



# Synthesis of benzenesulfonamides linked to quinazoline scaffolds as novel carbonic anhydrase inhibitors

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## ABSTRACT

Carbonic anhydrase (CA) inhibitory activities of newly synthesized quinazoline-linked benzenesulfonamides **10–29**, **31**, **32**, **35**, **36**, and **45–51** against human CA (hCA) isoforms I, II, IX, and XII were measured and compared to that of acetazolamide (AAZ) as a standard inhibitor. Potent selective inhibitory activity against hCA I was exerted by compounds **14**, **15**, **17**, **19**, **20**, **21**, **24**, **25**, **28**, **29**, **31**, **35**, **45**, **47**, **49**, and **51** with inhibition constant ( $K_i$ ) values of 39.4–354.7 nM that were nearly equivalent or even greater than that of AAZ ( $K_i$ , 250.0 nM). Compounds **15**, **20**, **24**, **28**, **29**, **45** and **47** proved to have inhibitory activities against hCA II with ( $K_i$ s, 0.73–16.5 nM) that were similar or improved to that of AAZ ( $K_i$ , 12.0 nM). Compounds **13–29**, **31–32**, and **45–51** displayed potent hCA IX inhibitory activities ( $K_i$ s, 1.6–32.2 nM) that were more effective than or nearly equal to AAZ ( $K_i$ , 25.0 nM). Compounds **14**, **15**, **20**, **21**, **26**, **45**, and **47** exerted potent hCA XII inhibitory activities ( $K_i$ s, 5.2–9.2 nM), indicating similar CAI activities as compared to that of AAZ ( $K_i$ , 5.7 nM).

## 1. Introduction

Most organisms possess multiple isoforms of carbonic anhydrase (CA, EC 4.2.1.1), a family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to bicarbonate and protons [1–4]. This hydration reaction is involved in several vital physiological pathways [1–4] and deregulation of CA activity is associated with disorders and diseases such as glaucoma [4], epilepsy [5], obesity [6], and cancer [7].

Human carbonic anhydrase inhibitors (hCAI) compounds have various therapeutic applications. For example, human CA (hCA) II, IV, and XII inhibitors are used as diuretics and in glaucoma. The hCA II and VII inhibitors are used as anti-epileptic drugs [1,2,8], whereas certain antitumor and anti-inflammatory CAIs target the isoforms hCA IX and XII. Recently, hCA IV, IX, and XII were shown to be involved in rheumatoid arthritis and their cognate inhibitors may constitute new pharmacological candidates for the management of this condition [1,9,10]. Furthermore, the transmembrane isoforms hCA IX/XII are overexpressed in many hypoxic tumors [11–15]. Sulfonamides are one of the best studied CAI classes [16–24], whereas some of them, such as

the coxibs, celecoxib, and valdecoxib, also inhibit cyclooxygenase (COX) [25].

The quinazolinone scaffold is frequently used in medicinal chemistry [25–44]. Several 2-[(3-substituted-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamides (**A**) are highly potent, nanomolar inhibitors of  $\alpha$ -CA from *Vibrio cholerae* (VchCA) and the human  $\alpha$ -CA isoforms hCA I and hCA II [45] (Fig. 1). Three 4-(quinazolin-4-ylamino) benzenesulfonamides and 4-(quinazolin-4-yloxy)benzenesulfonamide (**B**) exerted strong inhibitory effects against the cytosolic hCA I and II and the transmembrane hCA IX and XII, with  $K_i$  values in the low nanomolar range [46] (Fig. 1).

3-(3-Aminosulfonyl)-phenyl-2-mercapto-6-iodo-4(3H)quinazolinone and 3-(2-mercapto-7-flouro-4(3H)quinazolinon-3-yl)-benzenesulfonamide (**C**) were potent inhibitors of the tumor-associated isoforms hCA IX ( $K_i$ s, 1.5 and 2.7 nM, respectively) and XII ( $K_i$ s, 0.57 and 1.9 nM, respectively) [47].

3-(4-Aminosulfonyl)-phenyl-2-mercapto-substituted-4(3H)quinazolines (**C**) are highly potent inhibitors of hCA I ( $K_i$ s = 135–282 nM), hCA II ( $K_i$ s = 0.25–10.8 nM), IX ( $K_i$ s of 3.7–50.4 nM), and XII ( $K_i$ s of 0.60–52.9 nM) [48].

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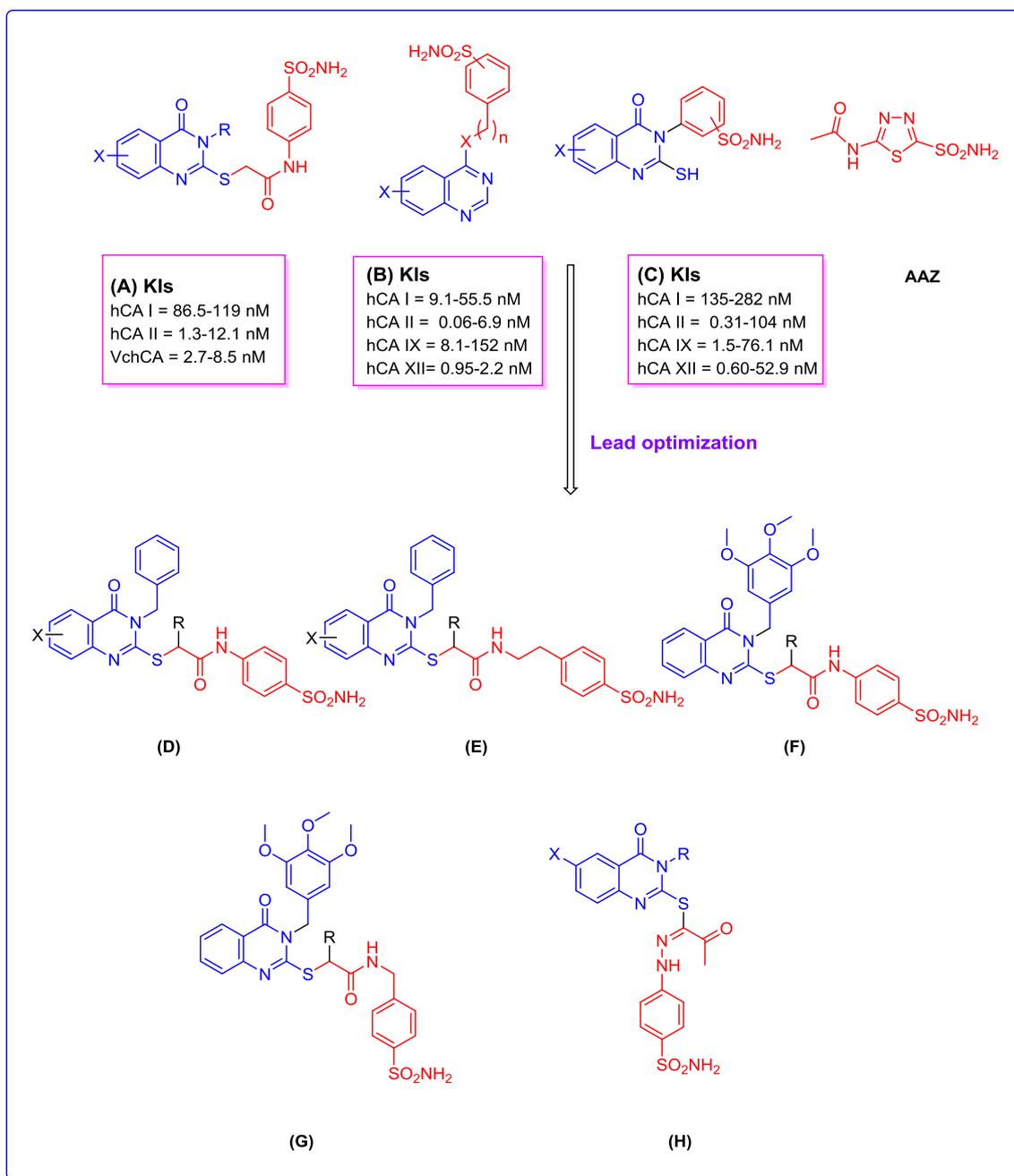


Fig. 1. Reported (A–C), AAZ and designed quinazoline derivatives (D–H) as CAIs.

