JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY
2020, VOL. 35, NO. 1, 598–609
https://doi.org/10.1080/14756366.2020.1722121

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RESEARCH PAPER

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Exploring structure-activity relationship of S-substituted 2-mercaptoquinazolin-4(3H)-one including 4-ethylbenzenesulfonamides as human carbonic anhydrase inhibitors

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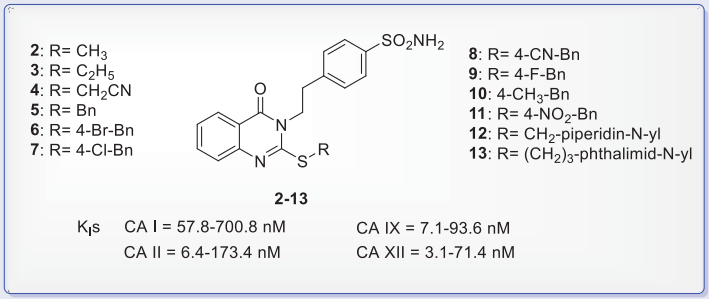
ABSTRACT

Inhibitory action of newly synthesised 4-(2-(2-substituted-thio-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamides compounds **2–13** against human carbonic anhydrase (CA, EC 4.2.1.1) (hCA) isoforms I, II, IX, and XII, was evaluated. hCA I was efficiently inhibited by compounds **2–13** with inhibition constants (K_Is) ranging from 57.8–740.2 nM. Compounds **2**, **3**, **4**, and **12** showed inhibitory action against hCA II with K_Is between 6.4 and 14.2 nM. CA IX exhibited significant sensitivity to inhibition by derivatives **2–13** with K_I values ranging from 7.1 to 93.6 nM. Compounds **2**, **3**, **4**, **8**, **9**, and **12** also exerted potent inhibitory action against hCA XII (K_Is ranging from 3.1 to 20.2 nM). Molecular docking studies for the most potent compounds **2** and **3** were conducted to exhibit the binding mode towards hCA isoforms as a promising step for SAR analyses which showed similar interaction with co-crystallized ligands. As such, a subset of these mercaptoquinazolin-4(3H)-one compounds represented interesting leads for developing new efficient and selective carbonic anhydrase inhibitors (CAIs) for the management of a variety of diseases including glaucoma, epilepsy, arthritis and cancer.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 21 December 2019
Revised 6 January 2020
Accepted 9 January 2020

KEYWORDS
Metalloenzyme; quinazolinone; sulphonamide; inhibition; selectivity; molecular docking study

1. Introduction

Carbonic anhydrases (CAs; EC 4.2.1.1) constitute the superfamily of metalloenzymes catalysing the CO_2 hydration/dehydration reaction. CAs are classified into eight genetically distinct families, named α-, β-, γ-, δ-, ζ-, η-, ϴ-, and ι-CAs[1,2]. 15 α-class isoenzymes have been detected in humans (h) and are sorted into four different subsets depending on their subcellular localisation: CA I, II, III, VII, VIII, X, XI, XIII are cytosolic proteins, CA VA and VB are present in the mitochondrial matrix, CA VI is a secreted enzyme, CA IV is a glycosylphosphatidylinositol (GPI)-anchored protein and CA IX, XII, and XIV are trans-membrane isoforms[1–3]. hCAs are spread in the human body and are implicated in a plethora of essential physiological processes. As a result, critical pathological conditions might occur upon their dysregulated expression and/or abnormal activity[2]. CA II is the most physiologically relevant isoform and is implicated in disorders such as cerebral oedema, glaucoma (such as CA XII), and epilepsy. It is conversely off-target like CA I, when targeting tumours where CA IX and XII are overexpressed and represent validated targets to combat the growth of both primary tumours and metastasis[4]. Significant similarity exists amongst the active site's architecture of hCAs making it difficult to produce inhibitors

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that exhibit net isoform-specificity in action and do not induce side effects as a consequence of CA inhibition[1]. Aromatic sulphonamides constitute the main subset of CAIs developed to date and have been clinically used for decades as diuretic, antiglaucoma, antiobesity, and antiepilepsy medications. The ureidobenzenesulfonamide SLC-0111 (Figure 1), a selective CA IX/XII inhibitor, is currently in Phase II/b clinical trials for the therapy of solid, metastatic tumours[2–6]. A wealth of sulphonamide derivatives have been reported as CAIs[5,7–19], COX-2 inhibitors, or antitumor[13,15,20–22]. The quinazolinone scaffold is also widely used in medicinal chemistry[23–39], such as COX-1/2 inhibitors[23,24] and antitumor[25–29,37–39]. 4–(2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2H)-yl)ethyl)benzenesulfonamide (**1**, Figure 1) showed effective inhibitory activity against a subset of hCA isoforms with subnanomolar inhibition constants[40]. Likewise, a series of 2-((3-benzyl-4-oxo-3,4-dihydroquinazolin-2-yl)thio)-N-(4-sulfamoylphenethyl)amides (**A**, Figure 1) showed nanomolar inhibitory action against a panel of hCAs[39]. Various quinazolin-4-yl-aminobenzenesulfonamide, quinazolin-4-yl-oxy-benzenesulfonamide derivatives (**B**, Figure 1) and 3-(6-iodo-4-oxo-2-thioxo-1,4-dihydroquinazolin-3(2H)-yl)benzenesulfonamide, 3-(2-mercapto-7-fluoro-4(3H)quinazolinon-3-yl)-benzenesulfonamide (**C**, Figure 1) were reported to exert potent inhibitory effect against CA I, II, IX and XII[41,42]. As observed from SAR analysis of the reported **C** derivatives (Figure 1), it is thought to add an ethyl linker between sulphonamide part and quinazoline scaffold and alkylation of free SH

