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Design, synthesis, and carbonic anhydrase inhibition activities of Schiff bases incorporating benzenesulfonamide scaffold: Molecular docking application

Adel S. El-Azab [a,*], Alaa A.-M. Abdel-Aziz [a], Silvia Bua [b], Alessio Nocentini [b], Ahmed H. Bakheit [a], Hamad M. Alkahtani [a], Mohamed M. Hefnawy [a], Claudiu T. Supuran [b,*]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[b] Department of Neurofarba, Sezione di Scienze Farmaceutiche Nutraceutiche, Università degli Studi di Firenze, Via U. Schiff 6, 50019 Sesto Fiorentino, Florence, Italy

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ABSTRACT

In this study, The inhibitory actions of human carbonic anhydrase (CA, EC 4.2.1.1) (hCA) isoforms I, II, IX, and XII are being examined using recently synthesized substituted hydroxyl Schiff derivatives based on the quinazoline scaffold **4**–**22**. Quinazolines **2**, **3**, **4**, **5**, **7**, **10**, **15**, and **18** reduce the activity of hCA I isoform effectively to a Ki of 87.6–692.3 nM, which is nearly equivalent to or more potent than that of the standard drug AAZ (Ki, 250.0 nM). Similarly, quinazolines **2**, **3**, and **5** and quinazoline **14** effectively decrease the inhibitory activity of the hCA II isoform to a K_I of 16.9–29.7 nM, comparable to that of AAZ (K_i, 12.0 nM). The hCA IX isoform activity is substantially diminished by quinazolines **2**–**12** and **14**–**21** (K_i, 8.9–88.3 nM against AAZ (K_i, 25.0 nM). Further, the activity of the hCA XII isoform is markedly inhibited by the quinazolines **3**, **5**, **7**, **14**, and **16** (K_i, 5.4–19.5 nM). Significant selectivity levels are demonstrated for inhibiting tumour-associated isoforms hCA IX over hCAI, for sulfonamide derivatives **6**–**15** (SI; 10.68–186.29), and **17**–**22** (SI; 12.52–57.65) compared to AAZ (SI; 10.0). Sulfonamide derivatives **4**–**22** (SI; 0.50–20.77) demonstrated a unique selectivity in the concurrent inhibition of hCA IX over hCA II compared to AAZ (SI; 0.48). Simultaneously, benzenesulfonamide derivative **14** revealed excellent selectivity for inhibiting hCA XII over hCA I (SI; 60.35), whereas compounds **5**–**8**, **12**–**14**, **16**, and **18**–**22** demonstrated remarkable selectivity for hCA XII inhibitory activity over hCA II (SI; 2.09–7.27) compared to AAZ (SI; 43.86 and 2.10, respectively). Molecular docking studies additionally support **8** to hCA IX and XII binding, thus indicating its potential as a lead compound for inhibitor development.

1. Introduction

Most living organisms contain different CA isoforms known as zinc metalloenzymes.(Supuran, 2008, Supuran, 2011, Alterio et al., 2012, Supuran 2017) The primary function of CA is to facilitate the reversible Conversion of carbon dioxide into protons and bicarbonate. Numerous critical pathophysiological pathways (Supuran, 2008, Supuran, 2011, Alterio et al., 2012, Supuran 2017), such as glaucoma (Supuran 2017), epilepsy (Mishra et al., 2017), obesity (Arechederra et al., 2013), and malignancies (Abdel Gawad et al., 2016), are affected by the hydration of carbon dioxide (Supuran, 2008, Supuran, 2011, Alterio et al., 2012, Supuran 2017). Human carbonic anhydrase inhibitors (hCAIs) have various therapeutic applications. For instance, hCA II and XII Inhibitors are used as diuretics to treat glaucoma and epilepsy treatments (Supuran, 2008, Alterio et al., 2012, Del Prete et al., 2014). CA I and II Inhibitors find therapeutic uses as antitumour and anti-inflammatory treatments (De Simone and Supuran, 2010, Neri and Supuran, 2011, Margheri et al., 2016, Supuran 2017, Nocentini and Supuran, 2018). The goals of glaucoma treatment involve CA IV, CA II, and CA XII inhibitors; however, CA II and XII inhibitors are used for rheumatoid arthritis, stroke, and retinitis pigmentosa (Matsui et al., 1996, Tang et al., 2006, Supuran, 2008). In numerous hypoxic malignancies, the transmembrane isoform CA IX showed a high level and was involved in a multitude of inflammation forms (De Simone and Supuran, 2010, Neri and Supuran, 2011, Margheri et al., 2016, Supuran 2017, Nocentini and Supuran, 2018). Sulfonamides are among the most abundant inhibitors of COX

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* Corresponding authors.
E-mail addresses: adelazab@ksu.edu.sa (A.S. El-Azab), claudiu.supuran@unifi.it (C.T. Supuran).