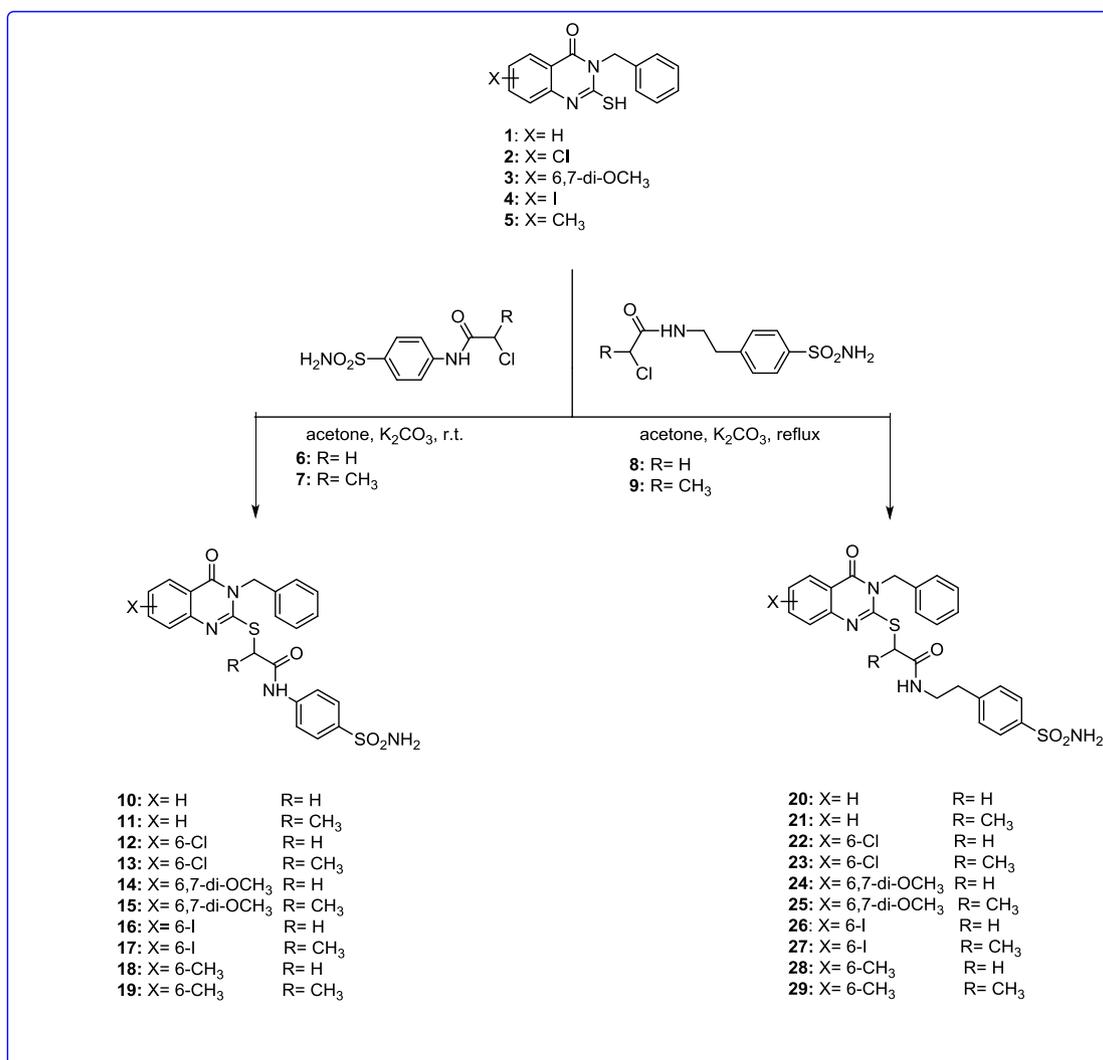
Based on the aforementioned rationale, we designed and synthesized a new series of 2-substituted-mercapto-3-substituted-4(3*H*)-quinazolinones by incorporating benzenesulfonamide moieties **10–29**, **31**, **32**, **35**, **36**, and **45–51** and assayed their inhibitory effects on four physiological significant hCA isoforms, the abundantly expressed, cytosolic hCA I and II and the tumor-associated hCA IX and XII (**D**, **E**, and **F**; Fig. 1) as CAI lead compounds.

## 2. Results and discussion

### 2.1. Chemistry

2-[(3-Benzyl-6-substituted-4(3*H*)-quinazolinon-2-yl)thio]-*N*-(4-sulfamoylphenyl)acetamides, 2-[(3-benzyl-6-substituted-4(3*H*)-quinazolinon-2-yl)thio]-*N*-(4-sulfamoylphenyl)propanamides (**10–19**), 2-[(3-benzyl-6-substituted-4(3*H*)-quinazolinon-2-yl)thio]-*N*-(4-

sulfamoylphenethyl)acetamides and 2-[(3-benzyl-6-substituted-4(3*H*)-quinazolinon-2-yl)thio]-*N*-(4-sulfamoylphenethyl)propanamides (**20–29**) were obtained with a yield of 87–96% by reacting the appropriate 6-substituted-2-mercapto-3-substituted-4(3*H*)-quinazolinones (**1–5**) with 2-chloro-*N*-(4-sulfamoylphenyl)amides (**6–7**) or 2-chloro-*N*-(4-sulfamoylphenethyl)amides (**8–9**) in acetone containing anhydrous potassium carbonate (Scheme 1). Multiple spectral analyses were performed to confirm the structures of target compounds **10–29**. The amide moiety (CONH) of compounds **10–29** was verified by <sup>1</sup>H NMR spectra with a peak for the amidic proton at 10.92–8.34 ppm and by <sup>13</sup>C NMR spectra with characteristic peaks at 170.80–166.63 ppm for the carbonyl group. The 3-benzylquinazolinone moiety was verified by a peak at 5.66–5.25 and at 49.09–47.20 ppm of CH<sub>2</sub>Ph group in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra, as well as a typical peak at 161.35–159.99 ppm of carbonyl group of quinazolinone moiety in the <sup>13</sup>C NMR spectra.



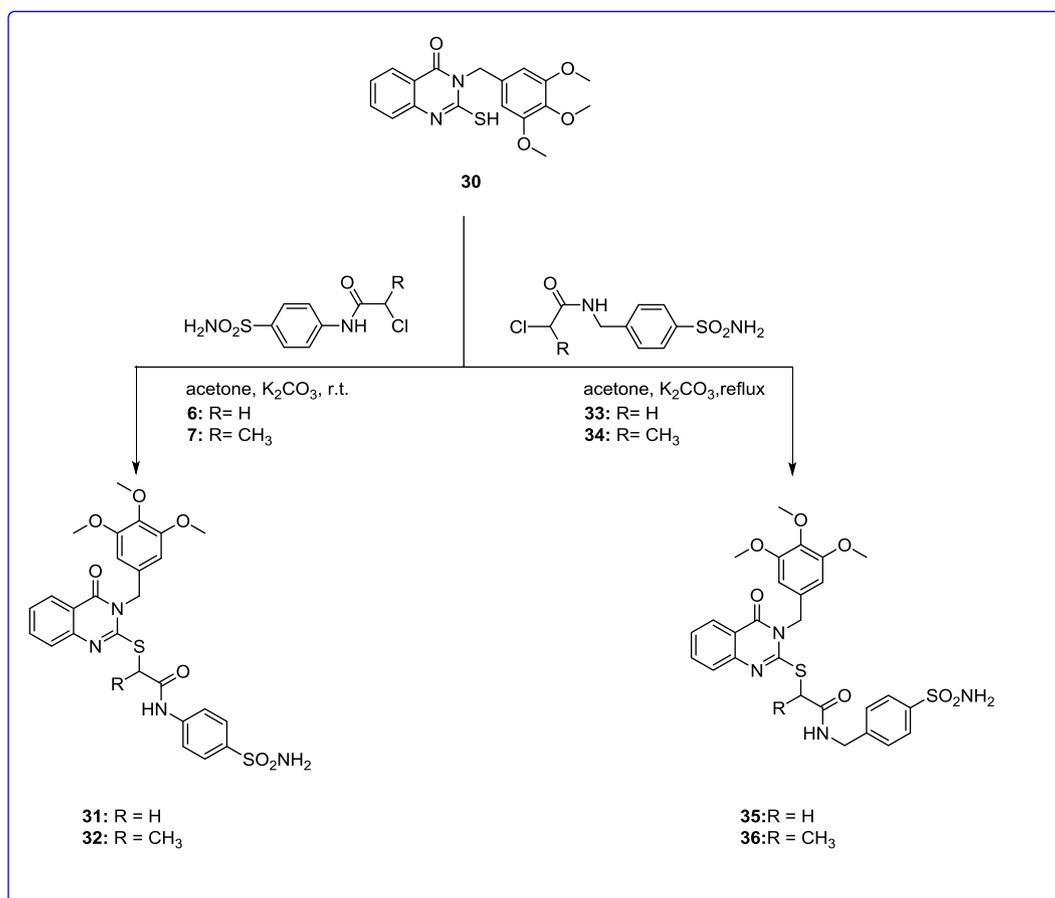
**Scheme 1.** Synthesis of 2-[(3-benzyl-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)anilides (10-19) and 2-[(3-benzyl-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)anilides (20-29).

Compounds **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, and **28** were confirmed by the presence of singlet peaks for two protons of the acetamide group (SCH<sub>2</sub>CONH) at 4.23–3.95 ppm and at 40.72–37.41 ppm in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra. Moreover, the propanamide moiety (S(CH)<sub>2</sub>CONH) of compounds **11**, **13**, **15**, **17**, **19**, **21**, **23**, **25**, **27**, and **29** was confirmed by a singlet proton peak at 4.77–4.51 ppm and a carbonyl group peak at 47.25–46.10 ppm in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra, respectively, as well as by verifying the methyl group with a doublet peak at 1.60–1.45 in the <sup>1</sup>H NMR and a peak at 18.77–17.57 ppm in the <sup>13</sup>C NMR. Compounds **20–29** were characterized by the presence of the ethylsulfonamide moiety indicated by two peaks at 2.97–2.77 and 3.38–3.28 ppm in the <sup>1</sup>H NMR spectra and at 35.24–35.03 and 40.50.38–36.24 ppm in the <sup>13</sup>C NMR spectra (Scheme 1). Moreover, the reaction of 2-mercapto-3-(3,4,5-trimethoxybenzyl)-4(3H)-quinazolinone (**30**) with either 2-chloro-N-(4-sulfamoylphenyl) amides (**6–7**) or 2-chloro-N-(4-sulfamoylmethyl)phenylamides (**33–34**) had a yield of 81% for either 2-[(3-(3,4,5-trimethoxybenzyl)-4(3H)quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (**31**) and 2-[(3-(3,4,5-trimethoxybenzyl)-4(3H)quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl) propanamide (**32**) or 2-[(3-(3,4,5-trimethoxybenzyl)-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (**35**) and 2-[(3-(3,4,5-trimethoxybenzyl)-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (**36**), respectively [44] (Scheme 2). Furthermore, 3-substituted-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-

