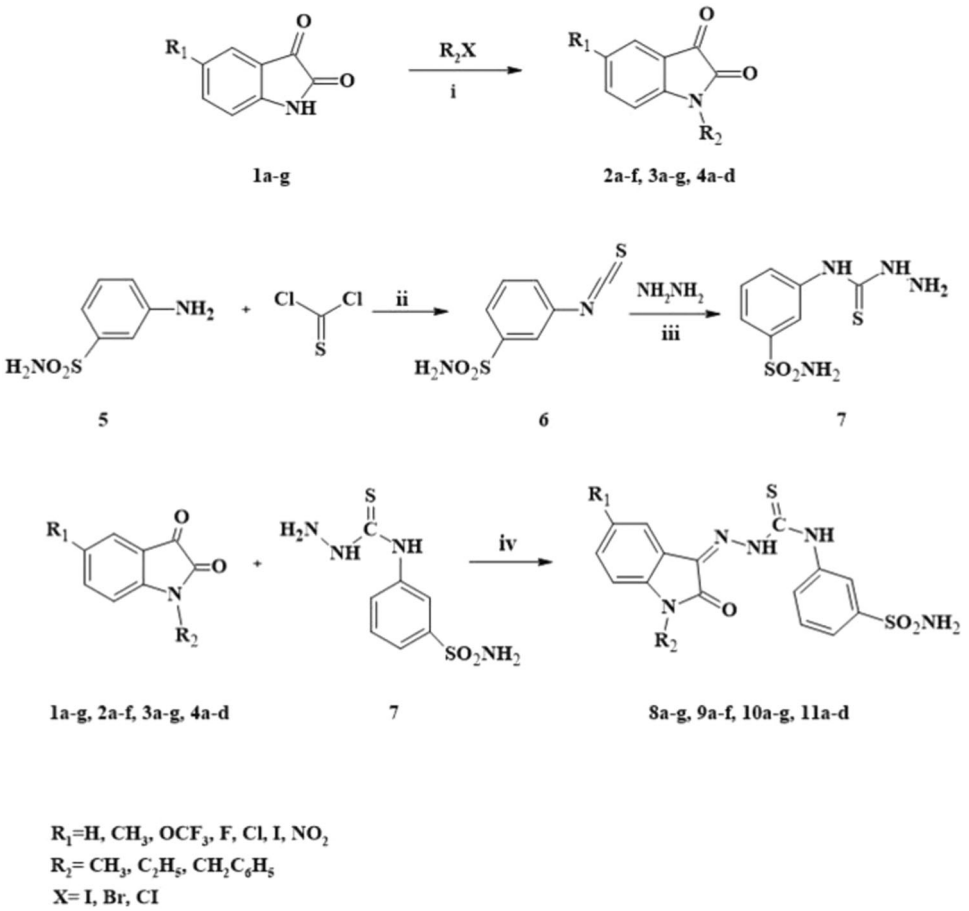

SCHEME 1 Syntheses of compounds **8**–**11**. Reagents and conditions: (i) DMF, K_2CO_3, KI, stirred, (ii) H_2O, HCl, stirred, (iii) EtOH, stirred, cooled, (iv) EtOH, H_2SO_4, reflux.

protons of the thiosemicarbazone moiety. The spectra of **8a**–**g** displayed a singlet at δ 11.26–12.11 ppm region corresponding to the indole NH proton. The phenyl C_2 protons, which are deshielded by the electron-attracting action of the sulfonamide and thioamide groups, were generally observed as double doublets in the δ 8.05–8.33 ppm area. Phenyl C_6 protons appeared as double doublets in the δ 7.86–8.12 ppm area. Phenyl C_4 protons usually resonated as triplet of doublets or doublets in the δ 7.70–7.97 ppm area. The phenyl C_5 protons at the *meta*-position of the sulfonamide and thioamide groups appeared as double doublets in the δ 7.60–7.87 ppm area. These values are consistent with the determined and calculated resonances of 3-sulfamoylphenylthiosemicarbazide **7**. In the spectrum of the compounds (**8a**, **9a**, **10a**, and **11a**) without substituents at position 5, the indole C_4 and C_6 protons were deshielded by the anisotropic effect of the azomethine group at the position 3 of the 2-indolinone ring. Whereas, the indole C_5 and C_7 protons were shielded by the mesomeric effect of the anilid NH group in the 2-indolinone ring. Thus, indole proton signals were observed in the order of C_7, C_5, C_6, and C_4. In the spectra of the R_1-substituted **8**–**11**, R_1 substituents shifted the indole C_4, C_6, and C_7 proton signals. However, the indole proton signals in all compounds were observed in the order of C_7, C_6, and C_4. The substituent effects observed splitting patterns and coupling constance confirmed these findings.

Further elucidation was done by HSQC-2D of **8a**, **8d**, **8g**, **9b**, and **11a**, and HMBC-2D of **8g**, **10g**, and **11a**, which showed signals at δ 160.90–163.77 and 130.05–132.73 ppm regions due to indole C_2 and C_3 signals, respectively. In addition, the C=S signals that confirmed the thiosemicarbazone structure were seen at the δ 176.54–177.35 ppm region. The HSQC-2D experiments confirmed the resonance of carbons bearing hydrogen in the indole and phenyl rings, while the HMBC-2D analysis confirmed the resonances of the quaternary carbons. Because the electron density of the 3-sulfamoylphenyl residue was reduced by the sulfonamide and thioamide groups, the phenyl carbon signals were observed in the order of C_3, C_1, C_5, C_6, C_4, and C_2 starting from downfield, as in the spectrum of **7**. The sulfonamide and thioamide groups shifted the phenyl C_2 carbon signals to a higher field. Indole C_2 and lactam NH-linked C_{7a} carbons showed the most downfield shift among the indole carbon signals. The indole C_7 and C_{3a} carbons in the *o*-position of the lactam group were found to be the most shielded among the indole carbons. In the spectra, shifts were observed in the indole signals based on the nature of the substituents at position 5 of the 2-indolinone ring. The indole carbon signals in the spectra of the R_1 fluorine-substituted **8d**, which showed that the ^{13}C–^{19}F connections were observed as separate doublets.

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Electron spray ionization (ESI) mass spectra of the compounds were obtained using the negative ionization technique for **8d**, **8e**, **9b**, **9d**, **10a**, **10c**, and **10e**, and the positive ionization technique for **8a**, **8d**, **9b**, **9d**, **10a**, **10e**, and **11d**. $M-H^-$ peaks confirming the molecular weights of all compounds run by the negative ionization technique were observed with various relative abundances. In the spectra of most of the compounds taken by this technique, the base peak was the *m/z* 213 ion. This ion may have formed by the loss of a proton from 3-sulfamoylphenylyisothiocyanate **6** after the cleavage of the N_2–CS bond. $[M-2H]^{2-}$–213 peaks formed by this major fragmentation pathway were also observed in the spectra of **8d**, **8e**, **9b**, **9d**, **10a**, **10c**, and **10e**. The spectra of R_1 chlorine-substituted **8e** and **10e** showed $[M-H]^-$+2 signals. $[M+Na]^+$ signals showed as base peaks in the spectra of the compounds obtained by the positive ionization technique. Moreover, $[M+H]^+$ peaks which verified the proposed structure of the compounds and $[M+H]^+$+2 peak in the spectra of R_1 chlorine-substituted **10e** and **11d** were also observed.

2.2 | Determination of inhibitory effects against hCA I, II, IX, and XII isoenzymes

In our previous study on 1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives, it was determined that compounds carrying a 4-sulfamoylphenyl moiety are strong and selective hCA IX and XII inhibitors compared to hCA I and II isoenzymes. The newly reported 3-imino/hydrazono-2-indolinone-based benzenesulfonamide derivatives are generally selective inhibitors of hCA IX and XII. In the present study, the CA inhibitory effects of a new class of 1*H*-indole-2,3-dione thiosemicarbazone containing a 3-sulfamoylphenyl moiety were determined and it was aimed to investigate the contribution of a sulfamoyl group at position 3 of the phenyl ring to CA inhibitory activity and selectivity. The CA inhibition assays were conducted on human cytosolic isoforms (hCAs I and II) and human tumor-associated isoforms (hCAs IX and XII) with all synthesized compounds and AAZ as a standard CAI by a stopped-flow CO_2 hydrase method. Compounds **8–11** carrying a 3-sulfamoylphenyl moiety showed an increase in inhibitory effect and selectivity against the hCA II isoenzyme, while a decrease in inhibitory effect against hCA IX and XII isoenzymes was observed. The results as inhibition constants K_i (nM) and selectivity ratios of the tested compounds are illustrated in Table 1.

2.3 | Molecular modeling

Most compounds in series **8–11** were able to adopt similar docked poses in the active site of hCA II. The negatively charged sulfonamide group of the ligands formed an interaction with the active site Zn^{2+}-ion and a hydrogen bond with the backbone of Thr199 in a similar way as the cocrystallized ligand acetazolamide (Figure 2). The other sulfur atom of the ligands formed a hydrogen bond with the sidechain of Asn62 and the carbonyl group formed a hydrogen bond with the sidechain of His64. The phenyl group of compound **11c** (K_i = 0.32 nM) formed additional H-arene interactions with the sidechain of His64, while the trifluoromethyl group was partly solvent-exposed and partly close to a hydrophobic region of the pocket. Compound **10e** (0.35 nM) contained an ethyl group instead of a benzyl group and did not form the H-arene interactions. The ligand's chlorine substituent was fully solvent-exposed.