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and zinc enzyme CA (Scozzafava et al., 1999, Abdel-Aziz et al., 2012, Abdel-Aziz et al., 2014, Abdel-Aziz et al., 2015, El-Azab et al., 2016, Abdel-Aziz et al., 2017, Angeli et al., 2017, Mohamed et al., 2017, Abdel-Aziz et al., 2018, Abdel-Aziz et al., 2019) (CA, EC 4.2.1.1). Conversely, the quinazolinone scaffold is essential in medicinal chemistry with various activities (Aziza et al., 1996, El-Azab, 2007, Alafeefy et al., 2008, Al-Obaid et al., 2009, Al-Omary et al., 2010, El-Azab et al., 2010, Alafeefy et al., 2011, El-Azab et al., 2011, El-Azab and ElTahir 2012, El-Azab and ElTahir 2012, Al-Suwaidan et al., 2013, Al-Suwaidan et al., 2013, Alanazi et al., 2013, El-Azab et al., 2013, Alanazi et al., 2014, Abdel-Aziz et al., 2016, Al-Suwaidan et al., 2016, Alaa et al., 2016, Alanazi et al., 2016, Mohamed et al., 2016, El-Azab et al., 2017, El-Azab et al., 2017, El-Sayed et al., 2018). Intriguingly, a group of mercaptoquinazolines may create new, sensitive CAIs that effectively combat various health problems and anticancer activities (Alanazi et al., 2013, El-Azab et al., 2017, El-Azab et al., 2019, Alkahtani et al., 2020, El-Azab et al., 2020, Alkahtani et al., 2022, El-Azab et al., 2023). Schiff derivatives with benzenesulfonamide moieties demonstrate adaptable inhibition of gainst CAs (Alaa et al., 2019, Alaa et al., 2019, El-Azab et al., 2019). Acetazolamide (AAZ) (Fig. 1), a strong inhibitor of numerous CA isozymes, modulates anticancer treatment when combined with different cytotoxic drugs (Supuran and Scozzafava 2000). Moreover, 4-Amino-*N*-(4-sulfamoylphenethyl)benzenesulfonamide (**I**), 4-(((dimethylcarbamothioyl)thio)amino)-*N*-(4-sulfamoylphenethyl) benzenesulfonamide, and 4-((((diethylcarbamothioyl)thio)amino)-*N*-(4-sulfamoylphenethyl)benzenesulfonamide (**II**) exhibit potent CAI activity (Supuran et al., 2001) comparable to that of AAZ (Fig. 1). Additionally, Schiff compounds with benzenesulfonamide moieties **III** preferentially inhibit the hCA II, with KI values of 79.9–236.0 nM (Nasr et al., 2009). The Schiff derivatives of 4-(2- aminoethyl)-benzenesulfonamide **IV** (Fig. 1) demonstrate varied inhibition against CAs I, II, IX, and XII with KI values of 393.0–453.0, 374.0–474, 39.1–138.0, and 46.8–3115 nM, respectively (Durgun et al., 2015). Furthermore, 2-mercaptoquinazoline **V** exhibits intense CAI activity against CAs I, II, and XII with K_I values of 31.5, 0.62, and 0.59 nM, respectively (Bozdag et al., 2016). Compared with AAZ, compound **VI** is robust and exhibits CAI activities against CAs I, II, IX, and XII with K_I values of 83.9–86.8, 0.73–6.2, 1.6–1.8, and 8.30–9.20 nM, respectively. Compound **VII** and **VIII** exhibit intense inhibition activity against CAs I, II, IX, and XII with K_I levels ranging from 39.4 to 494.5, 3.30–35.0, 16.4–24.2, and 5.20–8.90 nM, respectively, compared with that of AAZ (El-Azab et al., 2019).

In this study, physiologically appropriate hCA isoforms, such as the cytosolic and abundantly expressed I and II and tumour-associated IX and XII isoforms, were assessed for newly synthesized hydroxylated and substituted hydroxylated Schiff derivatives, including the benzenesulfonamide moiety (**4**–**22**) with SAR study of the synthesized compounds.