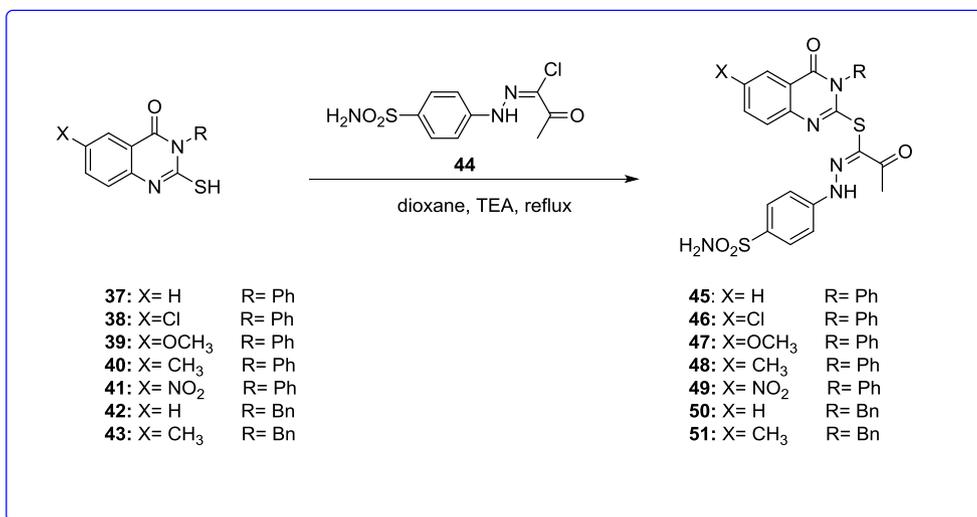
sulfamoylphenyl)propanehydrazonothioates (**45–51**) were obtained with a yield of 80–85% by heating 3-substituted-2-mercapto-4(3H)-quinazolinones (**37–43**) and 2-oxo-N-(4-sulfamoylphenyl)propanehydrazonoyl chloride (**44**) in dioxane containing triethylamine (Scheme 3). Compounds **45–51** were verified by a singlet peak for the NH group of the hydrazone moiety (–NH–N=C–) at 11.10–10.30 ppm in the <sup>1</sup>H NMR spectra, as well as a peak at 192.77–189.59 ppm for the carbonyl group of the propanehydrazonothioate moiety (–NH–N=CS–COCH<sub>3</sub>) in the <sup>13</sup>C NMR spectra. In addition, the methyl group of the propanehydrazonothioate moiety (–NH–N=CS–COCH<sub>3</sub>) was identified by a characteristic singlet peak at 2.61–2.55 and a peak at 26.30–25.48 ppm in the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra, respectively, as well as a typical peak at 165.57–161.45 ppm for the carbonyl group of the 3-substituted quinazolinone moiety in the <sup>13</sup>C NMR spectra.

## 2.2. CA inhibitory activity

The CAI activity of newly synthesized compounds (**10–29**, **31**, **32**, **35**, **36** and **45–51**) against isoforms hCA I, II, IV, and IX was measured and compared to that of acetazolamide (AAZ), a standard sulfonamide inhibitor. Regarding the CAI activity toward individual hCA isoforms, hCA I was effectively inhibited by compounds **14**, **15**, **17**, **19**, **20**, **21**, **24**, **25**, **28**, **29**, **31**, **35**, **45**, **47**, **49**, and **51** with inhibition constant (K<sub>i</sub>) values in the range of 39.4–353.8 nM, (AAZ: K<sub>i</sub>, 250.0 nM). Compounds



**Scheme 2.** Synthesis of 3-substituted quinazoline linked N-(4-sulfamoylphenyl)amide 31-32 and N-(4-sulfamoylbenzyl)amide 35-36.



**Scheme 3.** Synthesis of 3-substituted-4-(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (45-51).

**12, 13, 16, 18, 22, 23, 26, 27, 32, 35, 36, 46, 48,** and **50** showed moderate hCA I inhibitory activity with  $K_i$  values of 421.9–700.5 nM. In contrast, **10** and **11** had a weak inhibitory activity with a  $K_i$ s of 3665.4 and 6472.2 nM, respectively. Compounds **12, 13, 14, 15, 20, 21, 24, 25, 27, 28, 29, 45,** and **47** proved to be potent hCA II inhibitors, with  $K_i$  values of 0.73–28.4 nM, which were greater than or nearly equal to that of AAZ ( $K_i$ , 12.0 nM). Compounds **16, 17, 18, 19, 22, 23,** and **26** showed moderate hCA II inhibitory activity with  $K_i$ s ranging between 32.4 and 78.3 nM whereas compounds **10, 11, 31, 32, 35, 36, 46, 48,**

**49, 50,** and **51** showed a weak inhibitory activity with  $K_i$ s in the range of 151.7–833.1 nM. Compounds **13–29, 31–32,** and **45–51** displayed potent selective hCA IX inhibitory activity with  $K_i$  values ranging from 1.6 to 32.2 nM, being more effective than or nearly equivalent to AAZ. Against hCA IX, compounds **12, 35** and **36** exhibited moderate inhibitory activity, with  $K_i$  values of 65.5, 163.3, and 113 nM, respectively. Weak hCA IX inhibition was also observed for compounds **10** and **11** with  $K_i$ s values of 210.9 and 262.4 nM, respectively. Derivatives **14, 15, 20, 21, 26, 45,** and **47** possessed potent hCA XII inhibitory

**Table 1**

Inhibition constant values of quinazoline derivatives and standard sulfonamide inhibitor acetazolamide (AAZ) against human CA isoforms hCA I, II, IX and XII determined by a stopped flow, CO<sub>2</sub> hydrase assay.

Compound	Ki (nM) <sup>a</sup>				Compd. Type
	hCA I	hCA II	hCA IX <sup>b</sup>	hCA XII <sup>b</sup>	
10	3665.4	547.7	210.9	71.7	D
11	6472.2	591.1	262.4	75.5	D
12	645.7	27.6	65.2	32.0	D
13	764.1	22.8	28.3	34.6	D
14	225.6	28.4	23.8	7.60	D
15	39.4	3.30	24.2	8.90	D
16	642.2	78.3	32.2	44.8	D
17	254.4	62.7	27.8	61.6	D
18	421.9	53.0	17.9	50.1	D
19	219.0	32.4	20.1	42.5	D
20	78.2	15.5	22.1	8.80	E
21	136.5	21.2	18.4	9.20	E
22	617.4	55.3	26.9	12.1	E
23	453.1	50.9	15.2	40.6	E
24	307.1	16.5	28.6	15.2	E
25	353.8	22.6	29.2	28.4	E
26	494.5	35.0	16.4	5.20	E
27	488.6	26.3	13.2	19.0	E
28	106.3	6.90	24.3	12.2	E
29	268.1	5.20	24.6	16.6	E
31	215.5	218.7	31.6	73.0	F
32	609.3	151.7	29.2	46.8	F
35	354.7	484.8	163.3	90.5	G
36	747.7	833.1	113.0	58.8	G
45	86.6	0.73	1.80	8.30	H
46	428.7	195.5	22.5	73.5	H
47	83.9	6.20	1.60	9.20	H
48	631.0	188.3	12.3	75.2	H
49	328.4	320.1	17.1	149.4	H
50	700.5	251.1	20.3	51.1	H
51	201.1	237.5	23.1	23.8	H
AAZ	250.0	12.0	25.0	5.70	-

<sup>a</sup> Errors in the range of  $\pm$  5–10% of the reported values, from three different assays.

<sup>b</sup> Catalytic domain.

activities with K<sub>i</sub> values of 5.2–9.2 nM, exerting an activity that was improved or nearly equivalent to that of AAZ (K<sub>i</sub>, 5.7 nM). Compounds **12**, **13**, **22**, **23**, **24**, **25**, **27**, **28**, **29**, and **51** exerted weaker hCA XII inhibitory activities with K<sub>i</sub> values of 12.1–40.6 nM whereas compounds **10**, **11**, **16**, **17**, **18**, **19**, **31**, **32**, **35**, **36**, **46**, **48**, **49** and **50** had weak hCA XII inhibitory activities with K<sub>i</sub> values in the range of 42.5–149.4 nM (Table 1).

### 2.3. Structure-activity relationship analysis

- (I) The inhibition tests of hCA isoforms with compounds **10**–**29** and **45**–**51** (Table 1), showed that 6-substituted and 6,7-dimethoxyquinazoline derivatives **12**–**19** were more active than the unsubstituted derivatives **10** and **11** toward hCA isoforms; 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** and 6-methoxyquinazoline propanehydrazonothioate **47** were the most active derivatives among the propanehydrazonothioates (**45**–**51**) toward hCA isoforms.
- (II) Structure-activity relationship analysis for hCA I inhibition indicated that 1) the introduction of electron withdrawing groups such as 6-Cl or 6-I at the quinazoline moiety of compound **10** and **11** (K<sub>i</sub>s of 3665.4 and 6472.2 nM) produced compounds **12**, **13**, **16**, and **17** with strongly increased the CAI activity (K<sub>i</sub>s, 254.4–764.1 nM); 2) the introduction of electron donating groups such as the methyl group at position 6 or the methoxy groups at positions 6 and 7 of the quinazoline nucleus produced compounds **14**, **15**, **18**, and **19** with significantly improved CAI potency (K<sub>i</sub>s,

225.6, 39.4, 421.9, and 219.0 nM, respectively); 3) 6,7-dimethoxyquinazolines **14** and **15** (K<sub>i</sub>s, 225.6 and 39.4 nM) were more potent than 6-methylquinazolines **18** and **19** (K<sub>i</sub>s, 421.9 and 219.0 nM); 4) propanamides such as **15**, **17**, and **19** (K<sub>i</sub>s, 39.4, 254.4, and 219.0 nM, respectively) were more effective than the corresponding acetamides **14**, **16**, and **18** (K<sub>i</sub>s, 225.6, 642.2, and 421.9 nM, respectively); 5) unsubstituted quinazoline derivatives such as **20** and **21** (K<sub>i</sub>s, 78.2 and 136.5 nM) were more active than the corresponding 6-substituted and 6,7-dimethoxyquinazoline derivatives **22**–**29** (K<sub>i</sub>s, 106.3–617.4 nM); 6) the introduction of electron withdrawing groups such as 6-Cl and 6-I at the quinazoline moiety of compounds **20** or **21** produced compounds **22**, **23** and **26**, **27** with remarkably decreased CAI activity (K<sub>i</sub>s, 617.4, 453.1 and 494.5, 488.6 nM, respectively); 7) the introduction of electron donating groups such as the 6,7-dimethoxy groups or the 6-methyl group at the quinazoline nucleus of compounds **20** and **21** produced compounds **24**, **25** and **28**, **29** with non-significantly decreased CAI potency (K<sub>i</sub>s, 307.1, 353.8 and 106.3, 268.1 nM respectively); 8) unsubstituted and 6-substituted quinazoline ethylsulfonamide derivatives such as compounds **20**–**23**, **26**, and **28** (K<sub>i</sub>s, 78.2, 136.5, 617.4, 453.1, 494.5, and 106.3 nM, respectively) were more active than the corresponding sulfonamide derivatives **10**–**13**, **16**, and **18** (K<sub>i</sub>s, 3665.4, 6472.2, 645.7, 764.1, 642.2, and 421.9 nM, respectively); on the other hand, 6,7-dimethoxyquinazoline sulfonamides **14**, **15**, 6-iodo quinazoline sulfonamide **17**, and 6-methylquinazoline sulfonamide **19** (K<sub>i</sub>s, 225.6, 39.4, 254.4, and 219.0 nM, respectively) were more active than the corresponding ethylsulfonamide derivatives **24**, **25**, **27**, and **29** (K<sub>i</sub>s, 307.1, 353.8, 488.6, and 268.1 nM, respectively); 9) acetamide derivatives **31** and **35** (K<sub>i</sub>s, 215.5 and 354.7 nM) were more active than propanamides **32** and **36** (K<sub>i</sub>s, 609.3 and 747.7 nM); 10) the introduction of chloro, methyl or nitro groups at the 6-position of quinazoline moiety in propanehydrazonothioate **45** (K<sub>i</sub>, 86.6 nM) produced propanehydrazonothioates **46**, **48**, and **49** (K<sub>i</sub>s, 428.7, 631.0, and 328.4 nM, respectively) with remarkably decreased the hCA I inhibitory activity, whereas the introduction of the methoxy group at the 6-position of quinazoline moiety for propanehydrazonothioate **45** generated compound **47** (K<sub>i</sub>, 83.9 nM) with barely increased hCA I inhibitory activity; 11) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** (K<sub>i</sub>, 86.6 nM) was more active than 3-benzyl unsubstituted quinazoline propanehydrazonothioate **50** (K<sub>i</sub>, 700.5 nM) and 3-benzyl-6-methylquinazoline propanehydrazonothioate **51** (K<sub>i</sub>, 201.1 nM) was more active than 3-phenyl-6-methylquinazoline propanehydrazonothioate **48** (K<sub>i</sub>, 631.0 nM).