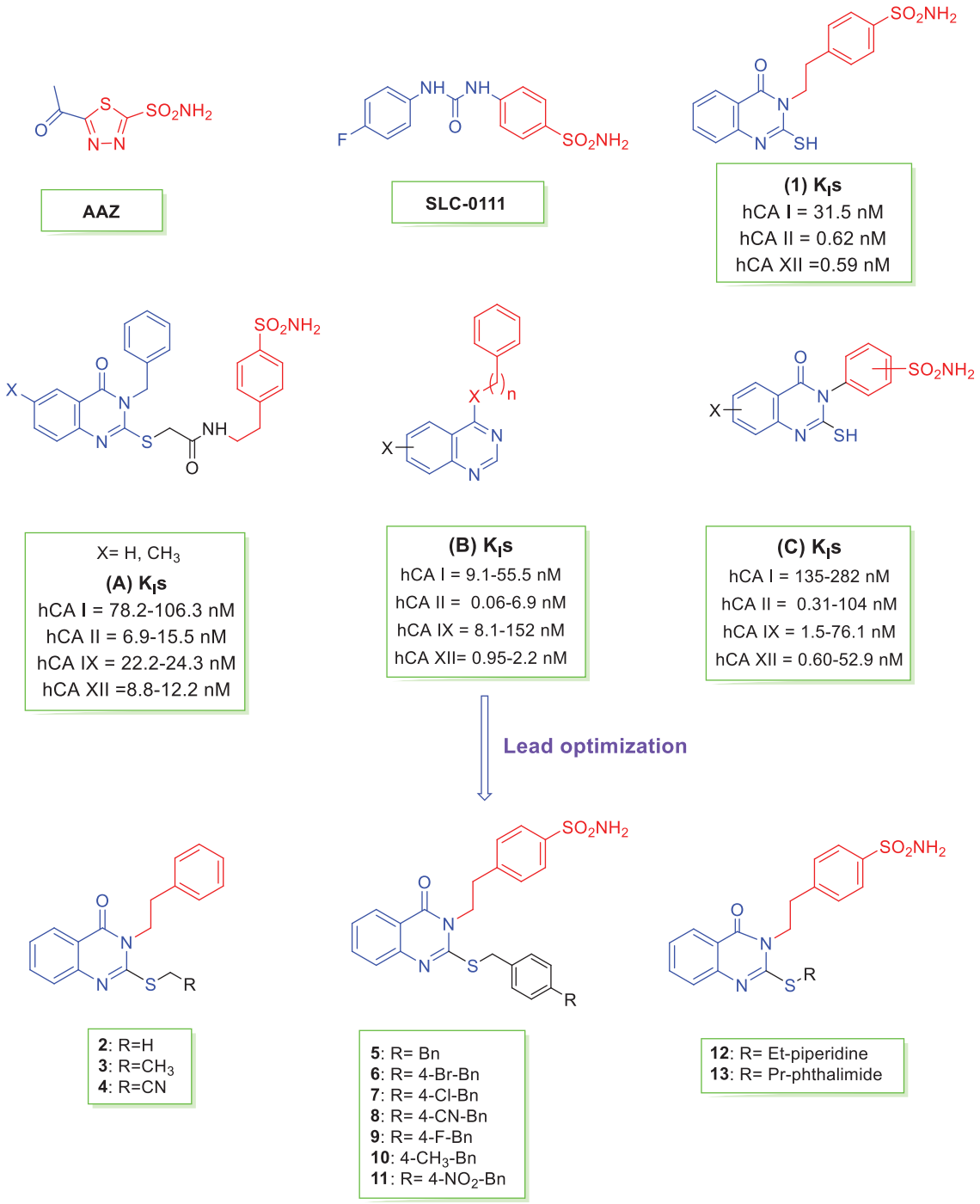


Figure 1. Structures of AAZ, SLC-0111, (**A–C**), and the herein designed quinazoline derivatives (**2–13**) as CAIs.

group to increase the lipophilicity as activity parameter in CA enzyme. Therefore, we report the synthesis of a new series of 4-(2-(2-(substituted-thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide compounds (**2–13**, Figure 1) and evaluated their inhibitory action against four pharmacologically relevant hCA isoforms, I, II, IX, and XII.

2. Materials and methods

2.1. 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., Waltham, MA). Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded with Bruker 500 or 700 MHz spectrometers (Zurich, Switzerland) using DMSO-d_6 as the solvent. Micro-analytical data (C, H, and N) were obtained using a Perkin-Elmer 240 analyser (Perkin Elmer Inc., MA) and agreed with the proposed structures within ±0.4% of the theoretical values. Mass spectra were recorded on a Varian TQ 320 GC/MS/MS mass spectrometer (Varian, Palo Alto, CA).

4-(2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl)ethyl)benzenesulfonamide compound **1** was prepared by heating anthranilic acid with 4-(2-isothiocyanatoethyl)benzenesulfonamide in ethanol in the presence of triethylamine[40].

2.1.1. General procedure for synthesis of 4-(2-(2-(substituted-thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamides (2–13)

A mixture of 4-(2-(4-oxo-2-thioxo-1,4-dihydroquinazolin-3(2H)-yl)ethyl)benzenesulfonamide (**1**) (1 mmol, 361 mg), appropriate halide (1 mmol) and potassium carbonate (3 mmol, 415 mg) in 7 ml acetone were stirred at room temperature for 8–13 h. The reaction mixture was filtered and the prepared solid was washed with water and dried.

2.1.1.1. 4-(2-(2-(Methylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (2).

M.P. 255–257 °C, 91% yield; IR (KBr, cm^{-1}) ν: 3285, 3237 (NH), 1655 (C=O), 1345, 1159 (O=S=O); ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.09 (d, 1H, J = 7.78 Hz), 7.89 (d, 3H, J = 8.17 Hz), 7.57 (d, 1H, J = 8.14), 7.48 (dd, 3H, J = 8.24 & 7.70 Hz), 7.35 (s, 2H), 4.27 (t, 2H, J = 15.99 Hz), 3.07 (t, 2H, J = 16.20 Hz), 2.65 (s, 3H); ^{13}C NMR (176 MHz, DMSO-d_6): δ 160.89, 157.38, 147.29, 143.07, 142.41, 135.21, 129.65, 126.89, 126.48, 126.43, 126.39, 119.16, 45.42, 33.58, 15.11; MS; *m/z* (375).

2.1.1.2. 4-(2-(2-(Ethylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (3).

M.P. 193–195 °C, 94% yield; ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.09 (d, 1H, J = 7.89 Hz), 7.80 (d, 3H, J = 8.12 Hz), 7.55 (d, 1H, J = 8.12 Hz), 7.47 (d, 3H, J = 8.19 Hz), 7.34 (s, 2H), 4.25 (t, 2H, J = 15.96 Hz), 3.27 (q, 2H, J = 7.30 Hz), 3.06 (t, 2H, J = 15.96 Hz), 1.36 (d, 3H, J = 7.32 Hz); ^{13}C NMR (176 MHz, DMSO-d_6): δ 160.97, 156.68, 147.33, 143.07, 142.41, 135.22, 129.66, 126.87, 126.46, 126.42, 126.38, 119.22, 45.38, 33.58, 26.49, 14.47; MS: *m/z* 389.

2.1.1.3. 4-(2-(2-((Cyanomethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (4).

M.P. 169–170 °C, 90% yield; IR (KBr, cm^{-1}) ν: 3287, 3232 (NH), 2193 (CN), 1700 (C=O), 1333, 1155 (O=S=O); ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.13 (d, 1H, J = 7.86 Hz), 7.86 (t, 1H, J = 7.61 Hz), 7.80 (d, 2H, J = 7.78 Hz), 7.63 (d, 1H, J = 8.11 Hz), 7.53 (t, 1H, J = 7.51 Hz), 7.49 (d, 2H, J = 7.82 Hz), 7.35 (s, 2H), 4.42 (s, 2H), 4.24 (t, 2H, J = 15.93 Hz), 3.08 (t, 2H, J = 15.95 Hz); ^{13}C NMR (176 MHz, DMSO-d_6): δ 160.76, 154.07, 146.88, 143.14, 142.19, 135.48, 129.69, 127.11, 127.01, 126.62, 126.50, 119.41, 117.91, 45.75, 33.62, 18.20; MS: *m/z* 400.

2.1.1.4. 4-(2-(2-(Benzylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (5).

M.P. 251–252 °C, 95% yield; IR (KBr, cm^{-1}) ν: 3289, 3233 (NH), 1686 (C=O), 1331, 1158 (O=S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.09 (d, 1H, J = 7.40 Hz), 7.28 (s, 1H), 7.78 (d, 2H, J = 6.80 Hz), 7.64 (d, 1H, J = 7.50 Hz), 7.53 (d, 2H, J = 6.10 Hz), 7.48 (d, 1H, J = 7.45 Hz), 7.44 (d, 2H, J = 6.75 Hz), 7.35 (s, 4H), 7.28 (d, 1H, J = 6.55 Hz), 4.57 (s, 2H), 4.25 (s, 2H), 3.04 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.93, 156.26, 147.18, 143.06, 142.33, 137.12, 135.28, 129.88, 129.65, 128.95, 127.90, 126.90, 126.52, 126.45, 45.41, 36.06, 33.61; MS: 451.

2.1.1.5. 4-(2-(2-((4-Bromobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (6).