2. Experimental

2.1. Chemistry

An electrothermal melting device (Barnstead 9100) was used to record the melting points (uncorrected). An FTIR Perkin-Elmer spectrometer (Perkin-Elmer Inc., Waltham, MA, USA) was used to record the IR spectra. Using Bruker 700 and 176 MHz instruments, ^{1}H and ^{13}C NMR spectra were acquired in DMSO-d_6 (Bruker, Billerica, MA, USA). An Agilent 6320 ion-trap mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to record the mass spectra. Additionally, C, H, and N were examined at King Saud University's Research Center in the College of Pharmacy in Saudi Arabia. The outcomes were ± 0.4 %, apart from the theoretical values. The reported method obtained compounds **1**

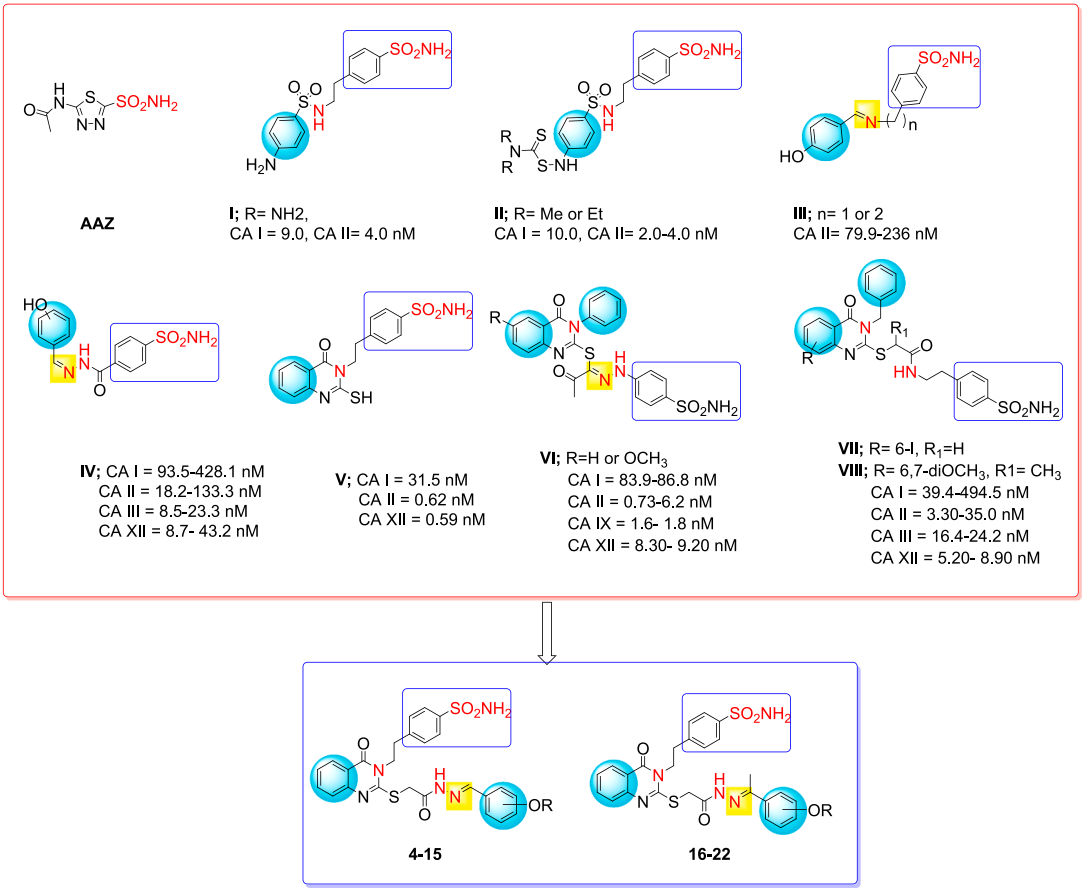


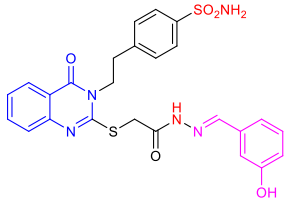
Fig. 1. The proposed Schiff bases compounds **4**–**22** and the reported CAI sulfonamides **(AAZ & I-VIII)**.

(El-Azab et al., 2020, El-Azab et al., 2020) **2**–**5**, **8**, **9**, and **16**–**18**. (El-Azab et al., 2023).

2.1.1. Preparation of benzylidenes 4–22

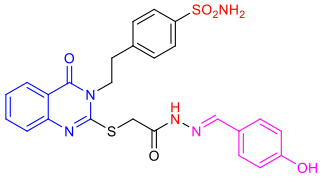
A solution of methanol (7 ml) acidified with five drops of acetic acid was used to stir a combination of 4-(2-(2-((2-hydrazineyl-2-oxoethyl) thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (**3**; 217 mg 5.0 mmol) and carbaldehyde or phenylethan-1-one derivatives (7.0 mmol) for 24 h at room temperature; the reaction mixture was filtered and dried. The ultimate pure-form product was achieved after washing with 50 % cold methanol (12 ml) and drying.