The sulfonamide group of compound **9b** (K_i = 8.4 nM) also interacted with the zinc ion and Thr199 sidechain as previously described (Figure 3a). The thiosemicarbazone group formed hydrogen bonds with the sidechains of Asn62 and Thr200, while the carbonyl group formed a hydrogen bond with the sidechain of Trp5. The ligand's indole moiety was close to the cationic sidechain of Arg60 (~3.7 Å) and cation–π interactions could occur. Similar poses were observed for the other tested compounds. Finally, the docked pose of compound **10b** (K_i = 9.2 nM) again showed the interactions between the zinc ion and Thr199 (Figure 3b). The thiosemicarbanoze group formed hydrogen bonds with the sidechain of Thr200 and the backbone of Pro201. This pose was also observed for the other compounds.

In general, the poses of the other compounds are similar to the ones shown in Figures 2 and 3. It is considered that the strong additional interactions with the thiosemicarbazone groups and enzyme active sites could be determinative of the affinity. For example, compared to membrane-bounded isoforms hCA IX/XII and cytosolic isoform hCA I, compounds such as **10e** and **11c** showed higher affinity to the other cytosolic isoform hCA II, which could be explained by the additional interactions with thiosemicarbazone group of the molecules and the active site residues (i.e., Asn62) of hCA II (Figures 2 and 4).

3 | DISCUSSION

As shown in Table 1, synthesized compounds had a more inhibitory activity with nanomolar values (K_i ranging between 0.32 and 83.3 nM) on hCA II isoenzyme than other isoenzymes. In particular, 1-benzyl-5-trifluoromethoxy-substituted **11c** (K_i = 0.32 nM), 1-ethyl-5-chlorine-substituted **10e** (K_i = 0.35 nM) and 1-benzyl-5-chlorine-substituted **11d** (K_i = 0.38 nM) were found to be the most potent inhibitors and 30-fold more active than AAZ (K_i = 12.0 nM) against hCA II. However, while activity against hCA IX and XII isoenzymes increased in R_2 benzyl-substituted compounds, selectivity for hCA II decreased. Among these compounds, **10e** showed potent inhibition against hCA II, as well as high selectivity for hCA II over hCAs I, IV, and IX. K_i values of **10e** were determined as >10.000, 126.5, and 5658.0 nM for hCAs I, IX, and XII, respectively. Furthermore, among 1-methyl- or ethyl-substituted compounds, 1-ethyl-5-fluorine-substituted **10d** (K_i = 0.46 nM), 1-methyl-5-chlorine-substituted **9e** (K_i = 0.49 nM), 1-ethyl-5-trifluoromethoxy-substituted **10c** (K_i = 0.57 nM), 1-ethyl-5-methyl-substituted **10b** (K_i = 0.72 nM), 1-methyl-substituted **9a** (K_i = 0.73 nM), and 1-methyl-5-iodine-substituted **9f** (K_i = 0.95 nM) possessed excellent inhibitory activities against hCA II. 5-Nitro-substituted **8g**, 5-iodine-substituted **8f**, and 5-fluorine-substituted **8d** among the unsubstituted compounds at the 5-position of indole ring exhibited

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TABLE 1 Human carbonic anhydrases inhibition data of compounds **8–11***.

			K_i (nM)				Selectivity ratio		
Compound	R_1	R_2	I	II	IX	XII	I/II	I/IX	I/XII
8a	H	H	>10,000	83.3	22.4	44.5	120.05	446.43	224.72
8b	CH_3	H	635.3	13.8	28.2	**9.6**	46.04	22.53	66.18
8c	OCF_3	H	692.2	5.9	36.6	24.1	117.32	18.91	28.72
8d	F	H	539.3	**0.92**	21.7	**10.5**	586.19	24.85	51.36
8e	Cl	H	776.1	9.0	33.4	47.9	86.23	23.24	16.20
8f	I	H	939.3	**0.90**	27.0	24.9	1043.67	34.79	37.72
8g	NO_2	H	538.3	**0.87**	22.3	55.6	618.73	24.14	9.68
9a	H	CH_3	3031.0	**0.73**	**12.8**	627.3	4152.05	236.80	4.83
9b	CH_3	CH_3	789.9	2.0	**8.4**	479.3	394.95	94.03	1.65
9c	OCF_3	CH_3	8481.0	2.1	**15.7**	76.4	4038.57	540.19	111.01
9d	F	CH_3	7839.0	1.9	29.6	770.4	4125.79	264.83	10.17
9e	Cl	CH_3	787.5	**0.49**	24.3	83.2	1607.14	32.41	9.46
9f	I	CH_3	810.0	**0.95**	25.1	55.8	852.63	32.27	14.52
10a	H	C_2H_5	654.0	1.9	41.5	78.2	344.21	15.76	8.36
10b	CH_3	C_2H_5	>10,000	**0.72**	968.7	**9.2**	13,888.89	10.32	1086.96
10c	OCF_3	C_2H_5	942.5	**0.57**	126.8	56.1	1653.51	7.43	16.80
10d	F	C_2H_5	2676.0	**0.46**	33.0	428.3	5817.39	81.09	6.25
10e	Cl	C_2H_5	>10,000	**0.35**	126.5	5658.0	28,571.43	79.05	1.77
10f	I	C_2H_5	>10,000	2.4	31.4	6872.0	4166.67	318.47	1.45
10g	NO_2	C_2H_5	>10,000	32.6	210.1	231.6	306.75	47.60	43.18
11a	H	$CH_2C_6H_5$	7927.0	27.4	122.6	311.9	289.31	64.66	25.41
11b	CH_3	$CH_2C_6H_5$	>10,000	31.1	258.7	341.6	321.54	38.65	29.27
11c	OCF_3	$CH_2C_6H_5$	>10,000	**0.32**	85.21	220.6	31,250.00	117.36	45.33
11d	Cl	$CH_2C_6H_5$	9619.0	**0.38**	34.3	191.6	25,313.16	280.44	50.20
Acetazolamide	-	-	250.0	12.0	25.0	5.7	20.83	10.00	43.86

Note: The lowest Ki values for hCAs II, IX, and XII are marked in bold.

significant inhibition hCA II profile with K_i values 0.87, 0.90, and 0.95 nM, respectively. The 1,5-unsubstituted **8a** (K_i = 83.3 nM) showed the least potent inhibition against hCA II isoform.

Based on the results, we are able to make the following observations about the structure–activity relationships of the compounds. Generally, 1-methyl-, 1-ethyl-, and 1-benzyl-substituted compounds **9–11** (K_i = 0.49–2.1, 0.35–32.6, and 0.32–31.1 nM, respectively) were determined to have K_i values lower than those of the compounds **8** (K_i = 0.87–83.3 nM) without substituents at the position 1. While elongation of the alkyl chain and the benzyl substitution increased the activity in 5-chlorine- and 5-trifluoromethoxy-substituted compounds, 1-alkylation decreased efficacy in 5-iodine- and 5-nitro-substituted compounds. The most effective ones among the 1-substituted compounds were 5-trifluoromethoxy- and 5-chlorine-substituted compounds. However, 1-benzyl substitution increased activity against hCA IX and XII isoenzymes while decreasing selectivity for hCA II isoenzyme compared to 1-ethyl-substituted compounds. Furthermore, it was apparent that the introduction of a chlorine atom at position 5 of the indole ring was remarkably beneficial for activity as in **9e**, **10e**, and **11d**, and the replacement of the chlorine atom by a methyl group leads to a decrease of activity and selectivity. It was determined that both high inhibitory effect and selectivity against hCA II isoenzyme were increased in the presence of the chlorine atom at position 5 and the ethyl group at position 1 of the indole ring.

Inhibition studies showed that **8–11** generally had less inhibitory effects on the tumor-associated isoform hCA IX than on hCA II. 1-Methyl-substituted compounds **9b**, **9a**, and **9c** (K_i = 8.4, 12.8, and 15.7 nM, respectively) showed the highest activity and better inhibition than AAZ (K_i = 25.0 nM) against hCA II. Generally, it was observed that the 1-methyl-substituted **9** (K_i = 8.4–29.6 nM) and the

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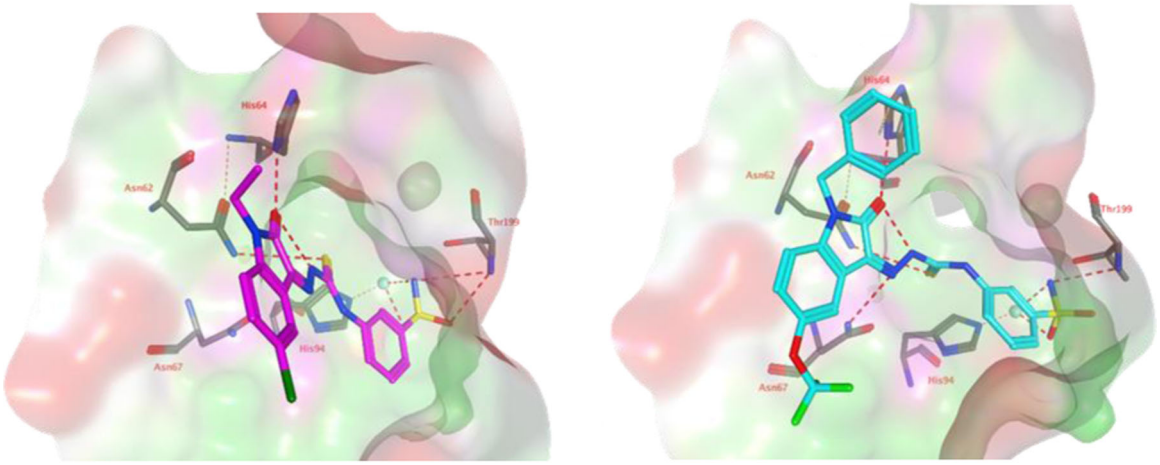

FIGURE 2 The docked pose of compounds **10e** (right, purple) and **11c** (left, turqouise) forming interactions in the active site of human carbonic anhydrases (hCA II). The Zn^{2+}-ion is indicated as a turquoise sphere. Hydrogen bonds and interactions with the Zn^{2+}-ion are indicated in red dashed lines. H-arene interactions are indicated in yellow dashed lines. The pocket surface has been colored according to its characteristics (polar region in purple, hydrophobic region in green, and solvent-exposed region in red).