M.P. 225–227 °C, 92% yield; IR (KBr, cm^{-1}) ν: 3291, 3226 (NH), 1645 (C=O), 1340, 1155 (O=S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (d, 1H, J = 7.26 Hz), 7.78 (t, 3H, J = 10. 05 & 7.10 Hz), 7.63 (d, 1H, J = 7.37 Hz), 7.48 (dd, 7H, J = 11.70 & 19.80 Hz), 7.36 (s, 2H), 4.52 (s, 2H), 4.23 (s, 2H), 3.04 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.91, 156.02, 147.11, 143.07, 142.34, 137.00, 135.28, 132.07, 131.74, 129.67, 126.90, 126.56, 126.46, 120.97, 119.27, 45.48, 35.21, 33.61; MS: *m/z* 530 and 532.

2.1.1.6. 4-(2-(2-((4-Chlorobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (7).

M.P. 244–245 °C, 95% yield; IR (KBr, cm^{-1}) ν: 3282, 3224 (NH), 1655 (C=O), 1333, 1155 (O=S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.04 (s, 1H), 7.78 (d, 3H, J = 6.51 Hz), 7.63 (s, 1H), 7.54 (s, 2H), 7.45 (s, 3H), 7.38 (s, 2H), 7.35 (s, 2H), 4.54 (s, 2H), 4.23 (s, 2H), 3.04 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.53, 156.04, 147.12, 142.36, 136.56, 135.30, 132.44, 131.71, 129.68, 128.81, 126.89, 126.58, 126.45, 119.26, 116.12, 45.47, 35.15, 33.60; MS; *m/z* 486 and 487.

2.1.1.7. 4-(2-(2-((4-Cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (8).

M.P. 265–267 °C, 95% yield; IR (KBr, cm^{-1}) ν: 3280, 3222 (NH), 2189 (CN), 1654 (C=O), 1334, 1155 (O=S=O); ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.08 (dd, 1H, J = 7.91 & 1.19 Hz), 7.82 (dd, 1H, J = 7.00 & 1.40 Hz), 7.79 (t, 4H, J = 17.70 Hz), 7.73 (d, 2H, J = 8.28 Hz), 7.62 (d, 1H, J = 8.19 Hz), 7.48 (d, 1H, J = 7.28 Hz), 7.45 (d, 2H, J = 8.19 Hz), 7.37 (s, 2H), 4.62 (s, 2H), 4.24 (t, 2H, J = 15.90 Hz), 3.05 (t, 2H, J = 15.87 Hz); ^{13}C NMR (176 MHz, $CDCl_3$/DMSO-d_6): δ 160.90, 155.79, 147.05, 143.83, 143.07, 142.35, 135.31, 132.70, 130.81, 129.70, 126.91, 126.63, 126.46, 126.41, 119.28, 119.23, 110.45, 45.58, 35.31, 33.60; MS: *m/z* 476.

2.1.1.8. 4-(2-(2-((4-Fluorobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (9).

M.P. 267–269 °C, 93% yield; IR (KBr, cm^{-1}) ν: 3285, 3220 (NH), 1663 (C=O), 1336, 1157 (O=S=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (d, 1H, J = 7.32 Hz), 7.78 (t, 3H, J = 12.70 & 6.69 Hz), 7.64 (d, 1H, J = 7.68 Hz), 7.57 (s, 2H), 7.44 (t, 3H, J = 9.65 & 6.56 Hz), 7.35 (s, 2H), 7.16 (d, 2H, J = 8.25 Hz), 4.55 (s, 2H), 4.23 (s, 2H), 3.04 (s, 2H);^{13}C NMR (125 MHz, DMSO-d_6): δ 160.92, 156.16, 147.15, 143.06, 142.35, 135.28, 133.55, 131.90, 131.83, 129.66, 126.90, 126.54, 126.45, 119.28, 115.76, 115.59, 45.44, 35.16, 33.60; MS: *m/z* 469.

2.1.1.9. 4-(2-(2-((4-Methylbenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (10).

M.P. 227–228 °C, 92% yield; IR (KBr,

cm^{-1}) ν: 3287, 3221 (NH), 1656 (C=O), 1334, 1156 (O=S=O); ^{1}H NMR (700 MHz, DMSO-d$_6$): δ 8.09 (d, 1H, J = 7.84 Hz), 7.81 (d, 1H, J = 6.79 Hz), 7.78 (d, 2H, J = 7.98 Hz), 7.64 (d, 1H, J = 8.12 Hz), 7.47 (t, 1H, J = 8.11 Hz), 7.44 (d, 2H, J = 7.91 Hz), 7.40 (d, 2H, J = 7.70 Hz), 7.35 (s, 2H), 7.14 (d, 2H, J = 7.70 Hz), 4.52 (s, 2H), 4.23 (t, 2H, J = 15.89 Hz), 3.03 (t, 2H, J = 15.92 Hz), 2.27 (s, 3H); ^{13}C NMR (176 MHz, DMSO-d$_6$): δ 160.91, 156.02, 147.11, 143.07, 142.34, 137.00, 135.28, 132.07, 131.74, 129.67, 126.90, 126.56, 126.46, 120.97, 119.27, 45.48, 35.21, 33.61; MS: *m/z* 465.

2.1.1.10. 4-(2-(2-((4-Nitrobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (11). M.P. 210–211 °C, 90% yield; IR (KBr, cm^{-1}) ν: 3279, 3226 (NH), 1661 (C=O), 1326, 1161 (O=S=O); ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 8.19 (d, 2H, J = 6.56 Hz), 8.08 (d, 1H, J = 6.10 Hz), 7.80 (t, 5H, J = 8.30 & 8.26 Hz), 7.64b (d, 1H, J = 5.55 Hz), 7.46 (s, 3H), 7.36 (s, 2H), 4.68 (s, 2H), 4.25 (s, 2H), 3.05 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d$_6$): δ 160.90, 155.71, 147.06, 146.06, 143.07, 142.36, 135.31, 131.08, 129.71, 126.89, 126.65, 126.45, 123.86, 119.27, 45.60, 35.02, 33.60; MS: *m/z* 496.

2.1.1.11. 4-(2-(4-Oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (12). M.P. 198–199 °C, 92% yield; ^{1}H NMR (700 MHz, DMSO-d$_6$): δ 7.08 (dd, 1H, J = 9.18 & 6.64 Hz), 7.79 (d, 3H, J = 8.22 Hz), 7.53 (d, 1H, J = 8.05 Hz), 7.47 (dd, 3H, J = 8.26 & 6.70 Hz), 7.36 (s, 2H), 4.26 (t, 2H, J = 16.04 Hz), 3.41 (t, 2H, J = 14.25 Hz), 3.07 (t, 2H, J = 16.01 Hz), 2.65 (s, 2H), 2.51 (t, 2H, J = 3.55 Hz), 2.48 (d, 2H, J = 6.54 Hz), 1.50 (d, 4H, J = 4.35 Hz), 1.38 (s, 2H); ^{13}C NMR (176 MHz, DMSO-d$_6$): δ 160.95, 156.81, 147.27, 143.08, 142.43, 135.24, 129.65, 126.88, 126.46, 126.38, 126.34, 119.19, 57.48, 54.15, 45.38, 33.59, 31.17, 29.37, 25.93, 24.36; MS: *m/z* 472.

2.1.1.12. 4-(2-(2-((3-(1,3-Dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (13). M.P. 205–207 °C, 91% yield; IR (KBr, cm^{-1}) ν: 3277, 3228 (NH), 1701 (C=O), 1328, 1159 (O=S=O); ^{1}H NMR (700 MHz, DMSO-d$_6$): δ 8.05 (d, 1H, J = 7.84 Hz), 7.88–7.83 (m, 4H), 7.79 (d, 2H, J = 7.91 Hz), 7.68 (p, 1H, J = 8.15, 14.14 Hz), 7.54 (d, 2H, J = 8.26 Hz), 7.42 (p, 1H, J = 17.15 & 7.03 Hz), 7.36 (s, 2H), 7.20 (d, 1H, J = 8.12 Hz), 4.21 (t, 2H, J = 15.86 Hz), 3.75 (t, 2H, J = 6.49 Hz), 3.31 (t, 2H, J = 7.18 Hz), 3.03 (t, 2H, J = 16.07 Hz), 2.10–2.06 (m, 2H); ^{13}C NMR (176 MHz, DMSO-d$_6$): δ 168.52, 160.88, 156.36, 147.11, 143.05, 142.37, 135.05, 134.87, 132.14, 129.62, 127.74, 126.84, 126.47, 126.36, 126.14, 123.51, 119.17, 45.28, 37.00, 33.54, 29.22, 28.21; MS: *m/z* 547.