2.1.1.1. 4-(2-(2-((2-(2-(3-Hydroxybenzylidene)hydrazineyl)-2-oxoethyl) thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (6)



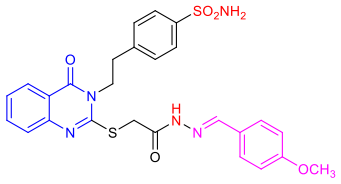
m.p 218-220°,87 % yield;^{1}H NMR: δ11.83 (s, 0.40H), 11.67 (s, 0.60H), 9.66 (s, 0.60H), 9.65 (s, 0.40H), 8.20 (s, 0.40H), 8.07 (t, 1H, J = 7.00 Hz), 8.02 (s, 0.600H), 7.810 (dd, 2.25H, 7.98 & 7.70 Hz), 7.75 (t, 0.75H, J = 7.59 Hz), 7.51 (t, 2.25H, J = 7.73 Hz), 7.450 (q, 1H, J = 7.77 Hz), 7.410 (d, 0.75H, J = 8.2 Hz), 7.360 (s, 2H), 7.24 (t, 1H, J = 7.78 Hz), 7.20 (s, 0.60H), 7.17 (s,0.40H), 7.14 (dd, 0.60H, J = 7.47 Hz), 7.09 (d,0.40H, J = 7.42 Hz), 6.48 (t, 1H, J = 9.29 Hz), 4.63 (s, 1.25H), 4.160 (s, 0.75H), 4.13 (q, 2H, J = 6.99 Hz), 3.13 (t, 2H, J = 14.45 Hz); ^{13}C NMR: δ 169.08, 163.99, 160.87, 160.83, 158.14, 158.11, 156.32, 156.18, 147.25, 147.08, 147.06, 144.08, 143.10, 143.08, 142.33, 135.84, 135.29, 135.25, 130.40, 130.35, 129.67, 129.65, 126.94, 126.61, 126.51, 126.38, 126.33, 119.35, 119.22, 119.17, 118.88, 117.96, 117.75, 113.11, 113.05, 45.55, 45.48, 35.42, 34.30, 33.65, 33.62; Ms; [*m/z*, 537].

2.1.1.2. 4-(2-(2-((2-(2-(4-Hydroxybenzylidene)hydrazineyl)-2-oxoethyl) thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (7)



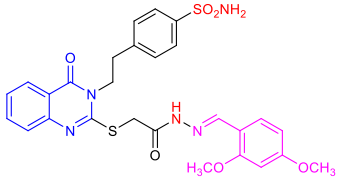
m.p 243-244°, 88 % yield;^{1}H NMR: δ 11.66 (s, 0.40H), 11.52 (s, 0.60H), 9.95 (s, 0.42H), 9.93 (s, 0.58H), 8.11 (s, 0.40H), 8.080 (d, 1H, J = 7.49 Hz), 7.99 (s, 0.60H), 8.81 (dd, 2.3H, 8.26 & 7.85 Hz), 7.750 (t, 0.70H, J = 7.59 Hz), 7.54 (dd, 2.2H, J = 19.11 Hz), 7.500 (t, 2H, J = 18.69 Hz), 7.42 (dd, 1.8H, J = 8.13 Hz), 7.360 (d, 2H, J = 2.66 Hz), 6.82 (t, 2H, J = 7.87 Hz), 4.611 (s, 1.2H), 5.31 (q, 2H, J = 7.31 Hz), 4.14 (s, 0.800H), 3.100 (t, 2H, J = 15.55 Hz); ^{13}C NMR: δ 168.75, 163.56, 160.88, 160.83, 159.92, 159.73, 156.35, 156.21, 147.49, 147.08, 144.22, 143.10, 143.08, 142.35, 142.33, 135.25, 129.67, 129.65, 129.36, 129.06, 126.93, 126.58, 126.53, 126.51, 126.39, 126.35, 125.57, 125.51, 119.22, 119.17, 116.17, 116.15, 45.54, 45.46, 35.43, 34.35, 33.64, 33.62.