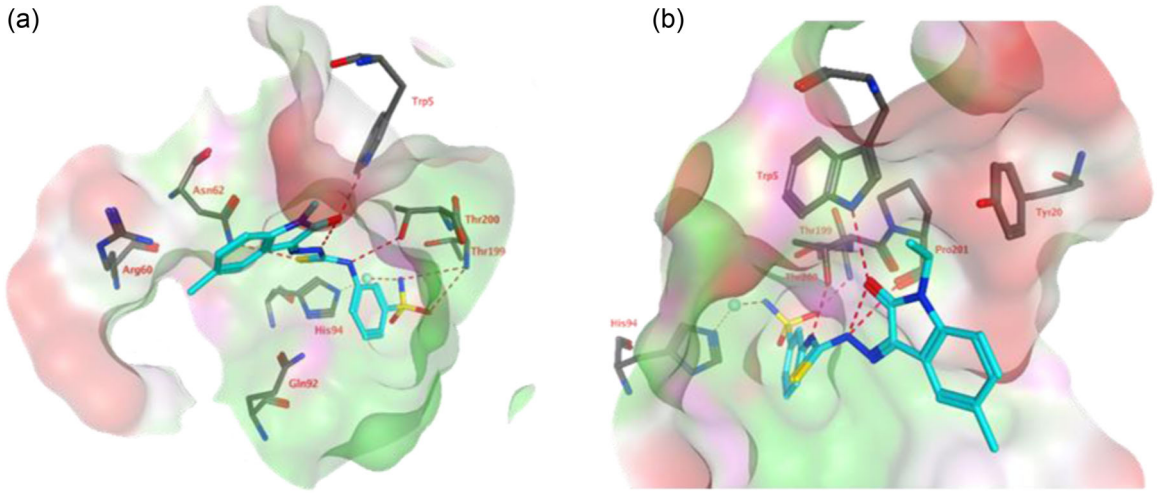

FIGURE 3 The docked poses of compound **9b** (right) in the active site of hCA IX (a) and compound **10b** (left) in the active site of hCA XII (Figure 3b) (b). The Zn^{2+}-ion is indicated as a turquoise sphere. Hydrogen bonds and interactions with the Zn^{2+}-ion are indicated in red dashed lines. The pocket surface has been colored according to its characteristics (polar region in purple, hydrophobic region in green, and solvent-exposed region in red).

compounds **8** (K_i = 21.7–36.6 nM) without substituents at the position 1 had higher activity than the 1-benzyl-substituted **11** (K_i = 34.3–258.7 nM) and 1-ethyl-substituted **10** (K_i = 31.4–968.7 nM) ones. These results demonstrated the importance of the 1-methyl substitution for hCA IX inhibition. Furthermore, it was determined that ethyl or benzyl substitution caused a significant decrease in the inhibitory effect against hCA IX. An increase in the inhibitory effect for hCA IX was observed in the 5-methyl-substituted derivatives, but their selectivity also decreased.

The transmembrane isoform hCA XII was inhibited by compounds **8–11** with K_i in the range of 9.2–6872.0 nM. It has been found that the compounds (**8a–g**) without substituents at position 1 have a generally higher inhibitory effect than that with 1-substituted compounds (**9–11**). However, 1-ethyl-5-methyl-substituted **10b** was an exception as it has the highest activity within the tested compounds against hCA II. 5-Methyl-substituted **8b**, 5-fluorine-substituted **8d**, and 1-ethyl-5-methyl-substituted **10b** are those that are the most effective on both hCA II and XII isoenzymes and have activity comparable to AAZ. Among these compounds, **10b** showed high selectivity for hCA II and XII isoenzymes over hCA I and IX isoenzymes. K_i values of **10b** were determined as >10.000, 0.72, 9.2, and 968.7 nM for hCA I, II, IX, and XII isoenzymes, respectively. These results showed that the hCA XII inhibitory activity increased at the methyl and fluorine substitution at position 5 of the indole ring and that the ethyl substitution at position 1 of the indole ring increased the selectivity for hCAs II and XII in these derivatives.

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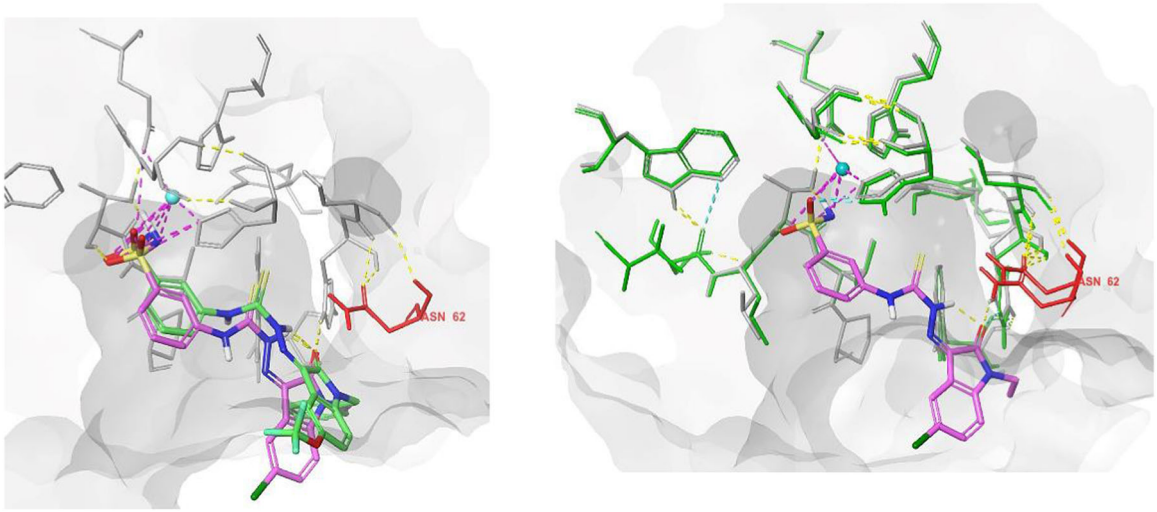

FIGURE 4 The docked poses of compounds **10e** (pink) and **11c** (green) in the active site of hCA I (right), the docked pose of compound **10e** (left) in the active site of hCA I (gray), and hCA IX (green). The Zn^{2+}-ion is indicated as a turquoise sphere. Hydrogen bonds are indicated with yellow and interactions with the Zn^{2+}-ion are indicated in pink dashed lines. The Asn62 is indicated with red.

To better understand the interactions between ligands and enzymes active sites, molecular modeling studies have been performed on hCAs I, II, IX, and XII. Generally, all of the synthesized molecules accommodate similarly at the active sites of the enzyme and could form the essential interactions with the Zn^{2+} atom, and their thiosemicarbazone parts contributed to the strong interactions, which could be an explanation for low enzyme inhibition values hCAs II, IX, and XII. Although there is no direct observed correlation between substitution patterns of R_1, formed bonds, and the enzyme inhibition values of the molecules, a bulky substitution like ethyl- or benzyl- (i.e., **10e** and **11c**) at the *N*-indole seems to be beneficial for the hCA II enzyme inhibition, compared with the un- or *N*-methyl-substituted derivatives. Such examples as compound **11c** (or **11d**) with *N*-benzyl substitution also could be able to form an additional H-arene interaction at the active site of the hCA II, which may explain the lowest inhibition value against hCA II.