2.2. CA inhibition

The hCA I, II, IX, and XII isoenzyme inhibition assay was performed according to the reported method using SX.18 MV-R stopped-flow instrument (Applied Photophysics, Oxford, UK)[43–45]. All CA isoforms were recombinant isoforms obtained in-house, as reported earlier[46,47].

2.3. Molecular docking method

Molecular docking was carried out according to the previously reported methods[24,28,29,37–39,48–52] using MOE 2008.10 from the Chemical Computing Group Inc[53].

3. Results and discussion

3.1. Chemistry

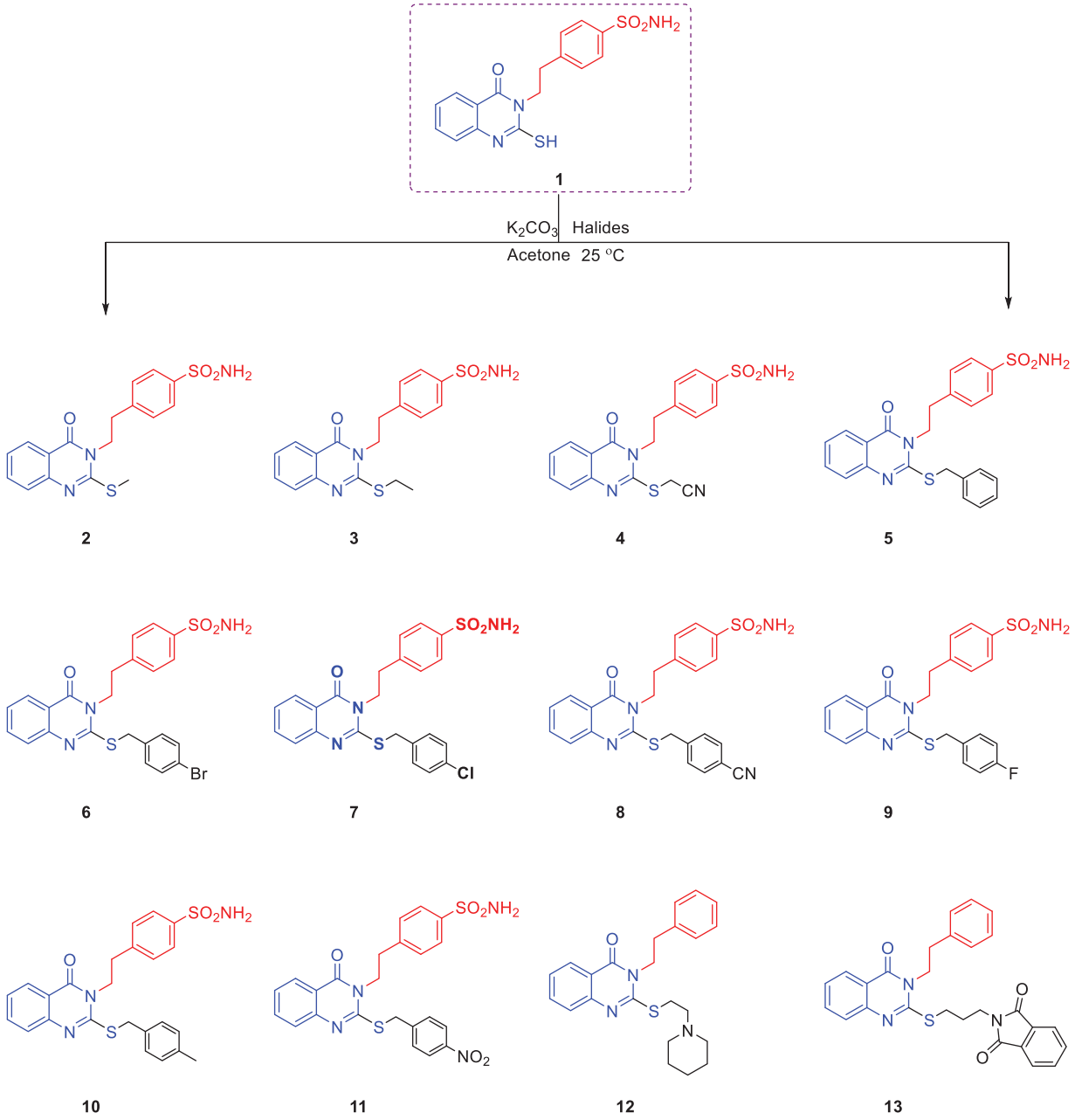
4-(2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2H)-yl)ethyl)benzenesulfonamide (**1**) was obtained through the reaction between anthranilic acid, 4-(2-isothiocyanatoethyl)benzenesulfonamide and triethylamine in ethanol[40,54] (Scheme 1). Its yield was 93%. Stirring of compound **1** with potassium carbonate in acetone and different alkyl-halides or aralkyl-halides produced the corresponding 4-(2-(2-(substituted-thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamides **2–13** with 90–95% yield. Various spectral analyses were performed to confirm the structures of compounds **2–13**. The formation of target compounds was assessed by the disappearance of thioamide proton (NH–C = S) at 13.03 ppm in ^{1}H NMR and thione moiety (NH–C = S) at 175.29 ppm in the ^{13}C NMR spectra, together with presence of the new thio-substituted moieties (S–R), that were confirmed by ^{1}H NMR and ^{13}C NMR spectra.

3.2. CA inhibitory activity

The CAI activity of newly produced 4-(2-(2-(substituted-thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamides **2-13** against hCA I, II, IV, and IX isoforms was measured by a Stopped-Flow kinetic assay[48] and compared to acetazolamide (AAZ), a standard sulphonamide inhibitor (Table 1, Figure 2). The hCA I was effectively inhibited by compounds **2–13** with inhibition constant (K_I) values in the range of 57.8–740.2 nM, (AAZ: K_I, 250.0 nM). Compounds **2**, **3**, **4**, and **12** showed to be potent hCA II inhibitors, with K_I values between 6.4 and 14.2 nM, which were greater than or nearly identical to that of AAZ (K_I, 12.0 nM). Compounds **7–11** and **13** showed modest hCA II inhibitory activity with K_Is ranging between 66.5 and 86.6 nM, whereas compounds **5** and **6** showed a weak inhibitory activity with K_Is values of 115.3 and 173.4 nM, respectively. Compounds **2–13** displayed potent hCA IX inhibitory activity with K_I values ranging from 7.1 to 93.6 nM (AAZ K_I, 25.0 nM). Quinazoline derivatives **2**, **3**, **4**, **8**, **9**, and **12** possessed potent hCA XII inhibitory activities with K_I values ranging between 3.1–20.2 nM (AAZ K_I, 5.7 nM). On the other hand, compounds **5**, **10**, and **11** exerted moderate hCA XII inhibitory activities with K_I values between 25.6–38.4 nM, whereas compounds **6**, **7**, and **13** had weak hCA XII inhibitory activities with K_I values in the range of 57.6–71.4 nM (Table 1, Figure 2).

The following structure-activity relationship (SAR) can be drawn on the basis of the inhibition data shown in Table 1.