2.1.1.3. 4-(2-(2-((2-(2-(4-Methoxybenzylidene)hydrazineyl)-2-oxoethyl) thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (10)



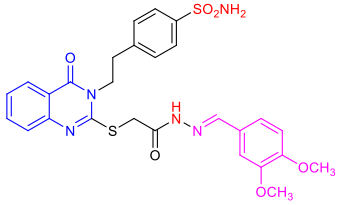
m.p 230-231°, 89 % yield; ^{1}H NMR: δ 11.88 (s, 0.36H), 11.75 (s, 0.64H), 8.26 (s, 0.36H), 8.08 (s, 0.640H), 8.080 (s, 1H), 7.81 (d, 1H, J = 7.87 Hz), 7.78 (d, 1.44H, J = 7.83 Hz), 7.75 (t, 0.66H, J = 7.35 & 7.56 Hz), 7.52–7.26 (m, 9H), 7.01 (d, 1H, J = 4.65 Hz), 4.65 (s, 1.23H), 4.31 (t, 2H, J = 14.63 Hz), 4.160 (s, 0.77H), 3.79 (s, 3H), 3.120 (t, 2H, J = 14.63 z); ^{13}C NMR: δ 169.18, 164.06, 160.86, 160.82, 160.02, 156.32, 156.19, 147.06, 143.73, 143.09, 142.32, 135.99, 135.25, 130.45, 130.40, 129.67, 129.63, 126.95, 126.61, 126.52, 126.37, 126.29, 120.48, 119.94, 119.23, 119.18, 116.77, 116.28, 112.06, 111.70, 55.62, 45.56, 45.47, 35.41, 34.35, 33.65; Ms; [*m/z*, 551].

2.1.1.4. 4-(2-(2-((2-(2-(2,4-Dimethoxybenzylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (11)



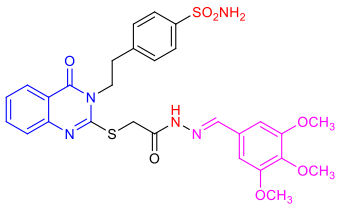
m.p 258-260°, 90 % yield;^{1}H NMR: δ 10.76 (s, 0.62H), 10.64 (s, 0.38H), 8.10 (dd, 1H, J = 7.70 Hz), 7.81 (t, 3H, J = 8.12 Hz), 7.52–7.7.41 (m, 4H),7.36 (ss, 3H), 7.21 (d, 0.61H, J = 8.33 Hz), 6.63 (s, 0.62H), 6.61 (s, 0.38H), 6.53 (d, 0.40H, J = 8.33 Hz), 6.50 (d, 0.60H, J = 0.33H), 6.32 (t, 0.39H, J = 6.65 & 850 Hz), 4.57 (s, 1.3H), 4.30 (d, 2H, J = 6.5 Hz), 4.27 (s, 0.7H), 3.84 (s, 1.85H), 3.81 (s, 1.55H), 3.78 (s, 2.60H), 3.111 (t, 2H, J = 7.70 Hz); ^{13}C NMR: δ 169.80, 164.30, 161.75, 161.72, 160.87, 160.84, 158.89, 158.78, 156.45, 156.42, 156.05, 154.78, 152.99, 150.62, 147.11, 147.08, 143.07, 142.36, 142.34, 135.27, 135.24, 130.54, 130.47, 129.67, 129.65, 129.62, 126.98, 126.91, 126.51, 126.49,126.46, 126.31, 122.10, 121.98, 119.28, 119.17, 115.56, 105.51, 105.36, 99.13, 99.05, 56.11, 56.09, 55.92, 55.81, 45.56, 45.41, 35.28, 34.82, 34.51, 33.610; Ms; [*m/z*, 581].

2.1.1.5. 4-(2-(2-((2-(2-(3,4-Dimethoxybenzylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (12)



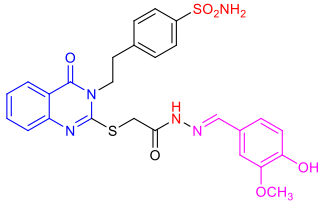
m.p 198-200°, 88 % yield;ν: 3385, 3257, 3140 (NH), 1754, 1683 (C = O), 1338, 1156 (O = S = O); ^{1}H NMR: δ 11.76 (s, 0.42H), 11.63 (s, 0.58H), 8.12 (s, 0.43H), 8.08 (s, 1H), 8.02 (s, 0.57H), 7.82–7.75 (m, 3H), 7.50–7.41 (m, 4H), 7.370 (s, 2.5H), 7.30 (s, 0.50H), 7.22 (ss, 1H), 7.01 (d, 1H, J = 2.87 Hz), 4.65 (s, 1.1H), 4.31 (s, 2H), 4.15 (s, 0.9H), 3.80 (s, 6H), 3.13 (s, 2H); ^{13}C NMR: δ 168.85, 163.75, 160.86, 160.82, 156.38, 156.19, 151.20, 151.02, 149.46, 147.36, 147.08, 144.05, 143.08, 143.07, 142.34, 142.31, 135.26, 135.23, 129.66, 129.62, 127.27, 127.22, 126.93, 126.58, 126.53, 126.51, 126.39, 126.27, 122.34, 121.64, 119.21, 119.17, 111.98, 111.86108.99, 108.69, 56.01, 55.86, 55.82, 45.55, 45.45, 35.40, 34.56, 33.64, 33.62; Ms; [*m/z*, 581].