- (III) Structure-activity relationship analysis for hCA II inhibition indicated that 1) anilides **12**–**15** (K<sub>i</sub>, 3.3–28.4 nM) were more effective than the corresponding compounds **16**–**19** (K<sub>i</sub>s, 32.4–78.3 nM); 2) 6,7-dimethoxyquinazoline **15** (K<sub>i</sub>, 3.3 nM) showed a higher hCA II inhibitory activity than 6-chloroquinazolines **12** and **13** (K<sub>i</sub>s, 27.6 and 22.8 nM); 3) propanamides derivatives such as compounds **13**, **15**, **17**, and **19** (K<sub>i</sub>s, 22.8, 3.3, 62.7, and 32.4 nM, respectively) were more effective than the corresponding acetamides **12**, **14**, **16**, and **18** (K<sub>i</sub>s, 27.6, 28.4, 78.3, and 53.0 nM, respectively); 4) the inhibitory activity of unsubstituted quinazoline derivatives **20** and **21** (K<sub>i</sub>s, 15.5 and 21.2 nM) was similar to that of AAZ (K<sub>i</sub>, 12.0 nM); 5) the introduction of electron withdrawing groups such as 6-Cl or 6-I at the quinazoline moiety produced compounds **22**, **23** and **26**, **27** with remarkably decreased the CAI activity (K<sub>i</sub>s, 55.3, 50.9 and 35.0, 26.3 nM, respectively); 6) the introduction of electron donating groups such as 6,7-dimethoxy groups at the quinazoline moiety generated compounds **24** and **25** without a change in CAI potency (K<sub>i</sub>s, 16.5 and 22.6 nM) whereas the addition of the 6-methyl function to the quinazoline nucleus produced compounds **28** and **29** with significantly increased CAI potency (K<sub>i</sub>s, 6.9 and

- 5.2 nM); 7) unsubstituted quinazoline ethylsulfonamides **20**, **21**, 6,7-dimethoxyquinazoline ethylsulfonamide **24**, 6-iodoquinazoline ethylsulfonamides **26**, **27**, and 6-methylquinazoline ethylsulfonamides **28**, **29** ( $K_{iS}$ , 15.5, 21.2, 16.5, 35.0, 26.3, 6.9, and 5.2 nM, respectively) were more active than the corresponding sulfonamide derivatives **10**, **11**, **14**, and **16–19** ( $K_{iS}$ , 547.7, 591.1, 28.4, 78.3, 62.7, 53.0, and 32.4 nM, respectively); on the other hand, 6-chloroquinazoline sulfonamides **12**, **13**, and 6–7-dimethoxyquinazoline sulfonamide **15** ( $K_{iS}$ , 27.6, 22.8, and 3.3 nM) were more active than the corresponding ethylsulfonamide derivatives **22**, **23**, and **25** ( $K_{iS}$ , 55.3, 50.9, and 22.6 nM, respectively); 8) the anilides of benzensulfonamides **31** and **32** ( $K_{iS}$ , 218.7 and 151.7 nM) were more potent than the corresponding anilides of benzylsulfonamides **35** and **36** ( $K_{iS}$ , 484.8 and 833.1 nM); 9) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** ( $K_i$ , 86.6 nM) was more active than 3-phenyl 6-substituted quinazoline propanehydrazonothioates **46–49** ( $K_{iS}$ , 6.2–320.1 nM); 10) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** and 3-phenyl-6-methylquinazoline propanehydrazonothioate **48** ( $K_{iS}$ , 0.73 and 188.3 nM) were more active than 3-benzyl unsubstituted quinazoline propanehydrazonothioate **50** and 3-benzyl-6-methylquinazoline propanehydrazonothioate **51** ( $K_{iS}$ , 251.1 and 237.5 nM).
- (IV) Structure-activity relationship analysis for hCA IX inhibition indicated that 1) 6-methylquinazolines **18** and **19** ( $K_{iS}$ , 17.9 and 20.1 nM) were more active against hCA IX than compounds **12–17** ( $K_{iS}$ , 23.8–65.2 nM); 2) compounds **20–29** showed high CAI selectivity toward hCA IX ( $K_{iS}$ , 15.2–29.2 nM) similar to that of AAZ ( $K_i$ , 25.0 nM); 3) 6-iodoquinazolines **26** and **27** ( $K_{iS}$ , 16.4 and 13.2 nM) were more potent than other 6-substituted derivatives such as compounds **22–27** and 6,7-dimethoxyquinazolines **28–29** ( $K_{iS}$ , 15.2–29.2 nM); 4) unsubstituted quinazoline ethylsulfonamides **20**, **21** and 6-substituted quinazoline ethylsulfonamide derivatives containing a deactivating group such as compounds **22**, **23**, **26**, and **27** ( $K_{iS}$ , 22.1, 18.4, 26.9, 15.2, 16.4, and 13.2 nM, respectively) were more active than the corresponding sulfonamide derivatives **10–13**, **16**, and **17** ( $K_{iS}$ , 210.9, 262.4, 65.2, 28.3, 32.2, and 27.8 nM, respectively); on the other hand, 6,7-dimethoxyquinazoline sulfonamides **14**, **15**, and 6-methylquinazoline sulfonamides **18**, **19** ( $K_{iS}$ , 23.8, 24.2, and 17.9, 20.1 nM, respectively) were more active than the corresponding ethylsulfonamide derivatives **24**, **25**, **28**, and **29** ( $K_{iS}$ , 28.6, 29.2, 24.3, and 24.6 nM); 5) anilides of benzensulfonamides **31** and **32** ( $K_{iS}$ , 31.6 and 29.2 nM) were more potent than the corresponding anilides of benzylsulfonamides **35** and **36** ( $K_{iS}$ , 163.3 and 113.0 nM); 6) compounds **45–51** were highly selective CAIs ( $K_{iS}$ , 1.6–23.1 nM) compared with AAZ ( $K_i$ , 25.0 nM); 7) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** ( $K_i$ , 1.8 nM) was more active than 3-phenyl 6-substituted quinazoline propanehydrazonothioates **46**, **48**, and **49** ( $K_{iS}$ , 22.5, 12.3, and 17.1 nM) whereas the CAI activity of 3-phenyl 6-methoxyquinazoline propanehydrazonothioate **47** ( $K_i$ , 1.6 nM) was similar to that of **45**; 8) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** and 3-phenyl-6-methylquinazoline propanehydrazonothioate **48** ( $K_{iS}$ , 1.8 and 12.3 nM) were more active than 3-benzyl unsubstituted quinazoline propanehydrazonothioate **50** and 3-benzyl-6-methylquinazoline propanehydrazonothioate **51** ( $K_{iS}$ , 20.3 and 23.1 nM).
- (V) Structure-activity relationship analysis for hCA XII inhibition indicated that 1) the CAI activity of 6,7-dimethoxyquinazoline derivatives **14** and **15** ( $K_{iS}$ , 7.6 and 8.9 nM) was higher than that of compounds **12–13** and **16–19** ( $K_{iS}$ , 32.0–61.6 nM); 2) acetamides such as compounds **20**, **22**, **24**, **26**, and **28** ( $K_{iS}$ , 8.8, 12.1, 15.2, 5.2, and 12.2 nM, respectively) were more effective than the corresponding propanamides **21**, **23**, **25**, **27**, and **29** ( $K_{iS}$ , 9.2, 40.6, 28.4, 19.0, and 16.6 nM, respectively); 3) unsubstituted quinazoline derivatives such as compounds **20** and **21** ( $K_{iS}$ , 8.8 and 9.2 nM) were more active than the corresponding 6-substituted and 6,7-dimethoxyquinazoline derivatives such as compounds **22–25** and **27–29** ( $K_{iS}$ , 12.1–40.6 nM); 4) unsubstituted and 6-substituted quinazoline ethylsulfonamide derivatives such as compounds **20–22**, **26–29** ( $K_{iS}$ , 8.8, 9.2, 12.1, 5.2, 19.0, 12.2, and 16.6 nM, respectively) were more active than the corresponding sulfonamide derivatives **10–12**, **16–19** ( $K_{iS}$ , 71.7, 75.5, 32.0, 44.8, 61.6, 50.1, and 42.5 nM, respectively); on the other hand, 6-chloroquinazoline sulfonamide **13** and 6,7-dimethoxyquinazoline sulfonamides **14** and **15** ( $K_{iS}$ , 34.6, 7.6 and 8.9 nM, respectively) were more active than the corresponding ethylsulfonamide derivatives **23**, **24**, and **25** ( $K_{iS}$ , 40.6, 15.2, and 28.4 nM, respectively); 5) propanamides **32** and **36** ( $K_{iS}$ , 46.8 and 58.8 nM) were more active than acetamides **31** and **35** ( $K_{iS}$ , 73.0 and 90.5 nM); 6) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** ( $K_i$ , 8.3 nM) was more active than 3-phenyl 6-substituted quinazoline propanehydrazonothioates **46–49** ( $K_{iS}$ , 9.2–149.4 nM); 7) 3-phenyl unsubstituted quinazoline propanehydrazonothioate **45** ( $K_i$ , 8.3 nM) was more active than 3-benzyl unsubstituted quinazoline propanehydrazonothioate **50** ( $K_i$ , 51.1 nM) and 3-benzyl-6-methylquinazoline propanehydrazonothioate **51** ( $K_i$ , 23.8 nM) was more active than 3-phenyl-6-methylquinazoline propanehydrazonothioate **48** ( $K_i$ , 75.2 nM).