I. SAR analysis for hCA I inhibition indicated that: (1) 2-(aliphatic-thio)quinazolin-4(3H)-one like compounds **2–4** (K_I values between 57.8 and 85.5 nM) were more active than the corresponding 2-(benzylthio)quinazolin-4(3H)-ones (K_I values between 229.4 and 740.2 nM) **5–11**, 4–(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) (K_I, 532.2 nM) and 4–(2-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**13**) (K_I, 320.5 nM); (2) 2-(benzylthio)quinazolin-4(3H)-one (**5**) (K_I, 229.4 nM) was more potent than the corresponding 4-substituted-2-(benzylthio)quinazolin-4(3H)-ones such as compounds **6–11** and **13** (K_I values ranging from 256.8 to 740.2 nM); (3) 4-(2-(2-((4-cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**8**) (K_I, 256.8 nM) was more effective than other 2-((4-substituted-benzyl)thio)-quinazolin-4(3H)-ones **6**, **7**, **9**, **10** and **11** (K_I values between 370.1 and 740.2 nM); (4) 4-(2-(2-(ethylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**2**) with K_I of 57.8 nM was



Scheme 1. Synthesis of 4–(2–(2-(substituted-thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamides **2–13.**

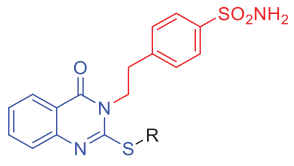
more potent than 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)-quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) with K_I of 532.2 nM; (5) hCA I inhibitory activity (K_I, 85.5 nM) of 4-(2-(2-((cyanomethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**4**) was more potent than 4-(2-(2-((4-cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**8**) with K_I of 256.8 nM; (6) substitution of benzyl group of compound **5** (K_I of 229.4 nM) by propylphthalimide moiety produced compound **13** with significant decrease of CA I activity (K_I, 320.5 nM).

II. SAR analysis for hCA II inhibition indicated that: (1) 2-(aliphatic-thio)quinazolin-4(3H)-one compounds such as compounds **2**, **3**, **4** and **12** (K_I values ranging from 6.4 to 14.2 nM) were more active than the corresponding 2-(benzylthio)quinazolin-4(3H)-ones **5–11** and 4-(2-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**13**) with K_I values between 66.5 and 173.4 nM; (2) the introduction of electron withdrawing groups such as 4-Cl, 4-CN, 4-F, 4-NO_2 or electron donating group such as 4-CH_3 at the benzyl moiety of compound **5** (K_I, 115.3 nM) produced compounds **7–11** with moderate increase in the CA I activity (K_I values ranging between 66.5 and 84.2 nM); (3) 4-(2-(2-(ethylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**2**) with K_I, 11.6 nM was more potent than 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) with K_I, 14.2 nM; (4) For hCA I, inhibitory activity (K_I, 13.5 nM) of 4-(2-(2-((cyanomethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**4**) was more potent than 4-(2-(2-((4-cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**8**) with K_I, 74.1 nM; (5) substitution of benzyl group in compound **5** (K_I, 115.3 nM) by propylphthalimide moiety produced compound **13** with significant increase in the CA activity (K_I, 86.6 nM).

III. SAR analysis for hCA IX inhibition indicated that: (1) 2-(aliphatic-thio)quinazolin-4(3H)-one such as compounds **2–4** (K_I values ranging between 7.1 and 12.6 nM) were more active than the corresponding 2-(benzylthio)quinazolin-4(3H)-one **5–11**, 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) and 4-(2-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**13**) (K_I values in the range of 19.3–93.6 nM); (2) the introduction of electron withdrawing groups such as 4-Cl, 4-F, or 4-NO_2 at the benzyl moiety in compound **5** (K_I, 50.7 nM) produced compounds **7**, **9**, and **11** with significant increase in the CA I activity (K_I values ranging between 19.3 and 34.2 nM); (3) 4-(2-(2-(ethylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**2**) with K_I of 7.1 nM was more potent than 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) with K_I value of 22.2 nM; (4) hCA I inhibitory activity of 4-(2-(2-((cyanomethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**4**) (K_I, 12.6 nM) was more potent than 4-(2-(2-((4-cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**8**) with K_I value of 47.3 nM; (5) substitution of benzyl group in compound **5** (K_I, 50.7 nM) by propylphthalimide moiety produced compound **13** with significant increase in the CA activity (K_I, 93.6 nM).

IV. SAR analysis for hCA XII inhibition indicated that: (1) 2-(aliphatic-thio)quinazolin-4(3H)-one compounds such as compounds **2-4** (K_I values between 3.1 and 8.6 nM) were more active than the corresponding 2-(benzylthio)quinazolin-4(3H)-one **5–11**, 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) and 4-(2-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**13**) (K_I values ranging from 17.2 to 71.4 nM); (2) the introduction of electron withdrawing groups such as 4-CN, 4-F, 4-NO_2 or electron donating group such as 4-CH_3 at the benzyl moiety in compound **5** (K_I, 115.3 nM) produced compounds **8–11** which significantly increased the CAI activity (K_I, 17.2–28.2 nM), while introduction of 4-Br and 4-Cl groups at the benzyl moiety in compound **5** gave compounds **6** and **7** which significantly increased the CAI activity (K_I values, 57.0 and 65.6 nM, respectively); 3) 4-(2-(2-(ethylthio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**2**) with K_I of 3.9 nM was more potent than 4-(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) with K_I of 20.2 nM; (4) hCA XII inhibitory activity of 4-(2-(2-((cyanomethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**4**) (K_I, 8.6 nM) was more potent than 4-(2-(2-((4-cyanobenzyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**8**) with K_I of 14.6 nM; (5) substitution of benzyl group in compound **5** (K_I of 38.4 nM) by propylphthalimide moiety

Table 1. Inhibition constant values of 2-ethylquinazoline derivatives **2–13** and standard sulphonamide inhibitor acetazolamide (AAZ) against human CA isoforms hCA I, II, IX, and XII as determined by a stopped flow, CO_2 hydrase assay[48].



		K_I (nM)[a]			
Cmpound	*R*	hCA I	hCA II	hCA IX	hCA XII
1	H	31.5	0.62		0.59
2	CH_3	67.0	6.4	9.5	3.1
3	C_2H_5	57.8	11.6	7.1	3.9
4	CH_2CN	85.5	13.5	12.6	8.6
5	Bn	229.4	115.3	50.7	38.4
6	4-Br-Bn	700.8	173.4	64.1	57.0
7	4-Cl-Bn	541.9	84.2	34.2	65.6
8	4-CN-Bn	256.8	74.1	47.3	17.6
9	4-F-Bn	370.1	69.3	28.9	17.2
10	4-CH_3-Bn	578.7	66.5	57.1	28.2
11	4-NO_2-Bn	740.2	81.7	19.3	25.6
12	CH_2CH_2-piperidin-N-yl	532.2	14.2	22.4	20.2
13	$(CH_2)_3$-phthalimid-N-yl	320.5	86.6	93.6	71.4
AAZ		250.0	12.0	25.0	5.7