2.1.1.6. 4-(2-(4-Oxo-2-((2-Oxo-2-(2-(3,4,5-trimethoxybenzylidene) hydrazineyl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (13)



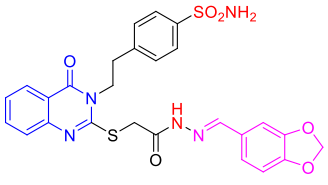
m.p 240-241°, 89 % yield;^{1}H NMR: δ 11.87 (s, 0.40H), 11.77 (s, 0.60H), 8.22 (s, 0.42H), 8.08 (s, 1H), 8.03 (s, 0.58H), 7.78 (ss, 3H), 7.51–7.7.37 (m, 6H), 7.09 (s, 1H), 7.03 (s, 1H), 4.68 (s, 1.1H), 4.310 (s, 2H), 4.160 (s, 0.9H), 3.83(s, 6H), 3.71 (3H), 3.13 (s, 2H); ^{13}C NMR: δ 169.02, 163.97, 160.85, 160.8, 156.39, 156.20, 153.67, 153.63, 147.08, 143.81, 143.09, 142.33, 142.30, 139.63, 139.52, 135.28, 135.25, 130.09, 129.67, 129.63, 126.95, 126.59, 126.50, 126.36, 126.24, 119.22, 119.17, 104.75, 104.55, 60.60, 60.57, 56.38, 56.34, 45.57, 45.46, 35.41, 34.63, 33.65, 33.62; Ms; [*m/z*, 611].

2.1.1.7. 4-(2-(2-((2-(2-(4-Hydroxy-3-methoxybenzylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (14)



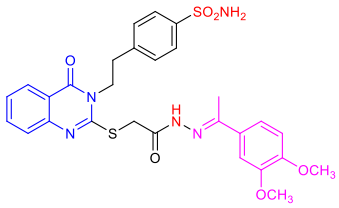
m.p 215-217°, 85 % yield;^{1}H NMR: δ 11.69 (s,0.40H), 11.56 (s, 0.60H), 9.57 (s, 0.44H), 9.55 (s, 0.56H), 8.16 (s, 0.43H), 8.080 (d, 1H, J = 7.77 Hz), 7.98 (s, 0.57H), 7.80 (p, 2.3H, J = 10.95 & 8.29 Hz), 7.56 (q, 0.7H, J = 7.63 Hz), 7.53 (d, 0.48H, J = 8.61 Hz), 7.500 (t, 2H, J = 13.44 Hz), 7.450 (q, 1H, J = 6.30 Hz), 7.410 (d, 0.52H, J = 8.13 Hz), 7.360 (d, 2H, J = 5.04 Hz), 7.33 (s, 0.60H), 7.27 (s, 0.40H), 7.13 (d, 0.57H, J = 7.91 Hz), 7.08 (d, 0.43H, J = 7.91 Hz), 6.83 (d, 1H, J = 8.05 Hz), 4.63 (s, 1.14H), 4.30 (p, 2H, J = 15.61 & 14.91 Hz), 4.13 (s, 0.84H), 3.80 (d, 3H, J = 3.78 Hz), 3.120 (t, 2H, J = 14.91 Hz); ^{13}C NMR: δ 168.71, 163.60, 160.87, 160.83, 156.41, 156.21, 149.46, 149.23, 148.45, 148.41, 147.68, 147.08, 144.38, 143.07, 142.34, 142.31, 135.24, 129.66, 129.63, 126.93, 126.57, 126.50, 126.37, 126.28, 125.98, 125.91, 122.58, 121.73, 119.21, 119.1672, 116.02, 115.85, 109.91, 109.43, 56.50, 55.94, 45.5513, 45.45, 35.40, 34.55, 33.64, 33.60; Ms; [*m/z*, 567].

2.1.1.8. 4-(2-(2-((2-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (15)