4 | CONCLUSION

We report here a novel class of thiosemicarbazones incorporating a 3-sulfamoylphenyl moiety, obtained by the reaction of 4-(3-sulfamoylphenyl)thiosemicarbazide with 1*H*-indole-2,3-diones. In our previous studies on 1*H*-indole-2,3-dione 3-thiosemicarbazone derivatives, it was determined that compounds carrying a 4-sulfamoylphenyl moiety were strong inhibitors, selective to hCAs IX and XII compared to hCAs I and II. In the present study, most of the compounds carrying a 3-sulfamoylphenyl moiety showed potent inhibitory action against hCA II, as well as high selectivity for hCA II over hCAs I, IV, and IX. In general, we determined that 1-substituted compounds had higher inhibitory efficacy than the compounds without substituents at position 1 against the hCA II. Elongation of the alkyl chain and benzyl substitution increased the inhibitory effect. However, 1-benzyl-substitution increased the activity against hCA IX and XII isoenzymes while decreasing the selectivity for hCA II isoenzyme compared to 1-ethyl-substituted compounds. It was determined that both high inhibitory effect and selectivity for hCA II isoenzyme were possible in the presence of the chlorine atom at position 5 and the ethyl group at position 1 of the indole ring. These findings may contribute to the development of selective new inhibitors and, as hCA II isoenzyme is an important target for antiglaucoma and diuretic agents, inhibitors selective to this isoform may be of interest for further pharmacological studies.

5 | EXPERIMENTAL

5.1 | Chemistry

5.1.1 | General remarks

All chemicals and reagents were purchased from Sigma-Aldrich or Merck (Germany). Melting points of the synthesized compounds were recorded in open capillaries using a Buchi 540 melting point apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV-1800 spectrophotometer. IR spectra (KBr discs) were measured with a Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR, HSQC-2D, and HMBC-2D spectra were obtained on Varian UNITY INOVA 500, Varian Mercury, Agilent 400, and Varian Mercury 300 NMR spectrophotometers in deuterated dimethyl sulfoxide (DMSO-d_6). Mass spectra were recorded on a Thermo Finnigan LCQ Advantage Max spectrometer. Analytical data were obtained on Thermo Finnigan Flash EA 1112 elemental analyzer.

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The NMR spectra and the InChI codes of the investigated compounds are provided as Supporting Information.

5.1.2 | Synthesis of 1-methyl/ethyl/benzyl-1*H*-indole-2,3-diones (**2–4**)

5-(Un)substituted 1*H*-indole-2,3-dione (**1a**–**g**) (5 mmol) and 0.97 g of anhydrous potassium carbonate (K_2CO_3) (7 mmol) were stirred in 10 ml dimethylformamide (DMF) at room temperature for 1 h. After the addition of methyl iodide, ethyl chloride, or benzyl bromide (15 mmol) and 0.17 g of anhydrous potassium iodide (KI) as a catalyst, the reaction was heated at 50–60°C until completed. The reaction mixture was evaporated to dryness under reduced pressure, washed with ice water, and crystallized from ethanol.[37–41]

*1-Ethyl-5-trifluoromethoxy-1*H*-indole-2,3-dione (**3c**)*

Orange powder (77%): mp: 70–72°C; UV λ $(EtOH)_{maks}$ nm (ε): 247.5 (10,574) and 293.5 (1088). IR (KBr): 1755, 1741 (C═O), 1602, 1485, and 1454 (C═C). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.16 (3H, t, *J*: 7.2 Hz, ind. N-CH_2CH_3), 3.71 (2H, q, *J*: 7.2 Hz, ind. N–CH_2CH_3), 7.29 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.55 (1H, d, *J*: 2.0 Hz, ind. C4–H), and 7.66 (1H, dd, *J*: 8.6, 1.6 Hz, ind. C6–H). Anal. calcd for $C_{11}H_8F_3NO_3$: C, 50.98; H, 3.11; N, 5.40 Found: C, 51.01; H, 3.09; N, 5.61.[41]

5.1.3 | Synthesis of 3-(isothiocyanato)-benzenesulfonamide (**6**)

3-Aminobenzenesulfonamide **5** (3 mmol) was dissolved in 50 ml of water containing 12 ml of concentrated hydrochloric acid. To the solution, thiophosgene ($CSCl_2$) (3 mmol) was added in one portion. Stirring was begun immediately and continued until the red color of thiophosgene disappeared and the product appeared as a white crystalline precipitate. The precipitate was filtered and washed thoroughly with water.[42]

5.1.4 | Synthesis of 4-(3-sulfamoylphenyl)-thiosemicarbazide (**7**)

To a solution of hydrazine (5 mmol) in ethanol (10 ml), a suspension of 3-(isothiocyanato)benzenesulfonamide (**6**) (5 mmol) in ethanol (10 ml) was added dropwise with vigorous stirring and cooling in an ice bath. The mixture was allowed to stand overnight. The crystals formed were recrystallized from ethanol. White powder (82%): mp: 153–155°C; UV λ $(EtOH)_{maks}$ nm (ε): 243.0 (17,906) and 268.5 (12,044). IR (KBr): 3305, 3255, 3221, 3190 (NH), 1637, 1598, 1546, 1500, 1477 (C═C), 1336, 1151 (S═O), and 1271 (C═S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 7.29 (2H, s, SO_2NH_2, D_2O exchange), 7.45 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.52 (1H, d, *J*: 7.1 Hz, phen. C4–H), 7.82 (1H, d, *J*: 6.8 Hz, phen. C6–H), 8.17 (1H, s, phen. C2–H), and 9.22 (1H, s, N2–H, D_2O exchange). ^{13}C NMR (DMSO-d_6/100 MHz) ppm: 121.15 (phen. C2), 121.55 (phen. C4), 128.95 (phen. C6,5), 140.28 (phen. C1), 144.41 (phen. C3), and 179.99 (C═S). Anal. calcd for $C_7H_{10}N_4O_2S_2$: C, 34.13; H, 4.09; N, 22.75; found: C, 34.14; H, 4.29; N, 22.43.[43,44]

5.1.5 | Synthesis of 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazones] (**8–11**)

4-(3-Sulfamoylphenyl)thiosemicarbazide (**7**) (3.5 mmol) was added to solutions of 1*H*-indole-2,3-diones (**1**–**4**) (3.5 mmol) in ethanol (20 ml) containing a catalytic amount of concentrated sulfuric acid. The mixture was refluxed in a water bath for 4 h. The product formed after cooling was filtered and washed with ethanol or recrystallized from ethanol.[43,44]

*1*H*-Indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8a**)*

Orange powder (98%): mp: 232–233°C; UV λ $(EtOH)_{maks}$ nm (ε): 249.0 (18221), 280.5 (9341), and 365.0 (22065). IR (KBr): 3244, 3221, 3182 (NH), 1695 (C═O), 1620, 1587, 1531, 1465 (C═N, C═C), 1332, 1153 (S═O), and 1205 (C═S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 6.94 (1H, d, *J*: 7.8 Hz, ind. C7–H), 7.11 (1H, dd, *J*: 7.8, 7.4 Hz, ind. C5–H), 7.37 (1H, td, *J*: 7.4, 1.2 Hz, ind. C6–H), 7.43 (2H, s, SO_2NH_2), 7.60 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5–H), 7.70 (1H, d, *J*: 8.2 Hz, phen. C4–H), 7.75 (1H, d, *J*: 7.4 Hz, ind. C4–H), 7.87 (1H, dd, *J*: 8.2, 1.9 Hz, phen. C6–H), 8.08 (1H, dd, *J*: 1.9, 1.8 Hz, phen. C2–H), 11.00 (1H, s, N4–H), 11.26 (1H, s, ind. N–H), 12.86 (1H, s, N2–H). HSQC-2D (DMSO-d_6/100 MHz): 111.14 (ind. C7), 119.82 (ind. C3a), 121.40 (ind. C4), 122.40 (ind. C5), 122.58 (phen. C2), 123.05 (phen. C4), 128.93 (phen. C6), 128.96 (phen. C5), 131.59 (ind. C6), 132.73 (ind. C3), 138.92 (phen. C1), 142.60 (ind. C7a), 144.31 (phen. C3), 162.70 (ind. C2), and 176.59 (CS). MSMS-ESI (+): *m/z* (%) 398 ($[M+Na]^+$, 100) and 399 ($[MH+Na]^{2+}$, 22). Anal. calcd for $C_{15}H_{13}N_5O_3S_2.{}^1/_2\ H_2O$: C, 46.86; H, 3.57; N, 16.68; found: C, 46.47; H, 3.57; N, 17.30.

*5-Methyl-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8b**)*

Deeply yellow powder (97%): mp: 228–229°C; UV λ $(EtOH)_{maks}$ nm (ε): 252.0 (19,628), 285.4 (10,631), and 364.5 (25,431). IR (KBr): 3346, 3309, 3219 (NH), 1685 (C═O), 1625, 1589, 1537, 1481 (C═N, C═C), 1325, 1159 (S═O), and 1207 (C═S). ^{1}H NMR (DMSO-d_6/300 MHz) ppm: 2.52 (3H, s, CH_3), 7.05 (1H, d, *J*: 7.9 Hz, ind. C7–H), 7.41 (1H, d, *J*: 7.9 Hz, ind. C6–H), 7.69 (2H, s, SO_2NH_2), 7.81–7.85 (2H, m, ind. C4–H, phen. C5-H), 7.93 (1H, d, *J*: 7.9 Hz, phen. C4–H), 8.10 (1H, d, *J*: 8.2 Hz, phen. C6–H), 8.30 (1H, s, phen. C2–H), 11.23 (1H, s, N4–H), 11.40 (1H, s, ind. N–H), and 13.07 (1H, s, N2–H). Anal. calcd for $C_{16}H_{15}N_5O_3S_2$: C, 49.34; H, 3.88; N, 17.98; found: C, 49.07; H, 3.44; N, 17.61.