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

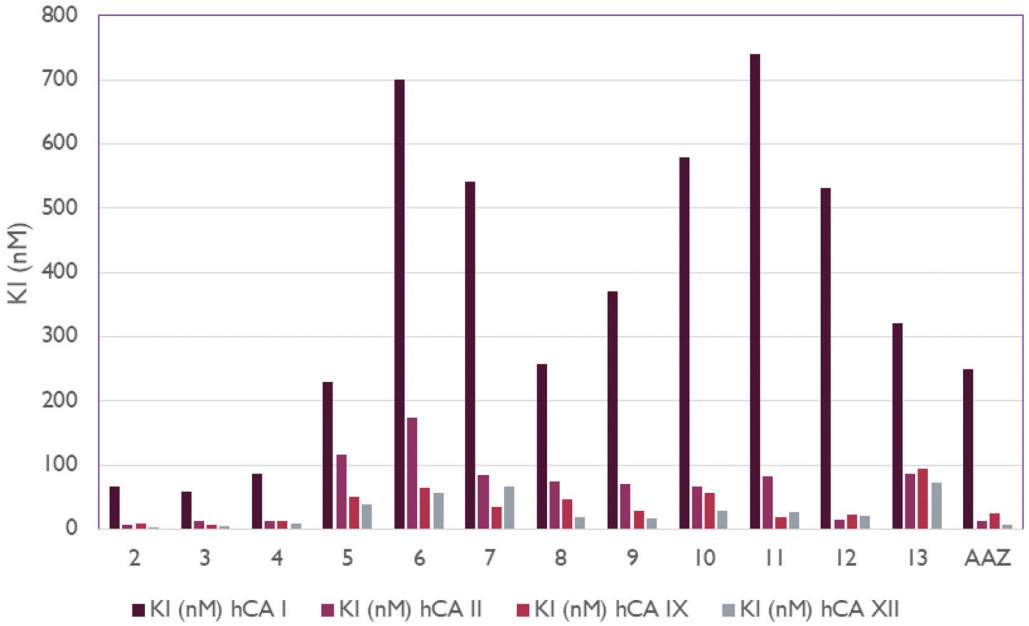


Figure 2. Carbonic anhydrase inhibition of 2-substituted-mercapto-4(3H|)-quinazolinone derivatives **2–13**.

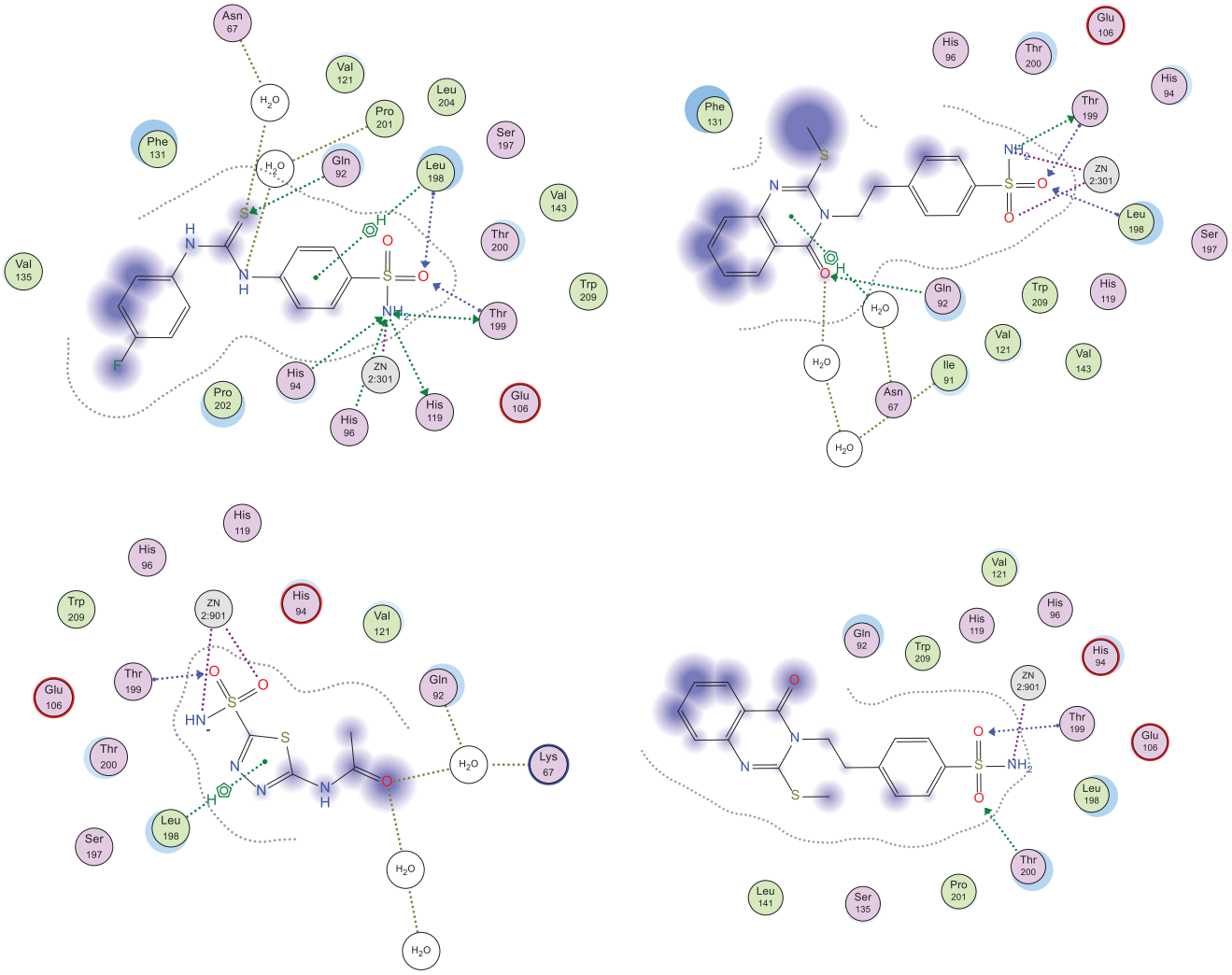


Figure 3. Docking modes of compound **2** in the binding pockets of CA isoenzymes II and XII. Interactions between the protein (PDB IDs: 5ULN and 1JD0). Predicted binding modes of co-crystallized inhibitor (upper left panel) and compound **2** (upper right panel) with hCA-II target as well as co-crystallized inhibitor (lower left panel) and compound **2** (lower right panel) with hCA-XII target.

produced compound **13** with a significant decrease in CAI activity (K_I, 71.4 nM).

3.3. Molecular docking studies

The docking simulations between the hCAs targets, and the most active compounds such as **2** and **3**, as well as least active compound such as **6** compared with the prototype **1** lead compound were performed using MOE Suite[53].

3.3.1. Docking of compounds 2, 3, and 6 with hCA isoenzymes

The most active methyl derivative **2** was docked with the binding pockets of the hCA isoforms II and XII, utilising the different protein crystal structures; 5ULN and 1JD0 downloaded from Protein Data Bank store[55,56]. As shown in Figure 3, and Table 2, the results suggested that the compound **2** displayed similar patterns to the co-crystallized ligands. Firstly, the docking pose of compound **2** in the complex with isoform II formed bidentate chelate with SO_2NH_2 fragment with zinc metal at a distance range of 2.45–2.01 Å. The sulphonyl part was stabilised by three strong hydrogen bonds through the residues, Thr199 and Leu198 at average bond distance of 2.35–2.22 Å. Moreover, the carbonyl moiety of 4(3H)quinazolinone was stabilised by direct and indirect hydrogen bonding between water and polar residues Gln92 and Asn67 at distance of 2.25 Å as co-crystallized inhibitor S-atom does. In addition, hydrophobic interactions were experienced with methylthioether fragment through Phe131 residue. Secondly, the docking poses of compound **2** in the complex with isoform XII exhibited interactions like the co-crystallized inhibitor especially the SO_2NH_2 part with Zinc metal and stabilising Thr199 and Thr200 residues. In addition, hydrophobic interactions were experienced with methylthioether fragment through Leu141 residue. The compound **3** was docked with the binding pockets in the hCA isoforms I and IX, utilising the different protein crystal structures; 4WR7 and 5FL4 downloaded from Protein Data Bank store[57,58]. As shown in Figure 4 and Table 2, the results suggested that the compound **3** interacted with both active sites in a similar fashion to the co-crystallized ligands in the pockets. Firstly, the docking pose of compound **3** in the complex with isoform I, consisted of a long, narrow tunnel, leading to a cavity that contained the catalytic Zn^{2+} ion chelated with SO_2NH_2 fragment (2.45–2.01 Å) and a sulphonyl part stabilised by two strong hydrogen bonds through the residues, Thr199 and His200 (2.38-2.41 Å). Moreover, different hydrophobic aromatic interactions were also formed with ethylthioether, phenethyl, and 4(3H)quinazolinone moieties through pockets of Leu131, Leu198, Pro202, Leu141, Trp209, Ala135, and Ala132 residues. Secondly, the docking pose

Table 2. Description of the docking data of selected target compounds **2** and **3**.