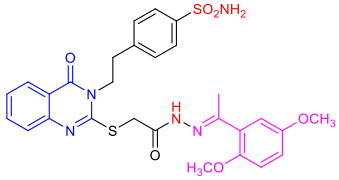
m.p 233-235°, 85 % yield; ν: 3385, 3261, 3142 (NH), 1753, 1682 (C = O), 1339, 1156 (O = S = O); ^{1}H NMR: δ 11.77 (s, 0.37H), 11.62 (s, 0.63H), 8.19 (s, 0.38H), 8.070 (t, 1H, J = 6.50 & 6.88 Hz), 8.001 (s, 0.62H), 7.80 (dd, 2.44H, J = 7.98 & 7.85 Hz), 7.750 (t, 0.66H, J = 7.95 Hz), 7.500 (t, 2.38H, J = 8.19 Hz), 7.450 (q, 1H, 7.91 Hz), 7.400 (d, 0.62H, J = 8.13 Hz), 7.350 (s, 2.58H), 7.25 (s,).42H), 7.17 (d, 1H, J = 7.98 Hz), 6.97 (t, 1H, J = 6.44 & 7.70 Hz), 6.08 (s, 2H), 4.62 (s, 1.27H), 4.300 (t, 2H, J = 15.91 Hz), 4.150 (s, 0.73H), 3.120 (t, 2H, J = 15.91 Hz); ^{13}C NMR: δ 168.98, 163.79, 160.87, 160.82, 156.32, 156.19, 149.59,149.40, 148.46, 148.42, 147.08, 147.06, 146.99, 143.61, 143.08, 142.33, 142.32, 135.25, 129.67, 129.63, 129.01, 128.95, 126.93, 126.58, 126.53, 126.51, 126.39, 126.29, 123.85, 123.52, 119.23, 119.17, 108.93, 105.60,105.49, 102.02, 45.55, 45.46, 35.41, 34.28, 33.65, 33.610; Ms; [*m/z*, 565].

2.1.1.9. 4-(2-(2-((2-(2-(1-(3,4-Dimethoxyphenyl)ethylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (19)



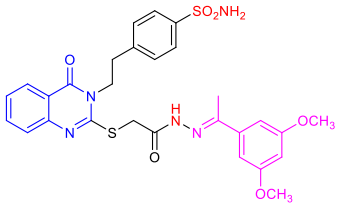
m.p 275-276°, 88 % yield;^{1}H NMR: δ 10.88 (s, 0.62H), 10.74 (s, 0.38H), 8.080 (dd, 1H, J = 8.17 & 7.85 Hz), 7.790 (dd, 3H, J = 7.42 & 7.56 Hz), 7.58–7.50 (m, 2H), 7.47 (dd, 2H, J = 7.47 & 7.56 Hz), 7.410 (d, 1H, J = 7.42 Hz), 7.38 (d, 0.63H, J = 8.68 Hz), 7.35 (d, 2H, J = 3.92 Hz), 7.33 (d, 0.37H, J = 8.68 Hz), 6.98 (dd, 1H, J = 8.19 Hz), 4.690 (s, 1.25H), 4.310 (t, 2H, J = 15.68 Hz), 4.28 (s, 0.75H), 3.979(dd, 6H, J = 8.19 & 9.87 Hz), 3.11 (q, 2H, J = 7.63 Hz), 2.30 (d, 3H, J = 10.57 Hz); ^{13}C NMR: δ 169.79, 166.63, 164.19, 160.87, 160.83, 156.46, 156.10, 152.95, 150.64, 150.53, 148.96,148.86, 148.49, 147.12, 147.10, 143.09, 143.07, 142.32, 135.30, 135.26, 131.07, 130.93, 129.67 129.60, 126.98, 126.94, 126.87, 126.61, 126.52, 126.48, 126.30, 126.27, 120.24, 119.93, 119.27, 119.20, 119.17, 111.57, 111.48, 109.63, 109.59, 55.99, 55.96, 55.87, 45.59, 45.42, 35.30, 35.18, 33.63, 14.65, 14.07; Ms; [*m/z*, 595].

2.1.1.10. 4-(2-(2-((2-(2-(1-(2,5-Dimethoxyphenyl)ethylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (20)



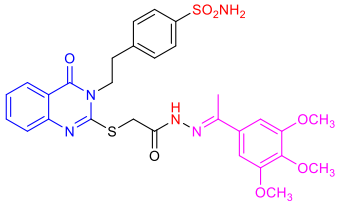
m.p 284-286°, 87 % yield;^{1}H NMR: δ 10.87 (s, 0.63H), 10.73 (s, 0.37H), 8.080 (dd, 1H, J = 7.83 & 7.70 Hz), 7.84–7.77 (m, 3H), 7.56 (d, 0.35H, J = 8.04 Hz), 7.500 (t, 2.4H, J = 8.82 & 8.33 Hz), 7.45 (t, 0.6H, J = 7.41 Hz), 7.400 (d, 0.65H, J = 8.08 Hz), 7.360 (s, 2H), 7.02 (t, 1H, J = 15.12 Hz), 7.00 (s, 0.70H), 6.96 (t, 1H, 9.52 Hz), 6.81 (s, 0.30H), 4.59 (s, 1.33H), 4.310 (dd, 2H, J = 16.08 & 8.29 Hz), 4.28 (s, 0.77H), 3.81 (s, 2H), 3.76 (s, 1H), 3.70 (d, 3H, J = 3.22 Hz), 3.11 (s, 2H), 2.23 (s, 3H); ^{13}C NMR: δ 170.01, 164.52, 160.86, 156.43, 154.50, 153.38, 153.26, 151.61, 150.36, 147.08, 143.07, 142.35, 135.28, 135.22, 130.13, 130.06, 129.67, 129.63, 126.99, 126.93, 126.64, 126.51, 126.28, 119.28, 119.17, 115.48115.43, 115.07, 113.43, 113.35, 56.59, 56.55, 56.50, 55.94, 55.90, 45.58, 45.44, 35.26, 34.91, 33.63, 19.03, 18.58, 18.13; Ms; [*m/z*, 595].