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*5-Trifluoromethoxy-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8c**)*

Yellow powder (90%): mp: 246–248°C; UV λ $(EtOH)_{maks}$ nm (ε): 249.0 (20,195), 280.5 (10,599), and 363.0 (23,728). IR (KBr): 3267, 3221, 3182 (NH), 1695 (C=O), 1627, 1589, 1521, 1481 (C=N, C=C), 1325, 1151 (S=O), and 1201 (C=S). ^{1}H NMR (DMSO-d_6/500 MHz) ppm: 7.04 (1H, d, *J*: 8.3 Hz, ind. C7–H), 7.39 (1H, dd, *J*: 8.3, 2.0 Hz, ind. C6–H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 8.3, 7.8 Hz, phen. C5–H), 7.73 (1H, d, *J*: 7.8 Hz, phen. C4–H), 7.79 (1H, d, *J*: 2.0 Hz, ind. C4-H), 7.88 (1H, d, *J*: 8.3 Hz, phen. C6–H), 8.07 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 11.07 (1H, s, N4–H), 11.43 (1H, s, ind. N-H), and 12.73 (1H, s, N2–H). Anal. calcd for $C_{16}H_{12}F_3N_5O_4S_2.H_2O$: C, 40.25; H, 2.96; N, 14.67; found: C, 40.05; H, 3.03; N, 14.23.

*5-Fluoro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8d**)*

Yellow powder (92%): mp 259°C; UV λ $(EtOH)_{maks}$ nm (ε): 249.5 (10,661), 282.1 (5468), and 363.0 (12,864). IR (KBr): 3342, 3259 (NH), 1707 (C=O), 1633, 1600, 1516, 1477, 1456 (C=N, C=C), 1344, 1143 (S=O), and 1199 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 6.95 (1H, dd, *J*: 8.4, 4.1 Hz, ind. C7–H), 7.22 (1H, td, *J*: 9.2, 2.8 Hz, ind. C6–H), 7.45 (2H, s, SO_2NH_2), 7.62 (1H, dd, *J*: 8.4, 2.8 Hz, ind. C4–H), 7.64 (1H, dd, *J*: 8.0, 8.0 Hz, phen. C5–H), 7.75 (1H, dd, *J*: 8.0, 1.6 Hz, phen. C4–H), 7.90 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 8.12 (1H, dd, *J*: 2.0, 2.0 Hz, phen. C2–H), 11.01 (1H, s, N4–H), 11.25 (1H, s, ind. N–H), and 12.76 (1H, s, N2–H). HSQC-2D (DMSO-d_6/100 MHz): 108.25 (d, *J*: 25.8 Hz, ind. C4), 112.19 (d, *J*: 8.4 Hz, ind. C7), 117.74 (d, *J*: 24.5 Hz, ind. C6), 121.22 (d, *J*: 8.9 Hz, ind. C3a), 122.47 (phen. C2), 123.14 (phen. C4), 128.84 (phen. C6), 128.96 (phen. C5), 132.03 (d, *J*: 3.2 Hz, ind. C3), 138.73 (phen. C1), 138.80 (d, *J*: 1.9 Hz, ind. C7a), 144.36 (phen. C3), 158,24 (d, *J*: 237.4 Hz, ind. C5), 162.73 (ind. C2), and 176.54 (CS). MSMS (ESI+): *m/z* (%) 439 ($[M+2Na]^{2+}$, 29), 416 ($[M+Na]^+$, 100), and 394 ($[M+H]^+$, 24). MSMS (ESI−): *m/z* (%) 392 ($[M-H]^-$, 30), 213 (100), 178 ($[M-2H]^{2-}$−213, 30). Anal. calcd for $C_{15}H_{12}FN_5O_3S_2$: C, 45.79; H, 3.07; N, 17.80; found: C, 45.56; H, 3.07; N, 17.65.

*5-Chloro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8e**)*

Deep yellow powder (93%): mp: 258–260°C; UV λ $(EtOH)_{maks}$ nm (ε): 253.5 (18,075), 285.4 (11,338), and 364.5 (25,903). IR (KBr): 3315, 3259, 3203 (NH), 1689 (C=O), 1622, 1589, 1537, 1483, 1463 (C=N, C=C), 1327, 1161 (S=O), and 1205 (C=S). ^{1}H NMR (DMSO-d_6/300 MHz) ppm: 7.16 (1H, d, *J*: 8.5 Hz, ind. C7–H), 7.61 (1H, dd, *J*: 8.5, 2.3 Hz, ind. C6–H), 7.67 (2H, s, SO_2NH_2), 7.86 (1H, dd, *J*: 8.5, 7.9 Hz, phen. C5–H), 7.93 (1H, d, *J*: 7.9 Hz, phen. C4–H), 8.07 (1H, d, *J*: 2.3 Hz, ind. C4–H), 8.09 (1H, d, *J*: 8.5 Hz, phen. C6–H), 8.28 (1H, s, phen. C2–H), 11.27 (1H, s, N4–H), 11.57 (1H, s, ind. N–H), 12.89 (1H, s, N2–H). MSMS (ESI−): *m/z* (%) 408, 410 ($[M-H]^-$, 28, 9), 407, 409 ($[M-2H]^{2-}$, 100, 29), 213 (18), 194, and 196 ($[M--2H]^{2-}$−213, 4, 1). Anal. calcd for $C_{15}H_{12}ClN_5O_3S_2.H_2O$: C, 42.10; H, 3.30; N, 16.37; found: C, 42.33; H, 3.10; N, 16.00.

*5-Iodo-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8f**)*

Orange powder (80%): mp: 254–255°C; UV λ $(EtOH)_{maks}$ nm (ε): 256.5 (14,839), 287.0 (9575), and 368.0 (20,353). IR (KBr): 3354, 3292, 3267 (NH), 1695 (C=O), 1612, 1593, 1541, 1487, 1462 (C=N, C=C), 1340, 1161 (S=O), and 1205 (C=S). ^{1}H NMR (DMSO-d_6/300 MHz) ppm: 6.98 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.66 (2H, s, SO_2NH_2), 7.79–7.93 (3H, m, ind. C6–H, phen. C5–H, phen. C4–H), 8.08 (1H, d, *J*: 7.9 Hz, phen. C6–H), 8.27 (1H, s, ind. C4–H), 8.33 (1H, d, *J*: 1.3 Hz, phen. C2–H), 11.26 (1H, s, N4–H), 11.54 (1H, s, ind. N–H), and 12.89 (1H, s, N2–H). Anal. calcd for $C_{15}H_{12}IN_5O_3S_2$: C, 35.94; H, 2.41; N, 13.97; found: C, 35.97; H, 2.50; N, 13.82.

*5-Nitro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**8g**)*

Yellow powder (85%): mp 238–239°C; UV λ $(EtOH)_{maks}$ nm (ε): 239.4 (23,588), 258.7 (20,475), 331.4 (18502), and 365.5 (21,615). IR (KBr): 3327, 3315, 3221 (NH), 1693 (C=O), 1622, 1604, 1558, 1483 (C=N, C=C), 1537, 1334 (N=O), 1348, 1159 (S=O), and 1205 (C=S). ^{1}H NMR (DMSO-d_6/300 MHz) ppm: 7.37 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.71 (2H, s, SO_2NH_2, D_2O exchange), 7.87 (1H, dd, *J*: 7.9, 7.9 Hz, phen. C5–H), 7.97 (1H, d, *J*: 7,9 Hz, phen. C4–H), 8.12 (1H, d, *J*: 7.9 Hz, phen. C6–H), 8.30 (1H, s, phen. C2–H), 8.52 (1H, dd, *J*: 8.8, 2.3 Hz, ind. C6–H), 8.92 (1H, d, *J*: 2.4 Hz, ind. C4–H), 11.49 (1H, s, N4-H, D_2O exchange), 12.11 (1H, s, ind. N–H, D_2O exchange), and 12.84 (1H, s, N2–H, D_2O exchange). HSQC-2D (DMSO-d_6/125 MHz): 112.05 (ind. C7), 117.47 (ind. C4), 121.56 (ind. C3a), 123.56 (phen. C2); 124.05 (phen. C4), 127.97 (ind. C6), 129.80 (phen. C6), 129.87 (phen. C5), 131.51 (ind. C3), 139.63 (phen. C1), 143.51 (ind. C5), 145.14 (phen. C3), 148.38 (ind. C7a), 163.77 (ind. C2), and 177.35 (CS). Anal. calcd for $C_{15}H_{12}N_6O_5S_2.H_2O$: C, 41.09; H, 3.22; N, 19.17; found: C, 41.13; H, 3.10; N, 19.08.