Compound	Target	Fragments	Residues (distance, Å)	Interactions	Binding energy (dG, kcal/mol)
1	hCA-II	SO_2NH_2	Thr199, 2.31	Hydrogen bonding	−24.11
			His119, 2.72		
			His94, 2.45		
			Zn metal, 2.42	Coordination bonding	
		Phenethyl	Trp209	Hydrophobic	
		4(3H)-Quinazolinone	Gln92, Lys67 (H_2O)	Hydrogen bonding	
	hCA-XII	SO_2NH_2	Thr199, 2.22	Hydrogen bonding	−23.65
			His119, 2.81		
			His94, 2.46		
			His96, 2.25		
		Phenethyl	Leu198	Hydrophobic	
		4(3H)-Quinazolinone	Gln92 (H_2O), Lys67 (H_2O)	Hydrogen bonding	
2	hCA-II	SO_2NH_2	Thr199, 2.31	Hydrogen bonding	−24.9
			Thr199, 2.42		
			Leu198, 2.46		
			Zn metal, 2.51	Coordination bonding	
		Phenethyl	His96	Aromatic stacking	
		4(3H)-Quinazolinone	Asn67 (H_2O)	Aromatic stacking	
			Gln92, 2.44	Hydrogen bonding	
			Ile91 (H^2O)		
			Phe131	Hydrophobic	
		Methylthioether	Phe131	Hydrophobic	
	hCA-XII	SO_2NH_2	Thr199, 2.50	Hydrogen bonding	−27.5
			Thr200, 2.47		
			Zn metal, 2.38	Coordination bonding	
		Phenethyl	Pro201, Ser128	Hydrophobic	
		4(3H)-Quinazolinone	Gln92, 2.44	Hydrogen bonding	
		Methylthioether	Leu141	Hydrophobic	
3	hCA-I	SO_2NH_2	His200, 2.57	Hydrogen bonding	−19.5
			Thr199, 2.45		
			Zn metal, 2.54	Coordination bonding	
		Phenethyl	Leu141, Trp202	Hydrophobic	
		4(3H)-Quinazolinone	Ala135, Ala132, Tyr204	Hydrophobic	
		Ethylthioether	Leu198, Leu131, Tyr204	Hydrophobic	
	hCA-IX	SO_2NH_2	Thr200, 2.47	Hydrogen bonding	−25.6
			His94, 2.36		
			His96, 2.48		
			Zn metal, 2.44	Coordination bonding	
		Phenethyl	Thr201 (H_2O)	Aromatic stacking	
			Trp210	Hydrophobic	
		4(3H)-Quinazolinone	Pro202 (H_2O)	Hydrogen bonding	
			Asp131, Leu134	Hydrophobic	
		Ethylthioether	Val130, Leu134	Hydrophobic	

[a]The data reported in the table were extracted from MOE programme showing the corresponding amino acid residues in enzyme pocket, corresponding fragment of ligands, interaction distances, types of interaction, and their binding energy to prototype 1 and selected active compounds.

of compound **3** in the complex with isoform IX, revealed that the cavity contained the catalytic Zn^{2+} ion forming bidentate chelate with SO_2NH_2 fragment (2.32-2.12 Å) and the sulphonyl part was stabilised by three hydrogen bonds through the residues, Thr200, His96 and His94 (2.44-1.99 Å). Different hydrophobic aromatic interactions were formed with ethylthioether, phenethyl, and 4(3H)quinazolinone moieties through pockets of Val130, Leu134, and Leu91 residues. The 4(3H)quinazolinone was modulated through molecules of H_2O in the pocket by polar interactions. In addition, the aryl moiety of benzene sulphonamide formed a H_2O-mediated π-π interactions with certain aromatic amino acids. Thr201 might also play an important role in increasing their binding affinity for the enzyme. In addition, the lease active compound **6** was placed in the hCA I binding cavity (Figure 5, right panel) and results showed that certain factors affecting the incorrect placement like the insertion of S-bromophenyl ring among polar Gln92 and Asn69 residues and disorienting of planer quinazolinone to Leu131 residue. Moreover, the docking of the least active compound **6** into the hCA-II pocket (Figure 5, left panel) revealed the intolerance of S side chain bromophenyl moiety into the His119 polar part leading to protrusion out of the pocket and so appeared incompatible with pocket residue that makes it low active.

However, the lead compound **1** was docked into the pockets of hCA II and XII (Figure 6, Table 2) for comparing its behaviour that showed the loss of SH role in the interactions compared to the potent active **2** and **3** derivatives. These overall docking findings proved that the S-alkylated derivatives exhibited good binding interactions better than the lead compound **1**.

4. Conclusions

A new series of 4-(2-(2-substituted-thio-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide derivatives **2–13** were synthesised and assessed *in vitro* for CA inhibition in comparison to AAZ as reference drug. 4–(2-(2-Aliphatic-thio-4-oxoquinazolin-3(4H)-yl)ethyl)-benzenesulfonamide derivatives **2–4** showed efficient inhibitory activity against hCA I, hCA II, h IX and hCA XII with K_I values ranging between 57.8–85.5, 6.4–13.5, 7.1–12.6, and 3.1–8.6 nM, respectively, which were better or had the same activity as AAZ as