### 3. Conclusion

A new series of substituted mercaptoquinazolinones (**10–29**, **31**, **32**, **35**, **36**, and **45–51**) was synthesized and assessed for *in vitro* CA inhibition as compared to that of AAZ as reference drug. Compounds **14**, **15**, **17**, **19**, **20**, **21**, **24**, **25**, **28**, **29**, **31**, **45**, **47**, **49**, and **51** showed strong inhibitory activity against hCA I ( $K_{iS}$ , 39.4–353.8 nM) as compared with that of AAZ ( $K_i$ , 250.0 nM). The hCA I isoform was moderately inhibited by compounds **12**, **13**, **16**, **18**, **22**, **23**, **26**, **27**, **32**, **35**, **36**, **46**, **48** and **50** with ( $K_{iS}$ , 421.9–700.5 nM) whereas compounds **10** and **11** showed weaker inhibitory activity with ( $K_{iS}$ , 3665.4 and 6472.2 nM, respectively). The hCA II isoform was inhibited by compounds **12**, **13**, **14**, **15**, **20**, **21**, **24**, **25**, **27**, **28**, **29**, **45**, and **47** with  $K_i$  values of 0.73–28.4 nM, which were comparable to that of AAZ, whereas compounds **16**, **17**, **18**, **19**, **22**, **23**, and **26** showed moderate inhibitory action towards hCA II with  $K_i$  values of 32.4–78.3 nM. Compounds **13–29**, **31–32**, and **45–51** exhibited strong hCA IX inhibitory activity with  $K_i$  values ranging between 1.6 and 32.2 nM, which were comparable to that of AAZ. Compounds **14**, **15**, **20**, **21**, **26**, **45**, and **47** were effective inhibitors of hCA XII with  $K_i$  values in the range of 5.2–9.2 nM, which was similar to that of AAZ. Compounds **12**, **13**, **22**, **23**, **24**, **25**, **27**, **28**, **29**, and **51** were less active hCA XII inhibitors with  $K_i$  values of 12.1–40.6 nM whereas compounds **10**, **11**, **16**, **17**, **18**, **19**, **31**, **32**, **35**, **36**, **46**, **48**, **49**, and **50** were weak hCA XII inhibitors ( $K_{iS}$ , 42.5–149.4 nM).

### 4. Materials and methods

#### 4.1. Chemistry

Melting points were recorded on a Barnstead 9100 electrothermal melting point apparatus (UK). IR spectra (KBr) were recorded on a FT-IR Perkin-Elmer spectrometer (Perkin Elmer Inc., MA). Nuclear magnetic resonance ( $^1\text{H}$  and  $^{13}\text{C}$  NMR) spectra were recorded with Bruker 500 or 700 MHz spectrometers (Zurich, Switzerland) using DMSO- $d_6$  as the solvent. Microanalytical data (C, H, and N) were obtained using a Perkin-Elmer 240 analyzer (Perkin Elmer Inc., MA) and agreed with the proposed structures within  $\pm 0.4\%$  of the theoretical values. Mass spectra were recorded on a Varian TQ 320 GC/MS/MS mass spectrometer (Varian, Palo Alto, CA).

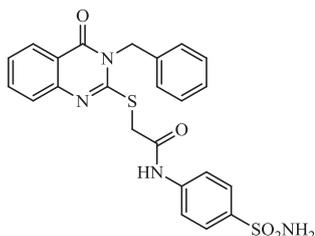
6-Substituted-2-mercapto-3-substituted-4(3*H*)-quinazolinones (**1–5**,

**30** and **37–43**) were prepared by heating of anthranilic acid derivatives with appropriate benzylisothiocyanate or phenylisothiocyanate in ethanol in the presence of triethylamine [30,32,33,35,36,41,42,49,50]. Anilides **6–9** and **33–34** were prepared by stirring an appropriate sulfonamide with 2-chloroacetyl chloride or 2-chloropropanoyl chloride in dichloromethane in the presence of triethylamine [44]. Previously reported protocols were followed to prepare compounds **31–32** (scheme 2; type F compounds), compounds **35–36** [44] (scheme 2; type G compounds), and **compound 44** [51].

#### 4.1.1. General procedure for the synthesis of 2-[(3-benzyl-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)anilides (10–19), (scheme 1; type D compounds)

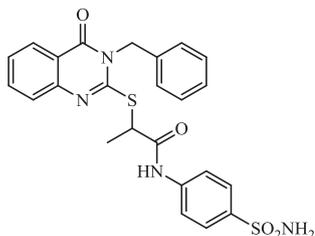
A mixture of appropriate 2-mercapto-3-benzyl-4(3H)-quinazolones (**1–5**) (2 mmol) and 2-chloro-N-(4-sulfamoylphenyl)acetamide (**6**) (2 mmol) or 2-chloro-N-(4-sulfamoylphenyl)propanamides (**7**) (2 mmol) in 15 ml acetone containing anhydrous potassium carbonate (3 mmol, 415 mg) was stirred at room temperature for 8–10 h. The reaction mixture was filtered and the solvent was removed under reduced pressure; the recovered solid was dried and recrystallized from ethanol, (scheme 1; type D compounds).

#### 4.1.2. 2-[(3-Benzyl-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (10)



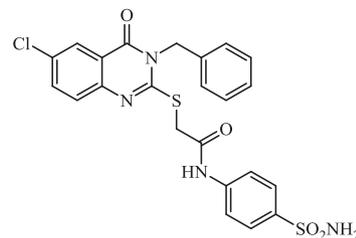
Yield, 96%; mp: > 350 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3397.0, 3271.49 (NH), 1682.0, 1666.0 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.76 (s, 1H), 8.11 (d, 1H,  $J = 4.2$  Hz), 7.79 (d, 5H,  $J = 7.0$  Hz), 7.45 (d, 2H,  $J = 11.2$  Hz), 7.36–7.30 (m, 5H), 7.27 (s, 2H), 5.37 (s, 2H), 4.23 (s, 2H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.75, 161.27, 157.00, 147.18, 142.33, 138.98, 136.04, 135.45, 129.09, 127.98, 127.37, 127.27, 127.14, 126.65, 126.24, 119.18, 119.13, 47.52, 37.50;  $m/z$ : 480

#### 4.1.3. 2-[(3-Benzyl-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (11)



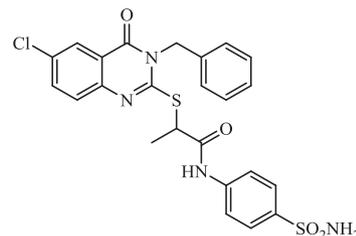
Yield, 95%; mp: 207–209 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3303.64 (br. NH) 1684.37, 1655.0 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.10 (d, 1H,  $J = 10.5$  Hz), 7.78 (dd, 4H,  $J = 13.3$  & 12.6 Hz), 7.68–7.57 (m, 1H), 7.54–7.42 (m, 3H), 7.37–7.33 (m, 2H), 7.29 (d, 3H,  $J = 10.5$  Hz), 7.24–7.21 (m, 1H), 5.33 (dd, 2H,  $J = 16.8$  Hz), 4.76 (dd, 1H,  $J = 9.8$  Hz), 1.60 (d, 3H,  $J = 9.8$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.55, 161.24, 156.63, 147.23, 142.32, 139.17, 136.00, 135.45, 129.10, 128.99, 127.95, 127.24, 127.19, 127.12, 126.70, 126.25, 119.31, 119.24, 112.89, , 47.51, 47.19, , 17.82;  $m/z$ : 494.

#### 4.1.4. 2-[(3-Benzyl-6-chloro-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (12)



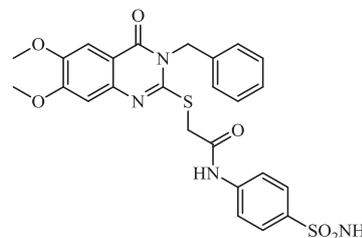
Yield, 95%; mp: 205–207 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3370.62, 3304.00, 3266.73 (NH), 1673.78 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.67 (s, 1H), 8.04 (d, 1H), 7.83 (dd, 1H,  $J = 2.0$ ), 7.79–7.75 (m, 4H), 7.47 (d, 1H,  $J = 9.0$  Hz), 7.37–7.30 (m, 5H), 7.27, s, 2H), 5.36 (s, 2H), 4.22 (s, 2H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.63, 160.36, 157.83, 145.89, 142.28, 139.01, 135.74, 135.57, 130.64, 129.10, 128.45, 128.04, 127.40, 127.26, 126.09, 120.43, 119.15, 47.73, 37.54;  $m/z$ : 514, (M + 2; 516).

#### 4.1.5. 2-[(3-Benzyl-6-chloro-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (13)



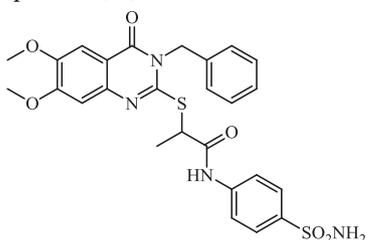
Yield, 93%; mp: 260–262 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3318.23, 3161.08 (NH), 1688.50, 1662.36 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.78 (s, 1H), 8.03 (d, 1H,  $J = 2.5$  Hz), 7.84 (dd, 1H,  $J = 2.0$  Hz), 7.77 (s, 4H), 7.52 (d, 1H,  $J = 8.5$ ), 7.35 (s, 1H), 7.33 (s, 1H), 7.29–7.28 (m, 5H), 5.31 (s, 2H), 4.74–4.70 (m, 1H), 1.60 (d, 3H,  $J = 7.5$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.42, 160.33, 157.42, 145.93, 142.18, 139.16, 135.67, 135.57, 130.73, 129.11, 128.43, 128.03, 127.27, 127.23, 126.08, 120.49, 119.34, 47.72, 47.24, 17.57;  $m/z$ : 528, (M + 2; 530).