*1-Methyl-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**9a**)*

Orange powder (97%): mp: 217–18°C; UV λ $(EtOH)_{maks}$ nm (ε): 251.0 (13,981), 283.2 (7516), and 364.5 (18,498). IR (KBr): 3354, 3321, 3201 (NH), 1670 (C=O), 1618, 1595, 1502, 1479 (C=N, C=C), 1336, 1151 (S=O), and 1186 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 3.30 (3H, s, ind. N–CH_3), 7.16 (1H, d, *J*: 7.8 Hz, ind. C7–H), 7.18 (1H, dd, *J*: 7.8, 7.4 Hz, ind. C5–H), 7.43 (2H, s, SO_2NH_2), 7.46 (1H, td, *J*: 7.8, 1.6 Hz, ind. C6–H), 7.60 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.70 (1H, dt, *J*: 8.0, 1.6 Hz, phen. C4–H), 7.79 (1H, d, *J*: 7.4 Hz, ind. C4–H), 7.87 (1H, dd, *J*: 8.0, 2.0 Hz, phen. C6-H), 8.08 (1H, dd, *J*: 1.8, 1.6 Hz, phen. C2–H), 11.03 (1H, s, N4–H), and 12.79 (1H, s, N2–H). Anal. calcd for $C_{16}H_{15}N_5O_3S_2$: C, 49.34; H, 3.88; N, 17.98; found: C, 49.48; H, 3.76; N, 17.72.

*1,5-Dimethyl-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**9b**)*

Orange powder (88%): mp: 237–239°C; UV λ $(EtOH)_{maks}$ nm (ε): 253.0 (16,421), 283.5 (10,490), and 364.0 (22,755). IR (KBr): 3307, 3244, 3207 (NH), 1681 (C=O), 1620, 1591, 1543, 1487, 1477 (C=N,

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C=C), 1332, 1163 (S=O), and 1180 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 2.32 (3H, s, CH_3), 3.19 (3H, s, ind. N–CH_3), 7.01 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.25 (1H, dd, *J*: 8.2, 2.0 Hz, ind. C6–H), 7.44 (2H, s, SO_2NH_2), 7.60 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.60 (1H, s, ind. C4–H), 7.71 (1H, dt, *J*: 8.0, 1.4 Hz, phen. C4–H), 7.88 (1H, dd *J*: 8.2, 2.0 Hz, phen. C6–H), 8.09 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 10.99 (1H, s, N4–H), and 12.75 (1H, s, N2–H). HSQC-2D (DMSO-d_6/100 MHz): 21.06 (CH_3), 26.22 (ind. N–CH_3), 110.16 (ind. C7), 119.57 (ind. C3a), 121.95 (ind. C4), 123.03 (phen. C2), 123.53 (phen. C4), 129.41 (phen. C5,6), 132.26 (ind. C6), 132.49 (ind. C3), 132.54 (ind. C5), 139.34 (phen. C1), 142.17 (ind. C7a), 144.77 (phen. C3), 161.32 (ind. C2), and 177.01 (CS). MSMS (ESI+): *m/z* (%) 449 ([M+2Na]$^{2+}$, 41), 426 ([M+Na]$^+$, 100), 404 ([M+H]$^+$, 26), and 190 (5). (ESI–): *m/z* (%) 402 ([M–H]$^-$, 10), 213 (100), and 188 ([M–2H]$^{2-}$–213, 7). Anal. calcd for $C_{17}H_{19}N_5O_3S_2$: C, 50.61; H, 4.25; N, 17.36; found: C, 50.77; H, 4.16; N, 17.09.

*1-Methyl-5-trifluoromethoxy-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazone] (**9c**)*

Deep yellow powder (90%): mp: 230–231°C; UV λ (EtOH)$_{maks}$ nm (ε): 239.5 (26,181), 250.5 (26,133), 283.0 (10,273), and 364.0 (38,585). IR (KBr): 3367, 3280, 3219 (NH), 1693 (C=O), 1620, 1593, 1544, 1473 (C=N, C=C), 1336, 1165 (S=O), and 1215 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 3.24 (3H, s, ind. N–CH_3), 7.26 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.44 (2H, s, SO_2NH_2), 7.48 (1H, dd, *J*: 8.6, 2.0 Hz, ind. C6–H), 7.62 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.72 (1H, dt, *J*: 7.8, 1.6 Hz, phen. C4–H), 7.81 (1H, d, *J*: 1.6 Hz, ind. C4–H), 7.86 (1H, dd, *J*: 7.8, 1.6 Hz, phen. C6-H), 8.05 (1H, dd, *J*: 2.0, 1.6 Hz, phen. C2–H), 11.08 (1H, s, N4–H), and 12.65 (1H, s, N2–H). Anal. calcd for $C_{17}H_{14}F_3N_5O_4S_2$: C, 43.13; H, 2.98; N, 14.79; found: C, 43.03; H, 2.92; N, 14.63.

*5-Fluoro-1-methyl-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**9d**)*

Orange powder (90%): mp: 237–239°C; UV λ (EtOH)$_{maks}$ nm ( ): 251.5 (13,323), 281.0 (8556), and 363.5 (20,657). IR (KBr): 3346, 3259 (NH), 1681 (C=O), 1618, 1595, 1543, 1473 (C=N, C=C), 1334, 1165 (S=O), and 1220 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 3.31 (3H, s, ind. N–CH_3), 7.16 (1H, dd, *J*: 8.6, 3.9 Hz, ind. C7–H), 7.31 (1H, td, *J*: 9.0, 2.8 Hz, ind. C6–H), 7.44 (2H, s, SO_2NH_2), 7.62 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5-H), 7.64 (1H, dd, *J*: 8.2, 2.8 Hz, ind. C4–H), 7.72 (1H, dt, *J*: 8.2, 2.0 Hz, phen. C4–H), 7.87 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 8.08 (1H, dd, *J*: 2.0, 1.6 Hz, phen. C2–H), 11.03 (1H, s, N4–H), and 12.66 (1H, s, N2–H). MSMS (ESI+): *m/z* (%) 430 ([M+Na]$^+$, 100), 408 ([M+H]$^+$, 44), and 194 (5). (ESI–): *m/z* (%) 406 ([M–H]$^-$, 14), 213 (100), and 192 ([M–2H]$^{2-}$–213, 20). Anal. calcd for $C_{16}H_{14}FN_5O_3S_2$: C, 47.17; H, 3.46; N, 17.19; found: C, 47.17; H, 3.52; N, 16.88.

*5-Chloro-1-methyl-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**9e**)*

Orange powder (88%): mp: 247–248°C; UV λ (EtOH)$_{maks}$ nm (ε): 254.5 (9707), 284.0 (6655), and 364.5 (14794). IR (KBr): 3336, 3307, 3259 (NH), 1681 (C=O), 1608, 1595, 1544, 1485 (C=N, C=C), 1336, 1165 (S=O), and 1219 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 3.21 (3H, s, ind. N–CH_3), 7.18 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.44 (2H, s, SO_2NH_2), 7.50 (1H, dd, *J*: 8.2, 2.0 Hz, ind. C6–H), 7.62 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5–H), 7.72 (1H, d, *J*: 7.8 Hz, phen. C4–H), 7.86-7.88 (2H, m, phen. C6–H, ind. C4–H), 8.07 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 11.06 (1H, s, N4–H), and 12.60 (1H, s, N2–H). Anal. calcd for $C_{16}H_{14}ClN_5O_3S_2$: C, 45.33; H, 3.33; N, 16.52; found: C, 45,39; H, 3.44; N, 16.05.

*5-Iodo-1-methyl-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**9f**)*

Orange powder (83%): mp 236–237°C; UV λ (EtOH)$_{maks}$ nm (ε): 244.0 (18,140), 255.5 (12,831), 286.5 (8863), and 369.0 (19,119). IR (KBr): 3336, 3311, 3255 (NH), 1681 (C=O), 1600, 1539, 1485, 1435 (C=C, C=N), 1336, 1161 (S=O), and 1217 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 3.33 (3H, s, ind. N–CH_3), 6.99 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.73 (1H, d, *J*: 7.8 Hz, phen. C4–H), 7.77 (1H, dd, *J*: 8.2, 1.6 Hz, ind. C6–H), 7.89 (1H, d, *J*: 8.2 Hz, phen. C6–H), 8.09 (1H, s, ind. C4–H), 8.14 (1H, d, *J*: 1.6 Hz, phen. C2–H), 11.07 (1H, s, N4–H), and 12.61 (1H, s, N2–H). Anal. calcd for $C_{16}H_{14}IN_5O_3S_2$: C, 37.29; H, 2.74; N, 13.59; found: C, 37.44; H, 2.92; N, 13.92.

*1-Ethyl-1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10a**)*

Yellow powder (85%): mp: 213–214°C; UV λ (EtOH)$_{maks}$ nm (ε): 251.0 (20,254), 287.4 (11,740), and 364.0 (28,323). IR (KBr): 3311, 3238, 3115 (NH), 1676 (C=O), 1614, 1583, 1517, 1490, 1469 (C=N, C=C), 1336, 1157 (S=O), and 1190 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.21 (3H, t, *J*: 7.0 Hz, ind. N–CH_2CH_3), 3.79 (2H, q, *J*: 7.0 Hz, ind. N–CH_2CH_3), 7.17 (1H, t, *J*: 7.4 Hz, ind. C5–H), 7.21 (1H, d, *J*: 7.8 Hz, ind. C7–H), 7.43 (2H, s, SO_2NH_2), 7.45 (1H, td, *J*: 7.4, 1.2 Hz, ind. C6–H), 7.60 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5–H), 7.70 (1H, dt, *J*: 7.8, 2.0 Hz, phen. C4–H), 7.80 (1H, d, *J*: 7.4 Hz, ind. C4–H), 7.87 (1H, dd, *J*: 7.8, 2.0 Hz, phen. C6–H), 8.08 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 11.02 (1H, s, N4–H), and 12.81 (1H, s, N2–H). MSMS (ESI+): *m/z* (%) 449 ([M+2Na]$^{2+}$, 10), 426 ([M+Na]$^+$, 100), 404 ([M+H]$^+$, 22). (ESI–): *m/z* (%) 402 ([M–H]$^-$, 8), 213 (100), and 188 ([M–2H]$^{2-}$–213, 9). Anal. calcd for $C_{17}H_{17}N_5O_3S_2$: C, 50.61; H, 4.25; N, 17.36; found: C, 50.60; H, 4.16; N, 17.04.