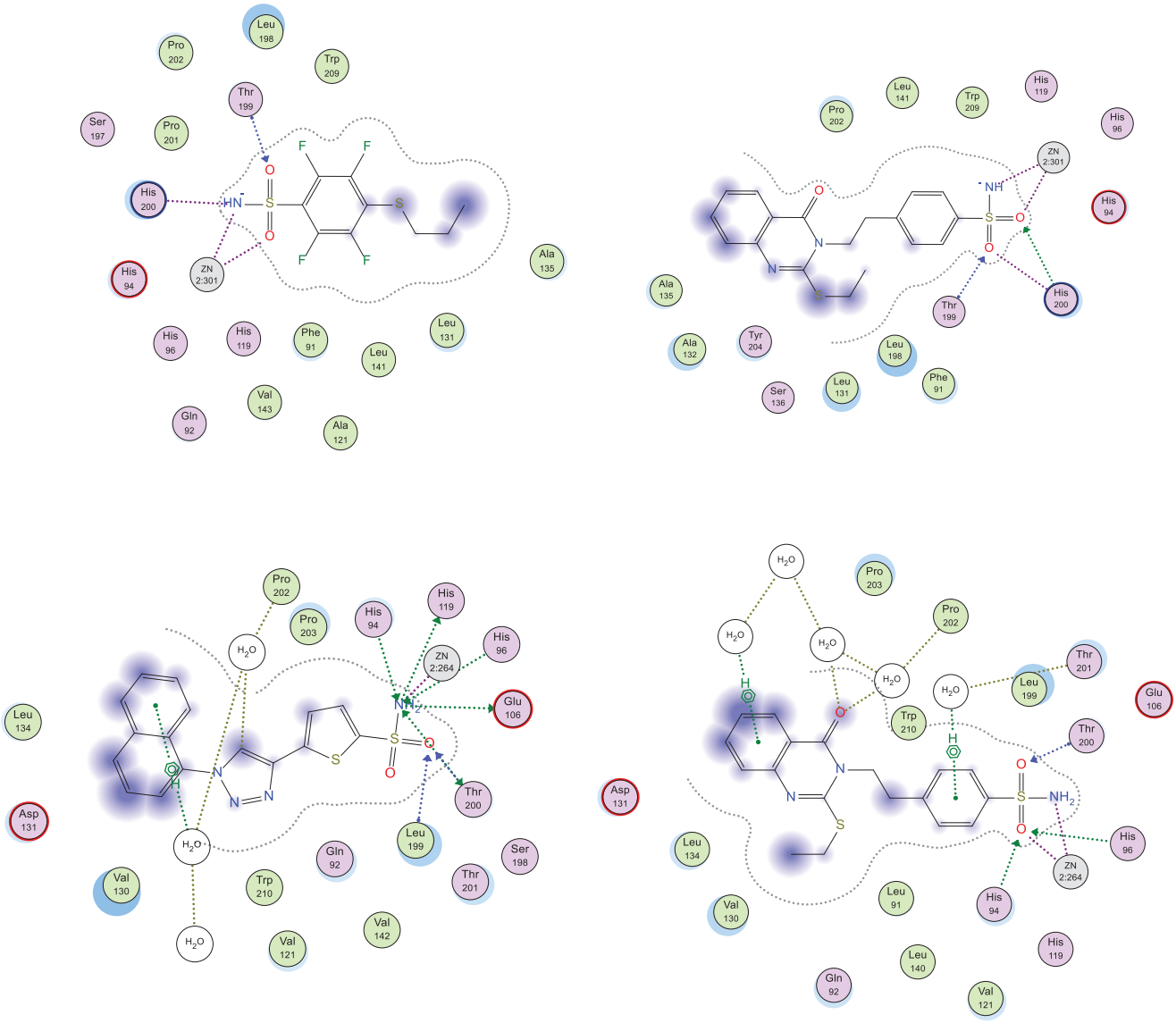


Figure 4. Docking modes of compound **3** in the binding pockets of CA isoenzymes I and IX. Interactions between the protein (PDB IDs: 4WR7, 5FL4). Predicted binding modes of co-crystallized inhibitor (upper left panel) and compound **3** (upper right panel) with hCA-I target as well as co-crystallized inhibitor (lower left panel) and compound **3** (lower right panel) with hCA-IX target.

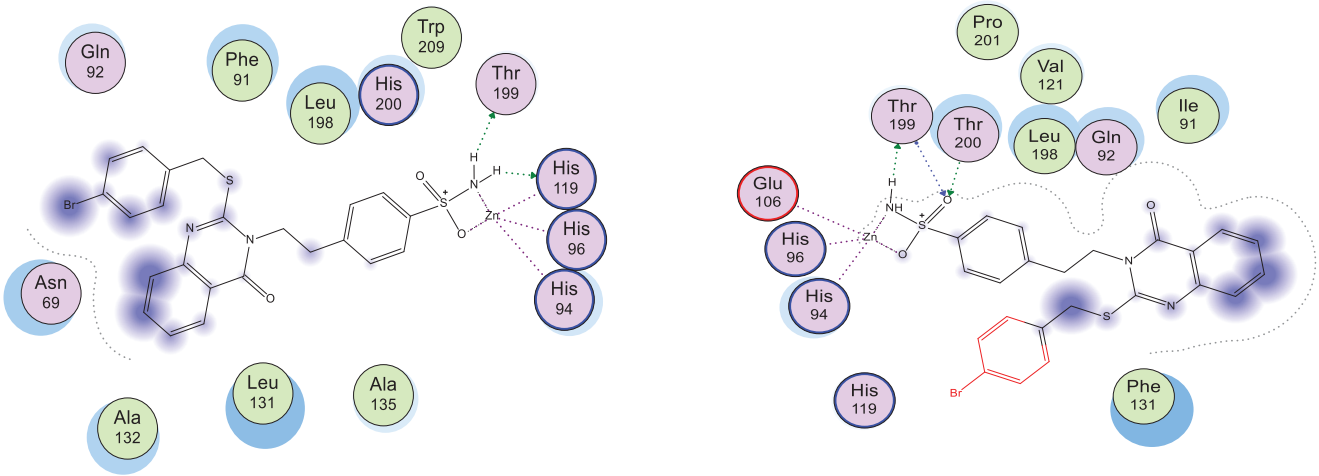


Figure 5. Docking modes of compound **6** as the least active example in the binding pockets of CA isoenzymes I and II. Interactions between the protein (PDB IDs: 4WR7, 5ULN). Predicted binding modes of compound **6** with CA-I; right panel, and CA-II; left panel.

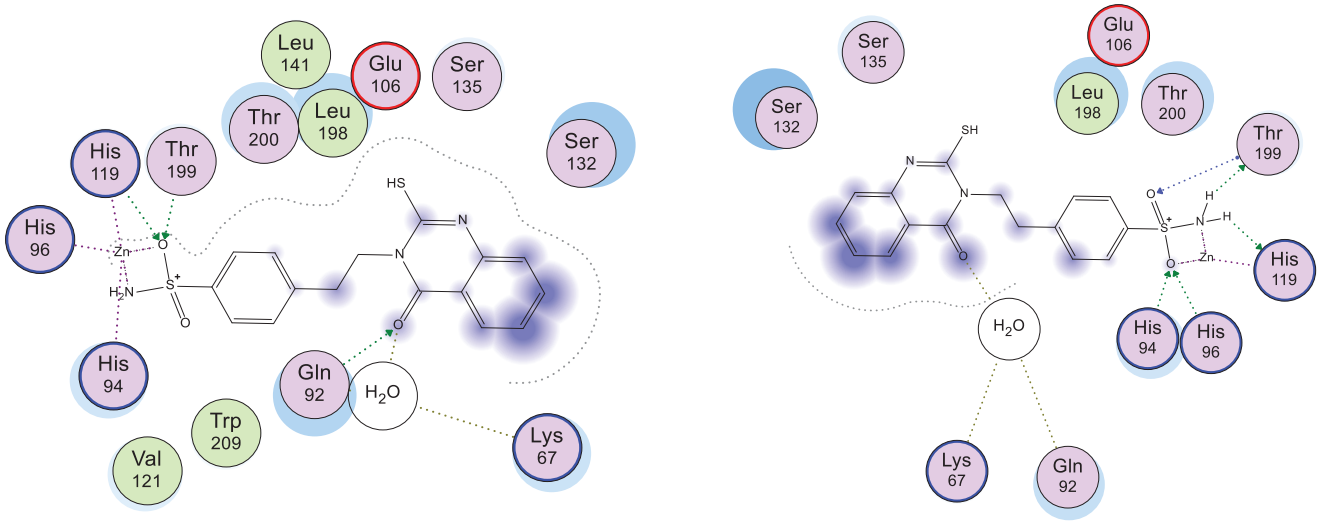


Figure 6. Docking modes of the lead compound **1** in the binding pockets of CA isoenzymes II (Right) and XII (Left) with (PDB IDs: 5ULN and 1JD0) respectively.

standard drug with K_I values of 250, 12.0, 25.0, and 5.7 nM, respectively. 4–(2-(2-Aliphatic-thio-4-oxoquinazolin-3(4H)-yl)ethyl)-benzenesulfonamide derivatives **2–4** were more active than the corresponding 2-(benzylthio)quinazolin-4(3H)-ones **5–11**, 4–(2-(4-oxo-2-((2-(piperidin-1-yl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**12**) and 4-(2-(2-((3-(1,3-dioxoisoindolin-2-yl)propyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**13**) but lower than 4-(2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2H)-yl)ethyl)benzenesulfonamide (**1**) as parent compound. Molecular docking studies for compounds **2** and **3** were done and exhibited specific binding modes for hCA isoforms as comparable interaction with lead compound **1**.

Acknowledgements

The authors thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-1435-046.

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