#### 4.1.6. 2-[(3-Benzyl-6,7-dimethoxy-4(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (14)



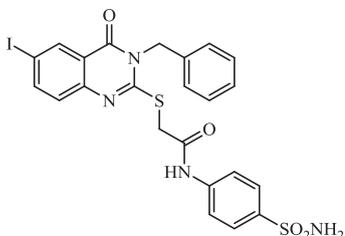
Yield, 92%; mp: > 350 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3331.09, 3254.34, 3105.46 (NH), 1663.84, 1645.42 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.75 (s, 1H), 7.79 (s, 4H), 7.41–7.28 (m, 8H), 6.87 (s, 1H), 5.34 (s, 2H), 4.19 (s, 2H), 3.85 (s, 3H), 3.77 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  167.06, 160.65, 155.38, 154.89, 148.58, 143.48, 142.41, 138.98, 129.06, 127.94, 127.35, 127.28, 119.07, 112.01, 107.06, 106.10, 56.29, 56.19, 47.35, 37.4139;  $m/z$ : 540.

#### 4.1.7. 2-[(3-Benzyl-6,7-dimethoxy-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (15)



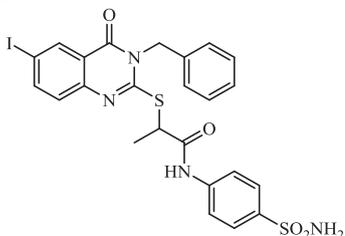
Yield, 92%; mp: 155–157 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3413.43, 3312.0, 3214.0 (NH), 1685.71, 1654.27 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.34 (s, 1H), 7.89 (s, 1H), 7.72 (s, 2H), 7.67 (s, 2H), 7.59–7.31 (m, 8H), 5.37 (s, 2H), 4.77 (s, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.36 (s, 2H), 2.79 (s, 2H), 1.60 (d, 3H,  $J = 6.5$  Hz);  $m/z$ : 554.

#### 4.1.8. 2-[(3-Benzyl-6-iodo-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (16)



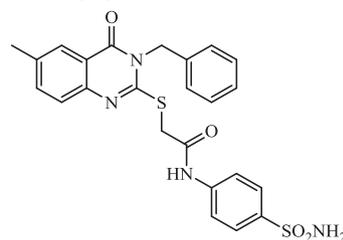
Yield, 93%; mp: 219–220 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3435.67, 3261.88 (NH), 1678.24, 1654.44 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.81 (s, 1H), 8.35 (s, 1H), 8.04–7.33 (m, 13H), 5.34 (s, 2H), 4.23 (s, 2H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.64, 160.02, 157.93, 146.42, 143.75, 142.29, 138.98, 135.76, 135.31, 129.11, 128.40, 128.04, 127.37, 127.27, 121.02, 119.14, 91.15, 47.69, 37.53;  $m/z$ : 605.

#### 4.1.9. 2-[(3-Benzyl-6-iodo-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (17)



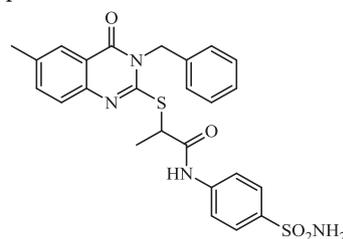
Yield, 91%; mp: 333–334 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3367.27, 3290.0, 3192.12 (NH), 1692.08, 1666.0 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.58 (s, 1H), 8.36 (d, 1H,  $J = 1.5$  Hz), 8.10 (dd, 1H,  $J = 1.5$  Hz), 7.77 (s, 4H), 7.35 (t, 3H,  $J = 5.0$  & 5.5 Hz), 7.30 (s, 1H), 7.28 (d, 3H,  $J = 2.0$  Hz), 7.27 (s, 1H), 5.30 (d, 2H,  $J = 5.5$ ), 4.74–4.71 (m, 1H), 1.60 (d, 3H,  $J = 5.0$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.42, 159.99, 157.53, 146.47, 143.75, 142.22, 139.14, 135.71, 135.30, 129.11, 128.40, 128.01, 127.25, 127.22, 121.09, 119.32, 91.25, 47.68, 47.25, 17.79;  $m/z$ : 620.

#### 4.1.10. 2-[(3-Benzyl-6-methyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)acetamide (18)



Yield, 94%; mp: > 350 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3413.37, 3328.04, 3238.67 (NH), 1685.57, 1670.79 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.76 (s, 1H), 7.90 (s, 1H), 7.78 (s, 4H), 7.60 (d, 1H,  $J = 5.5$  Hz), 7.36–7.28 (m, 8H), 5.36 (s, 2H), 4.21 (s, 2H), 2.40 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.80, 161.25, 155.87, 145.30, 142.34, 138.97, 136.68, 136.33, 136.12, 129.07, 127.95, 127.33, 127.26, 126.41, 126.14, 119.13, 118.92, 47.43, 37.45, 21.21;  $m/z$ : 494.

#### 4.1.11. 2-[(3-Benzyl-6-methyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenyl)propanamide (19)

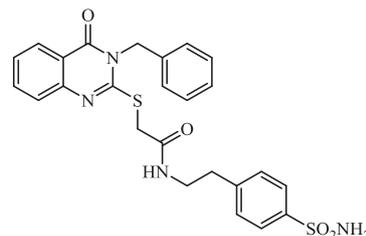


Yield, 94%; mp: 252–254 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3421.06, 3286.33 (NH), 1700.50, 1686.25 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.92 (d, 1H,  $J = 11.5$ ), 7.90 (s, 1H), 7.79 (d, 4H,  $J = 6.5$  Hz), 7.61 (d, 1H,  $J = 7.0$ ), 7.42–7.27 (m, 8H), 5.31 (d, 2H,  $J = 5.0$  Hz), 4.75 (d, 1H,  $J = 5.5$  Hz), 2.41 (s, 3H), 1.58 (d, 3H,  $J = 5.0$  Hz);  $m/z$ : 508.

#### 4.1.12. General procedure for the synthesis of 2-[(3-benzyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)anilides (20–29), (scheme 1; type E compounds).

A mixture of appropriate 2-mercapto-3-benzyl-4(3H)-quinazolinones (**1–5**) (2 mmol) and 2-chloro-*N*-(4-(2-sulfamoylethyl)phenyl)acetamide (2 mmol) (**8**) or 2-chloro-*N*-(4-(2-sulfamoylethyl)phenyl)propanamide (2 mmol) (**9**) in 15 ml acetone containing anhydrous potassium carbonate (3 mmol, 415 mg) was refluxed for 4–6 h. The reaction mixture was cooled down and filtered; the solvent was removed under reduced pressure and the recovered solid was dried and recrystallized from ethanol, (scheme 1; type E compounds).

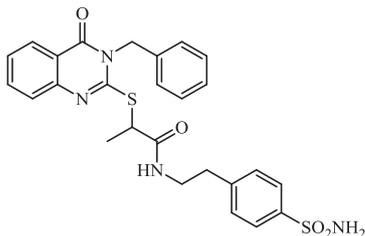
#### 4.1.13. 2-[(3-Benzyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)acetamides (20)



Yield, 92%; mp: 173–175 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3399.76, 3279.85 (NH), 1673.94, 1651.41 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.39 (t, 1H,  $J = 4.9$  & 5.6 Hz), 8.13 (d, 1H,  $J = 7.7$  Hz), 7.83 (t, 1H,  $J = 7.0$  &

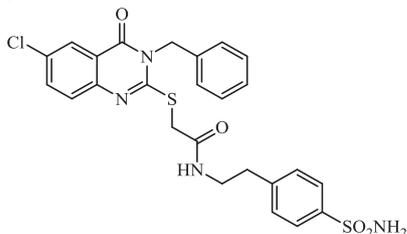
7.7 Hz), 7.73 (d, 2H,  $J = 7.7$  Hz), 7.51 (d, 1H,  $J = 8.4$  Hz), 7.49 (t, 1H,  $J = 7.0$  & 7.7 Hz), 7.38 (d, 2H,  $J = 7.7$  Hz), 7.36 (d, 2H,  $J = 8.4$  Hz), 7.32 (s, 2H), 7.29 (d, 3H,  $J = 7.7$  Hz), 5.35 (s, 2H), 3.97 (s, 2H,  $J = 6.3$  & 7.0 Hz), 3.36 (dd, 2H), 2.79 (t, 2H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  167.06, 161.35, 157.00, 147.23, 143.98, 142.53, 136.05, 135.43, 129.56, 129.09, 127.93, 127.26, 127.11, 126.62, 126.40, 126.16, 119.14, 36.31, 35.16, 47.39;  $m/z$ : 508

4.1.14. 2-[(3-Benzyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)propanamide (21)



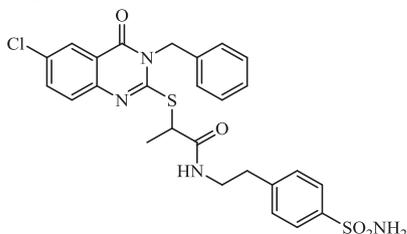
Yield, 88%; mp: 198–199 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3421.76, 3290.98 (NH), 1687.00, 1654.58 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.45 (t, 1H,  $J = 5.6$  Hz), 8.12 (dd, 1H,  $J = 1.4$  Hz), 7.85–7.83 (m, 1H), 7.72 (d, 2H,  $J = 8.4$  Hz), 7.55 (d, 1H,  $J = 7.7$  Hz), 7.50–7.48 (m, 1H), 7.37–7.34 (m, 4H), 7.31 (s, 2H), 7.28 (d, 1H,  $J = 7.7$  Hz), 7.26 (d, 2H,  $J = 7.7$  Hz), 5.34 (d, 1H,  $J = 16.8$  Hz), 5.28 (d, 1H,  $J = 16.8$  Hz), 4.55 (d, 1H,  $J = 7.0$  Hz), 3.34–3.29 (m, 2H), 2.78 (t, 2H,  $J = 7.0$  Hz), 1.47 (d, 3H,  $J = 7.0$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.73, 161.32, 156.72, 147.28, 143.91, 142.55, 136.03, 135.45, 129.59, 129.10, 127.90, 127.12, 126.67, 126.39, 126.10, 119.20, 35.05, 18.77, 47.33, 46.23, 40.47, 18.77;  $m/z$ : 522.1