*1-Ethyl-5-methyl 1*H*-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10b**)*

Orange powder (90%): mp: 242°C; UV λ (EtOH)$_{maks}$ nm (ε): 241.6 (10,270), 254.5 (4133), 284.5 (2880), and 367.0 (6805). IR (KBr): 3394, 3242 (NH), 1681 (C=O), 1622, 1593, 1541, 1475 (C=N, C=C), 1328, 1166 (S=O), and 1180 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.19 (3H, t, *J*: 7.0 Hz, ind. N–CH_2CH_3), 2.31 (3H, s, ind. C_5–CH_3), 3.74 (2H, q, *J*: 7.0 Hz, ind. N–CH_2CH_3), 7.06 (1H, d, *J*: 7.8 Hz, ind. C7–H), 7.24 (1H, d, *J*: 7.8 Hz, ind. C6–H), 7.44 (2H, s, SO_2NH_2), 7.58–7.62 (2H, m, phen. C5–H, ind. C4–H), 7.71 (1H, d, *J*: 7.8 Hz, phen. C4–H), 7.89 (1H, d, *J*: 8.2 Hz, phen. C6–H), 8.10 (1H, s, phen. C2–H), 10.98 (1H, s, N4–H), and 12.77 (1H, s, N2–H). Anal. calcd for

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$C_{18}H_{19}N_5O_3S_2$: C, 51.78; H, 4.59; N, 16.77; found: C, 51.79; H, 4.71; N, 16,21.

*1-Ethyl-5-trifluoromethoxy-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazone] (**10c**)*

Orange powder (61%): mp 233–235°C; UV λ $(EtOH)_{maks}$ nm (ε): 239.5 (18,036), 251.0 (17,841), 285.4 (5654), and 365.0 (27,591). IR (KBr): 3387, 3250 (NH), 1697 (C=O), 1620, 1597, 1541, 1473 (C=N, C=C), 1334, 1157 (S=O), and 1213 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.21 (3H, t, *J*: 7.0 Hz, ind. N–CH_2CH_3), 3.81 (2H, q, *J*: 7.0 Hz, ind. N–CH_2CH_3), 7.32 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.45 (2H, s, SO_2NH_2), 7.45–7.47 (1H, m, ind. C6–H), 7.62 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.73 (1H, dt, *J*: 7.8, 1.5 Hz, phen. C4–H), 7.82 (1H, d, *J*: 1.5 Hz, ind. C4–H), 7.87 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 8.06 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 11.08 (1H, s, N4–H), 12.67 (1H, s, N2–H). MSMS (ESI–): *m/z* (%) 486 ($[M-H]^-$, 100), 485 ($[M-2H]^{2-}$, 88), 213 (29), 272 ($[M-2H]^{2-}$–213, 1). Anal. calcd for $C_{18}H_{16}F_3N_5O_4S_2$: C, 44.35; H, 3.31; N, 14.37; found: C, 44.20; H, 3.41; N, 14.27.

*1-Ethyl-5-fluoro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10d**)*

Deep yellow powder (92%): mp: 243–244°C; UV λ $(EtOH)_{maks}$ nm (ε): 240.5 (15,383), 251.5 (15,931), 281.5 (9777), and 364.0 (23,391). IR (KBr): 3417, 3257, 3105 (NH), 1672 (C=O), 1620, 1585, 1519, 1485, 1463 (C=N, C=C), 1334, 1161 (S=O), and 1209 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.19 (3H, t, *J*: 7.0 Hz, ind. N–CH_2CH_3), 3.78 (2H, q, *J*: 7.0 Hz, ind. N–CH_2CH_3), 7.22 (1H, dd, *J*: 8.6, 4.3 Hz, ind. C7–H), 7.30 (1H, td, *J*: 9.0, 2.8 Hz, ind. C6–H), 7.44 (2H, s, SO_2NH_2), 7.61 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5–H), 7.65 (1H, dd, *J*: 7.8, 2.8 Hz, ind. C4–H), 7.71 (1H, dt, *J*: 8.2, 1.6 Hz, phen. C4–H), 7.87 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 8.08 (1H, dd, *J*: 2.0, 1.6 Hz, phen. C2–H), 11.03 (1H, s, N4–H), and 12.69 (1H, s, N2–H). Anal. calcd for $C_{17}H_{16}FN_5O_3S_2.H_2O$: C, 46.46; H, 4.13; N, 15.94; found: C, 46.21; H, 4.70; N, 16.21.

*5-Chloro-1-ethyl-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10e**)*

Orange powder (85%): mp: 251°C; UV λ $(EtOH)_{maks}$ nm (ε): 255.5 (9502), 284.5 (6481), and 367.0 (14,801). IR (KBr): 3390, 3244, (NH), 1689 (C=O), 1612, 1593, 1541, 1508, 1463 (C=N, C=C), 1328, 1178 (S=O), and 1211 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.19 (3H, t, *J*: 7.2 Hz, ind. N–CH_2CH_3), 3.79 (2H, q, *J*: 7.2 Hz, ind. N–CH_2CH_3), 7.25 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.44 (2H, s, SO_2NH_2), 7.49 (1H, dd, *J*: 8.2, 2.3 Hz, ind. C6–H), 7.61 (1H, dd, *J*: 8.6, 7.8 Hz, phen. C5–H), 7.72 (1H, dt, *J*: 7.8, 1.6 Hz, phen. C4-H), 7.87 (1H, dd, *J*: 7.8, 2.0 Hz, phen. C6–H), 7.90 (1H, d, *J*: 2.3 Hz, ind. C4-H), 8.07 (1H, dd, *J*: 2.0, 1.9 Hz, phen. C2–H), 11.07 (1H, s, N4–H), 12.63 (1H, s, N2–H). MSMS (ESI+): *m/z* (%) 460, 462 ($[M+Na]^+$, 100, 38), 438, 440 ($[M+H]^+$, 34, 16), 224 (3). (ESI–): *m/z* (%) 436, 438 ($[M-H]^-$, 25, 8), 213 (100); 222, and 224 ($[M-2H]^{2-}$–213, 25, 9). Anal. calcd for $C_{17}H_{16}ClN_5O_3S_2$: C, 46.63; H, 3.68; N, 15.99; found: C, 46.88; H, 3.58; N, 16.17.

*1-Ethyl-5-iodo-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10f**)*

Deep yellow powder (72%): mp 246–247°C; UV λ $(EtOH)_{maks}$ nm (ε): 255.5 (21,969), 287.5 (15,087), and 369.0 (32,397). IR (KBr): 3388, 3244 (NH), 1689 (C=O), 1635, 1598, 1539, 1463 (C=N, C=C), 1332, 1159 (S=O), and 1178 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.19 (3H, t, *J*: 7.2 Hz, ind. N–CH_2CH_3), 3.77 (2H, q, *J*: 7.2 Hz, ind. N–CH_2CH_3), 7.08 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.73 (1H, d, *J*: 8.2 Hz, phen. C4–H), 7.77 (1H, dd, *J*: 8.2, 1.6 Hz, ind. C6–H), 7.89 (1H, d, *J*: 7.8 Hz, phen. C6–H), 8.08 (1H, dd, *J*: 2.0, 1.6 Hz, phen. C2–H), 8.17 (1H, d, *J*: 2.0 Hz, ind. C4–H), 11.09 (1H, s, N4–H), 12.65 (1H, s, N2–H). Anal. calcd for $C_{17}H_{16}IN_5O_3S_2$: C, 38.57; H, 3.05; N, 13.23; found: C, 38.53; H, 3.09; N, 13.62.

*1-Ethyl-5-nitro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**10g**)*

Deep yellow powder (78%): mp: 247°C; UV λ $(EtOH)_{maks}$ nm (ε): 236.5 (24,755), 258.7 (11,660), 332.3 (21,078), and 365.0 (26,848). IR (KBr): 3381, 3244 (NH), 1695 (C=O), 1614, 1595, 1539, 1469, (C=N, C=C), 1523, 1338 (N=O), 1332, 1174 (S=O), and 1205 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 1.24 (3H, t, *J*: 7.0 Hz, ind. N–CH_2CH_3), 3.88 (2H, q, *J*: 7.0 Hz, ind. N–CH_2CH_3), 7.46 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.47 (2H, s, SO_2NH_2), 7.64 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.74 (1H, d, *J*: 7.8 Hz, phen. C4–H), 7.90 (1H, d, *J*: 7.8 Hz, phen. C6–H), 8.07 (1H, dd, *J*: 2.0, 1.5 Hz, phen. C2–H), 8.36 (1H, dd, *J*: 8.6, 2.3 Hz, ind. C6–H), 8.71 (1H, d, *J*: 2.3 Hz, ind. C4–H), 11.28 (1H, s, N4–H), and 12.56 (1H, s, N2–H). HMBC-2D (DMSO-d_6/125 MHz): 13.04 (ind. N–CH_2CH_3), 35.19 (ind. N–CH_2CH_3), 110.64 (ind. C7), 116.91 (ind. C4), 120.75 (ind. C3a), 123.29 (phen. C2), 123.84 (phen. C4), 127.57 (ind. C6), 129.57 (phen. C5), 129.59 (phen. C6), 130.35 (ind. C3), 139.15 (phen. C1), 143.52 (ind. C5), 144.91 (phen. C3), 148.01 (ind. C7a), 161.46 (ind. C2), and 177.05 (CS). Anal. calcd for $C_{17}H_{16}N_6O_5S_2$: C, 45.53; H, 3.60; N, 18.74; found: C, 45.29; H, 3.84; N, 18.60.