4.1.15. 2-[(3-Benzyl-6-chloro-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)acetamide (22)



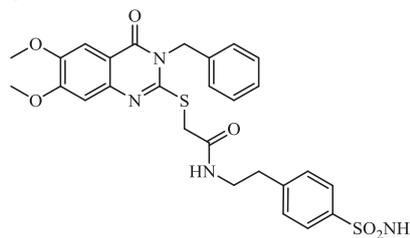
Yield, 89%; mp: 210–212 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3352.77, 3298.39 (NH), 1696.86, 1631.64 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.37 (t, 1H,  $J = 5.5$  Hz), 8.05 (d, 1H,  $J = 2.5$  Hz), 7.86 (dd, 1H,  $J = 2.0$  & 2.5 Hz), 7.73 (s, 1H), 7.72 (s, 1H), 7.52 (d, 1H,  $J = 9.0$  Hz), 7.39–7.28 (m, 9H), 5.34 (s, 2H), 3.96 (s, 2H), 3.34 (t, 2H,  $J = 5.0$  & 7.0 Hz), 2.97 (t, 2H,  $J = 7.0$  & 7.5 Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.93, 160.42, 157.84, 145.94, 143.95, 142.55, 135.76, 135.502, 130.60, 129.54, 129.09, 128.61, 127.99, 127.31, 126.1514, 126.04, 120.39, 47.61, 40.49, 36.38, 35.14;  $m/z$ : 542; (M + 2); 544).

4.1.16. 2-[(3-Benzyl-6-chloro-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)propanamide (23)



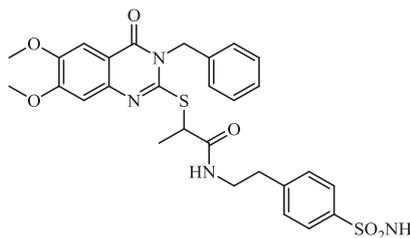
Yield, 87%; mp: 124–126 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3389, 3330.83, 3247.72 (NH), 1684.54, 1654.15 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.44 (s, 1H), 8.05 (d, 1H,  $J = 2.0$  Hz), 8.85 (dd, 1H,  $J = 2.0$  & 2.5 Hz), 7.75 (d, 1H,  $J = 8.0$  Hz), 7.72 (s, 1H), 7.70 (s, 1H), 7.57 (d, 1H,  $J = 9.0$  Hz), 7.40 (d, 1H,  $J = 7.0$ ), 7.37 (s, 1H), 7.35 (d, 1H,  $J = 8.0$  Hz), 7.33 (s, 1H), 7.30 (d, 2H,  $J = 5.5$  Hz), 7.26 (s, 1H), 7.25 (s, 1H), 5.35–5.25 (m, 2H), 5.57–4.51 (m, 1H), 3.32–3.28 (m, 2H), 2.80–2.77 (m, 2H), 1.47–1.45 (m, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.65, 160.40, 157.53, 145.99, 143.88, 142.55, 135.73, 135.49, 130.65, 129.65, 129.57, 129.09, 128.62, 127.96, 127.17, 126.11, 126.09, 126.03, 120.44, 47.56, 46.34, 40.48, 35.03, 18.69;  $m/z$ : 556, (M + 2); 558).

4.1.17. 2-[(3-Benzyl-6,7-dimethoxy-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)acetamide (24)



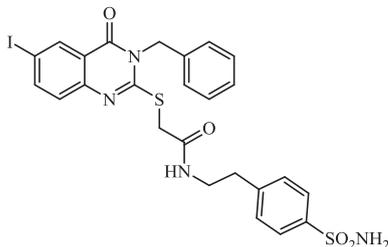
Yield, 90%; mp: 158–159 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3398.21, 3302.51 (NH), 1670.07, 1641.77 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.38 (s, 1H), 7.72 (d, 2H,  $J = 4.0$  Hz), 7.44–7.27 (m, 10H), 7.03 (s, 1H), 5.34 (s, 2H), 3.96 (s, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 3.35 (s, 2H), 2.80 (s, 2H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  167.12, 160.75, 155.45, 154.88, 148.59, 143.94, 143.63, 142.53, 136.28, 129.54, 129.07, 127.88, 127.24, 126.15, 112.00, 107.27, 106.07, 56.45, 56.18, 47.20, 40.72, 36.24, 35.24;  $m/z$ : 568

4.1.18. 2-[(3-Benzyl-6,7-dimethoxy-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)propanamide (25)



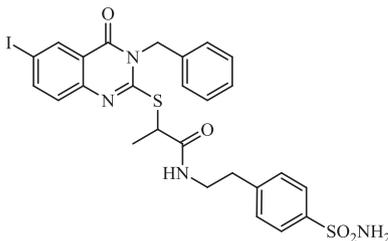
Yield, 88%; mp: 255–257 °C; IR (KBr)  $\nu_{\text{max}}/\text{cm}^{-1}$  3421.03, 3181.47, 3121.62 (NH), 1717.96, 1686.68 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.43 (s, 1H), 7.70 (s, 3H), 7.60–7.30 (m, 9H), 6.95 (s, 1H), 5.66 (s, 2H), 4.54–4.45 (m, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.35 (s, 2H), 2.78 (s, 2H), 1.47 (dd, 3H,  $J = 5.0$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.80, 159.35, 155.85, 155.47, 147.25, 143.88, 143.67, 142.54, 137.28, 135.58, 129.57, 129.07, 127.37, 127.10, 126.10, 108.45, 107.19, 98.25, 56.46, 56.21, 49.09, 40.46, 35.10, 29.53, 18.96;  $m/z$ : 582.

4.1.19. 2-[(3-Benzyl-6-iodo-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)acetamide (26)



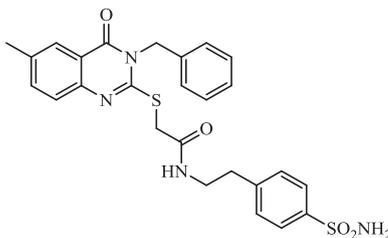
Yield, 89%; mp: 210–212 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3355.02, 3303.79 (NH), 1694.13, 1654.06 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.37 (s, 2H), 8.11 (d, 1H,  $J = 8.0$  Hz), 7.74 (d, 2H,  $J = 7.0$ ), 7.38 (d, 2H,  $J = 7.0$  Hz), 7.34–7.29 (m, 8H), 5.33 (s, 2H), 3.96 (s, 2H), 3.36 (s, 2H), 2.79 (s, 2H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  166.92, 160.07, 157.95, 146.48, 143.95, 143.67, 142.55, 135.78, 135.28, 129.55, 129.08, 128.56, 127.97, 127.29, 126.16, 121.00, 91.05, 47.57, 40.71, 36.40, 35.16;  $m/z$ : 634.

4.1.20. 2-[(3-Benzyl-6-iodo-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)propanamide (27)



Yield, 87%; mp: 225–227 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3340.79, 3250.30, 3116.61 (NH), 1670.39, 1654.31 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.40 (d, 2H,  $J = 22.0$  Hz), 8.11 (d, 1H,  $J = 7.0$  Hz), 7.72 (d, 2H,  $J = 7.5$  Hz), 7.35–7.25 (m, 10H), 5.29 (d, 2H,  $J = 14.5$  Hz), 4.52 (d, 1H,  $J = 6.5$  Hz), 3.38 (s, 2H), 2.78 (s, 2H), 1.45 (d, 3H,  $J = 6.0$  Hz);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.65, 160.05, 157.64, 146.53, 143.89, 143.69, 142.54, 135.74, 135.28, 129.58, 129.09, 128.57, 127.95, 127.14, 126.10, 121.06, 91.10, 47.53, 46.34, 40.47, 35.05, 18.70;  $m/z$ : 648.

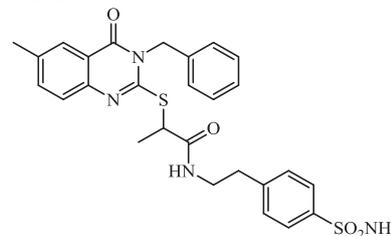
4.1.21. 2-[(3-Benzyl-6-methyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)acetamide (28)



Yield, 88%; mp: 169–170 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3394.93, 3282.68 (NH), 1717.95, 1654.33 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  2.36 (s, 1H), 7.91 (s, 1H), 7.73 (s, 2H), 7.65 (s, 1H), 7.42–7.26 (m, 9H), 5.34 (s, 2H), 3.95 (s, 2H), 3.36 (s, 2H), 2.78 (s, 2H), 2.43 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  167.09, 161.31, 155.90, 145.35, 143.97, 142.55, 136.67, 136.27, 136.14, 129.54, 129.06, 127.90, 127.23,

126.39, 126.29, 126.16, 118.90, 47.30, 40.71, 36.27, 35.17, 21.23;  $m/z$ : 522.

4.1.22. 2-[(3-Benzyl-6-methyl-4-(3H)-quinazolinon-2-yl)thio]-N-(4-sulfamoylphenethyl)propanamide (29)

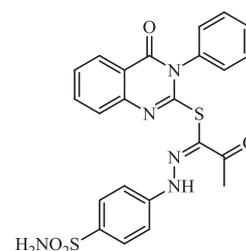


Yield, 87%; mp: 160–162 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3336.28, 3258.37 (NH), 1677.23, 1654.10 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  8.41 (s, 1H), 7.91 (s, 1H), 7.71 (d, 2H,  $J = 6.5$  Hz), 7.65 (d, 1H,  $J = 8.0$  Hz), 7.46 (d, 1H,  $J = 8.5$  Hz), 7.36–7.24 (m, 9H), 5.30 (dd, 2H,  $J = 16.0$  & 16.5 Hz), 4.54 (d, 1H,  $J = 6.5$  Hz), 3.35 (s, 2H), 2.78 (s, 2H), 2.44 (s, 3H), 1.47 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  170.75, 161.28, 155.63, 145.40, 143.91, 142.56, 136.68, 136.34, 136.12, 129.56, 129.06, 127.86, 127.09, 126.39, 126.27, 126.10, 118.95, 47.26, 46.10, 40.50, 35.06, 21.23, 18.72;  $m/z$ : 536.

4.1.23. General procedure for the synthesis of 3-substituted-4-(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (45–51) (scheme 1; type H compounds).

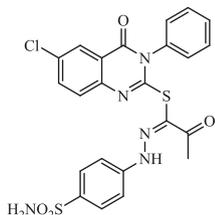
A mixture of appropriate 3-substituted-2-mercapto-4(3H)-quinazolinone (37–43) (2 mmol) and 2-oxo-N-(4-sulfamoylphenyl)propanehydrazonoyl chloride (44) (2 mmol, 550 mg) in 15 ml dioxane containing triethylamine (3 mmol, 303 mg) was refluxed for 4–5 h. The reaction mixture was cooled down and the solvent was removed under reduced pressure; the recovered solid was filtered, washed with water, dried, and recrystallized from ethanol (scheme 1; type H compounds).

4.1.24. 3-Phenyl-4-(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (45)



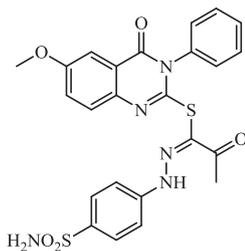
Yield, 85%; mp: 310–312 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3348.71, 3234.84 (NH), 1686.10, 1654.34 (CO);  $^1\text{H}$  NMR (700 MHz, DMSO- $d_6$ ):  $\delta$  10.34 (s, 1H), 8.19 (d, 2H,  $J = 1.4$  & 7.0 Hz), 7.94 (d, 2H,  $J = 7.0$  Hz), 7.84 (d, 1H,  $J = 7.7$  Hz), 7.62–7.57 (m, 1H), 7.54 (s, 2H), 7.35–7.30 (m, 2H), 7.28 (d, 2H,  $J = 4.9$  Hz), 7.23 (t, 2H,  $J = 7.0$  Hz), 7.02 (t, 1H,  $J = 6.3$  & 7.0 Hz), 2.58 (d, 3H,  $J = 8.4$  Hz);  $^{13}\text{C}$  NMR (175 MHz, DMSO- $d_6$ ):  $\delta$  189.95, 165.07, 157.13, 148.58, 148.34, 143.39, 140.86, 139.12, 132.93, 130.29, 129.21, 129.17, 128.73, 127.22, 125.37, 124.00, 119.60, 119.52, 25.50;  $m/z$ : 493.

4.1.25. 6-Chloro-3-phenyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (46)



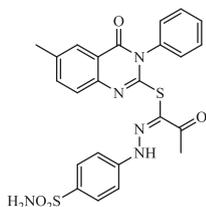
Yield, 83%; mp: 333–335 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3290.00, 3166.99 (NH), 1705.07, 1689.03 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.39 (s, 1H), 8.15 (d, 2H,  $J = 9.0$  Hz), 7.91 (d, 2H,  $J = 8.5$  Hz), 7.82 (d, 1H,  $J = 2.0$ ), 7.66 (dd, 1H,  $J = 2.5$  Hz), 7.51 (s, 2H), 7.39 (d, 1H,  $J = 8.5$  Hz), 7.32 (d, 2H,  $J = 7.5$  Hz), 7.27 (d, 2H,  $J = 7.0$  Hz), 7.05 (d, 1H,  $J = 6.0$  Hz), 2.59 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  189.94, 163.79, 157.65, 149.01, 146.85, 143.15, 140.68, 139.14, 136.98, 132.45, 129.65, 129.24, 129.07, 127.20, 124.24, 124.06, 121.58, 119.76, 25.51;  $m/z$ : 527; (M + 2); 529).

4.1.26. 6-Methoxy-3-phenyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (47)



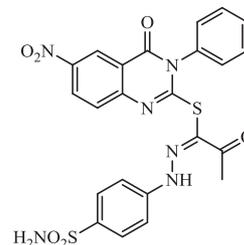
Yield, 82%; mp: 330–332 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3567.95, 3448.06 (NH), 1684.62, 1654.29 (CO);  $^1\text{H}$  NMR (700 MHz, DMSO- $d_6$ ):  $\delta$  10.84 (s, 1H), 7.82 (d, 2H,  $J = 8.4$  Hz), 7.58 (d, 2H,  $J = 8.4$  Hz), 7.55 (s, 1H), 7.47 (t, 2H,  $J = 7.0$  & 7.7 Hz), 7.40 (d, 2H,  $J = 7.7$  Hz), 7.29 (s, 3H), 7.19 (d, 2H,  $J = 8.4$  Hz), 3.87 (s, 3H), 2.59 (s, 3H);  $^{13}\text{C}$  NMR (175 MHz, DMSO- $d_6$ ):  $\delta$  192.77, 161.51, 160.68, 154.75, 147.59, 145.59, 138.38, 135.48, 130.95, 130.13, 129.21, 127.80, 126.87, 126.68, 124.03, 121.05, 120.27, 115.34, 115.25, 114.31, 56.01, 26.30;  $m/z$ : 523.

4.1.27. 6-Methyl-3-phenyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (48)



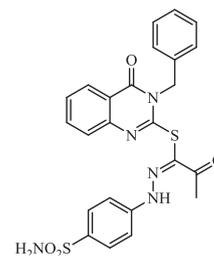
Yield, 81%; mp: > 350 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3358, 3292.43, 3219.00 (NH), 1689.98, 1654.00 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  10.30 (s, 1H), 8.18 (d, 2H,  $J = 11.5$  Hz), 7.94 (d, 2H,  $J = 9.5$  Hz), 7.66 (s, 1H), 7.52 (s, 2H), 7.42 (d, 1H,  $J = 7.5$  Hz), 7.23 (d, 5H,  $J = 6.0$  Hz), 7.01 (s, 1H), 2.58 (s, 3H), 2.38 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ):  $\delta$  190.01, 165.04, 156.91, 148.51, 145.97, 143.38, 140.91, 139.14, 134.67, 133.34, 130.62, 129.20, 128.39, 127.23, 124.00, 119.59, 119.37, 25.48, 20.91;  $m/z$ : 507.

4.1.28. 6-Nitro-3-phenyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (49)



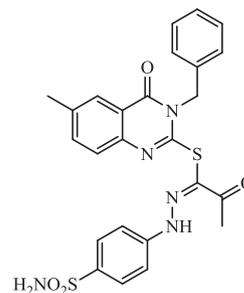
Yield, 80%; mp: > 350 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3318.23, 3161.08 (NH), 1688.50, 1662.36 (CO);  $^1\text{H}$  NMR (700 MHz, DMSO- $d_6$ ):  $\delta$  10.51 (s, 1H), 8.62 (d, 1H,  $J = 2.8$  Hz), 8.45 (dd, 1H,  $J = 2.1$  & 2.8 Hz), 8.14 (d, 2H,  $J = 8.4$  Hz), 7.93 (d, 2H,  $J = 8.4$  Hz), 7.65 (d, 1H,  $J = 9.1$  Hz), 7.54 (s, 2H), 7.28–7.25 (m, 4H), 7.07 (dd, 1H,  $J = 2.8$  & 3.5 Hz), 2.61 (s, 3H);  $^{13}\text{C}$  NMR (175 MHz, DMSO- $d_6$ ):  $\delta$  189.95, 163.16, 158.85, 153.62, 149.34, 143.86, 143.75, 140.51, 138.67, 129.71, 129.30, 127.98, 127.25, 125.73, 124.55, 124.47, 120.69, 119.86, 25.58;  $m/z$ : 538.

4.1.29. 3-Benzyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (50)



Yield, 84%; mp: 343–344 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3313.86, 3197.23 (NH), 1667.39, 1652.26 (CO);  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ ):  $\delta$  11.10 (s, 1H), 8.10 (d, 1H,  $J = 7.0$  Hz), 7.80 (d, 2H,  $J = 8.5$  Hz), 7.75 (s, 1H), 7.58 (d, 2H,  $J = 8.5$  Hz), 7.48–7.28 (m, 9H), 5.50 (s, 2H), 2.59 (s, 3H);  $m/z$ : 507.

4.1.30. 3-Benzyl-6-methyl-4(3H)-quinazolinon-2-yl-2-oxo-N-(4-sulfamoylphenyl)propanehydrazonothioate (51)



Yield, 85%; mp: 240–242 °C; IR (KBr)  $\nu_{\max}/\text{cm}^{-1}$  3384.92, 3256.60, 3182.99 (NH), 1670.47, 1662.11 (CO);  $^1\text{H}$  NMR (700 MHz, DMSO- $d_6$ ):  $\delta$  11.10 (s, 1H), 7.90 (s, 1H), 7.81 (d, 2H,  $J = 9.1$  Hz), 7.59 (s, 1H), 7.58 (d, 1H,  $J = 3.5$ ), 7.56 (d, 1H,  $J = 1.4$  Hz), 7.40 (d, 4H,  $J = 3.5$  Hz), 7.35–7.32 (m, 1H), 7.30 (s, 2H), 7.28 (s, 1H), 5.51 (s, 2H), 2.59 (s, 3H), 2.40 (s, 3H);  $^{13}\text{C}$  NMR (175 MHz, DMSO- $d_6$ ):  $\delta$  192.61, 161.45, 152.95, 145.60, 145.39, 138.47, 136.81, 136.74, 136.15, 129.40, 129.16, 128.09, 127.81, 127.48, 126.59, 126.33, 119.34, 115.34, 48.76, 26.26, 21.21;  $m/z$ : 521.

## 4.2. Carbonic anhydrase inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO<sub>2</sub> hydration activity [52]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na<sub>2</sub>SO<sub>4</sub> (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO<sub>2</sub> hydration reaction for a period of 10–100 s. The CO<sub>2</sub> concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionised 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 prior to assay, in order 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 [52,53]. All CA isoforms were recombinant ones obtained in-house as reported earlier [52–55].

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2019.03.007>.

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