*1-Benzyl-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**11a**)*

Deep yellow powder (93%): mp 218–220°C; UV λ $(EtOH)_{maks}$ nm (ε): 251.5 (23,556), 287.0 (12,755), and 367.5 (31,703). IR (KBr): 3315, 3246 (NH), 1687 (C=O), 1612, 1587, 1533, 1485 (C=N, C=C), 1336, 1153 (S=O), and 1226 (C=S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 4.99 (2H, s, ind. N–CH_2), 7.05 (1H, d, *J*: 8.2 Hz, ind. C7–H) 7.16 (1H, dd, *J*: 7.8, 7.4 Hz, ind. C5–H), 7.25–7.40 (6H, m, ind. C6–H/benz.H), 7.45 (2H, s, SO_2NH_2), 7.61 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5-H), 7.71 (1H, d, *J*: 8.2 Hz, phen. C4–H), 7.82 (1H, d, *J*: 7.8 Hz, ind. C4–H), 7.88 (1H, d, *J*: 7.8 Hz, phen. C6-H), 8.09 (1H, s, phen. C2–H), 11.07 (1H, s, N4–H), and 12.80 (1H, s, N2–H). HSQC-2D (DMSO-d_6/100 MHz): 42.61 (ind. N–CH_2), 110.45 (ind. C7), 119.37 (ind. C3a), 121.28 (ind. C4), 122.60 (phen. C2), 123.08 (ind. C5), 123.14 (phen. C4), 127.46 (benz. C3,5), 127.65 (benz. C4), 128.73 (benz. C2,6), 128.98 (phen. C6), 129.00 (phen. C5), 131.39 (ind. C6), 131.69 (ind. C3), 135.66 (benz. C1) 138.91 (phen. C1), 142.78 (ind. C7a), 144.36 (phen. C3),

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160.90 (ind. C2), and 176.61 (CS). Anal. calcd for $C_{22}H_{19}N_5O_3S_2$: C, 56.76; H, 4.11; N, 15.04; found: C, 56.61; H, 4.36; N, 14.84.

*1-Benzyl-5-methyl-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**11b**)*

Orange powder (65%): mp: 222–223°C; UV λ $(EtOH)_{maks}$ nm (ε): 254.0 (21149), 285.5 (13691), and 369.0 (31,699). IR (KBr): 3325, 3302, 3244 (NH), 1693 (C═O), 1624, 1591, 1539, 1479 (C═N, C═C), 1340, 1161 (S═O), and 1180 (C═S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 2.31 (3H, s, ind. C_5–CH_3), 4.99 (2H, s, ind. N–CH_2), 6.95 (1H, d, *J*: 8.2 Hz, ind. C7–H), 7.20 (1H, d, *J*: 8.2 Hz, ind. C6–H), 7.27–7.41 (5H, m, benz.H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.68 (1H, s, ind. C4–H), 7.73 (1H, dt, *J*: 7.8, 1.6 Hz, phen. C4–H), 7.90 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 8.10 (1H, dd, *J*: 2.0, 1.9 Hz phen. C2–H), 11.08 (1H, s, N4–H), and 12.80 (1H, s, N2–H). Anal. calcd for $C_{23}H_{21}N_5O_3S_2$: C, 57.60; H, 4.41; N, 14.60; found: C, 57.20; H, 4.56; N, 14.45.

*1-Benzyl-5-trifluoromethoxy-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)thiosemicarbazone] (**11c**)*

Yellow powder (87%): mp: 190–192°C; UV λ $(EtOH)_{maks}$ nm (ε): 251.5 (21,761), 281.4 (12,254), and 367.5 (31,873). IR (KBr): 3354, 3296, 3280 (NH), 1687 (C═O), 1622, 1589, 1521, 1481 (C═N, C═C), 1342, 1159 (S═O), and 1215 (C═S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 5.01 (2H, s, ind. N–CH_2), 7.13 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.26-7.41 (6H, m, ind. C6–H/benz.H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 7.8, 7.8 Hz, phen. C5–H), 7.73 (1H, d, *J*: 8.2 Hz, phen. C4-H), 7.85 (1H, d, *J*: 1.6 Hz, ind. C4-H), 7.88 (1H, d, *J*: 8.6 Hz, phen. C6–H), 8.06 (1H, dd, *J*: 1.9, 1.6 Hz, phen. C2–H), 11.12 (1H, s, N4–H), and 12.66 (1H, s, N2–H). Anal. calcd for $C_{23}H_{18}F_3N_5O_4S_2$: C, 50.27; H, 3.30; N, 12.74; found: C, 50.32; H, 3.41; N, 12.54.

*1-Benzyl-5-chloro-1H-indole-2,3-dione 3-[4-(3-sulfamoylphenyl)-thiosemicarbazone] (**11d**)*

Deep yellow powder (63%): mp 222–224°C; UV λ $(EtOH)_{maks}$ nm (ε): 241.6 (15,949), 253.5 (18,349), 284.0 (11,199), and 364.5 (25,349). IR (KBr): 3332, 3307 (NH), 1693 (C═O), 1613, 1593, 1537, 1471 (C═N, C═C), 1334, 1155 (S═O), and 1176 (C═S). ^{1}H NMR (DMSO-d_6/400 MHz) ppm: 4.99 (2H, s, ind. N–CH_2), 7.05 (1H, d, *J*: 8.6 Hz, ind. C7–H), 7.25–7.39 (5H, m, benz.H), 7.42 (1H, dd, *J*: 8.6, 2.0 Hz, ind. C6–H), 7.46 (2H, s, SO_2NH_2), 7.63 (1H, dd, *J*: 8.2, 7.8 Hz, phen. C5–H), 7.73 (1H, dt, *J*: 7.8, 1.5 Hz, phen. C4–H), 7.88 (1H, dd, *J*: 8.2, 2.0 Hz, phen. C6–H), 7.92 (1H, d, *J*: 2.0 Hz, ind. C4–H), 8.08 (1H, dd, *J*: 2.0, 1.9 Hz phen. C2–H), 11.11 (1H, s, N4–H), 12.62 (1H, s, N2–H). MSMS (ESI+): *m/z* (%) 522 and 524 ($[M+Na]^+$, 100, 43). Anal. calcd for $C_{22}H_{18}ClN_5O_3S_2$: C, 52.85; H, 3.63; N, 14.01; found: C, 52.58; H, 3.80; N, 14.24.

5.2 | Determination of inhibitory effects against hCA I, II, IX, and XII isoenzymes

An Applied Photophysics (Leatherhead, UK) stopped-flow instrument was used for assaying the CA-catalyzed CO_2 hydration activity.[45] Phenol red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 20 mM HEPES (pH 7.5) as a buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5%–10% of the reaction were used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of the inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature before assay, to allow for the formation of the E–I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng–Prusoff equation, as reported earlier, and represent the mean from at least three different determinations.[46–49]

5.3 | Molecular modeling studies

5.3.1 | Preparation of ligand structures

The three-dimensional (3D) structures of synthesized compounds were generated using the MOE software package (v2016.08; Chemical Computing Group, Inc.). Protonation states of the compounds at physiological pH were assigned. All compounds were minimized using a steepest-descent protocol (MMFF94x force field). Finally, a database was created in which all the compound structures were present in 3D format and saved as a mol2 file.

5.3.2 | Preparation of protein structures

The crystal structures of hCA I (pdb: 3LXE, 1.90 Å), hCA II (pdb: 4E3D, 1.60 Å), hCA IX (pdb: 3IAI; 2.20 Å), and hCA XII (pdb: 1JD0; 1.50 Å) were obtained from the Protein Data Bank (PDB). The hydrogen atoms were added and reagents, ligands, and water molecules were removed from the crystal structures. The remaining structure was protonated using the MOE software package and subsequently the obtained structure was energy-minimized (AMBER12: EHT force field).

5.3.3 | Docking protocols

The molecular docking method was performed using the FlexX docking tool (v2.3.2; BioSolveIT GmbH) to emulate the binding interactions between the investigated compounds and the hCA structures (default settings). The sulfonamide tail of the ligands was forced to adopt a similar orientation and interactions to the

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Zn^{2+} ion as observed for AAZ in the hCA IX and XII structures using a pharmacophore model. The best scoring three poses were subjected to refinement calculations. To this end, the ligand and binding pocket residues (defined as residues within 6.5 Å of the docked ligand) were energy-minimized using the GBVI/WSA force field.[36]

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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