2.1.1.11. 4-(2-(2-((2-(2-(1-(3,5-Dimethoxyphenyl)ethylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3(4H)-yl)ethyl)benzenesulfonamide (21)



m.p 309-310°, 88 % yield; ^{1}H NMR: δ 10.98 (s, 0.67H), 10.82 (s, 0.333H), 8.080 (t, 1H, J = 12.95 & 7.57 Hz), 7.80 (s, 1.60H), 7.78 (d, 1.40H, J = 7.56 Hz), 7.540 (d, 0.37H, J = 8.12 Hz), 7.42 (d, 0.63H, J = 8.05 Hz), 7.47 (ddd, 4H, J = 7.63, 7.91 & 7.35 Hz), 7.350 (s, 2.5H), 7.02 (s, 1H), 6.93 (s, 0.50H), 6.58 (s, 0.64H), 6.55 (s, 0.36H), 4.70 (s, 1H), 4.30 (t,3H, J = 7.42 & 10.15 Hz), 3.77 (ss, 6H), 3.11 (s, 2H), 2.30 (ss, 3H); ^{13}C NMR: δ 170.06, 164.50, 160.89, 156.41, 152.44, 148.18, 147.10, 143.07, 142.33, 140.55, 135.24, 126.92, 126.50, 126.29, 119.18, 105.01, 101.63, 101.13, 55.73, 45.44, 35.23, 33.64, 14.96, 14.32; Ms; [*m/z*, 595].

2.1.1.12. 4-(2-(4-Oxo-2-((2-oxo-2-(2-(1-(3,4,5-trimethoxyphenyl)ethylidene)hydrazineyl)ethyl)thio)quinazolin-3(4H)-yl)ethyl)benzenesulfonamide (22)



m.p 282-283°, 85 % yield;^{1}H NMR: δ 10.94 (s, 0.63H), 1.79 (s, 0.37H), 8.080 (dd, 1H, J = 9.91 & 7.77 Hz), 7.810 (dd, 3H, J = 7.21 & 7.49 Hz), 7.53 (dd, 1.4H, J = 8.12 & 7.56 Hz), 7.47 (dd, 2H, J = 7.49 & 8.12 Hz), 7.40 (0.6H, J = 8.05 Hz), 7.350 (s, 2H), 7.16 (s, 1.25H), 7.08 (s, 0.75H), 4.700 (s, 1.25H), 4.310 (t, 2H, J = 6.37 & 7.56 Hz), 3.81 (ss, 6H), 3.69 (s, 3H), 3.110 (t, 2H, J = 7.91 & 7.21 Hz), 2.340 (s, 1.25H), 2.32 (s, 1.75H); ^{13}C NMR: δ 169.895, 164.345, 160.861, 160.838, 156.469, 156.447, 153.181, 153.088, 152.913, 148.516, 147.107, 143.099, 143.078, 142.334, 139.235, 139.192, 135.310, 135.253, 134.108, 133.985, 129.676, 129.604, 126.991, 126.947, 126.618, 126.527, 126.489, 126.295, 126.242, 119.278, 119.172, 104.408, 104.326, 60.589, 60.555, 56.497, 56.401, 56.375, 45.614, 45.442, 35.298, 35.269, 33.638, 14.993, 14.352; Ms; [*m/z*, 625].

2.2. Carbonic anhydrase evaluation

the inhibition assay for the hCA I, II, IX, and XII isozymes was performed in accordance with the previously published protocol (Khalifah, 1971).

2.3. Docking method

The crystal structures of human carbonic anhydrases IX (PDB code: 5FL4) (Leitans et al., 2015) and XII (PDB code: 1JD0) (Whittington et al., 2001) were obtained from the Protein Data Bank (PDB). The docking protocol used for the molecular docking of compound **8** with human carbonic anhydrase IX and XII proteins followed the same methodology described earlier (Hamdi et al., 2022, Abuelizz et al., 2023, Eskandrani et al., 2023).

3. Results

3.1. Chemistry

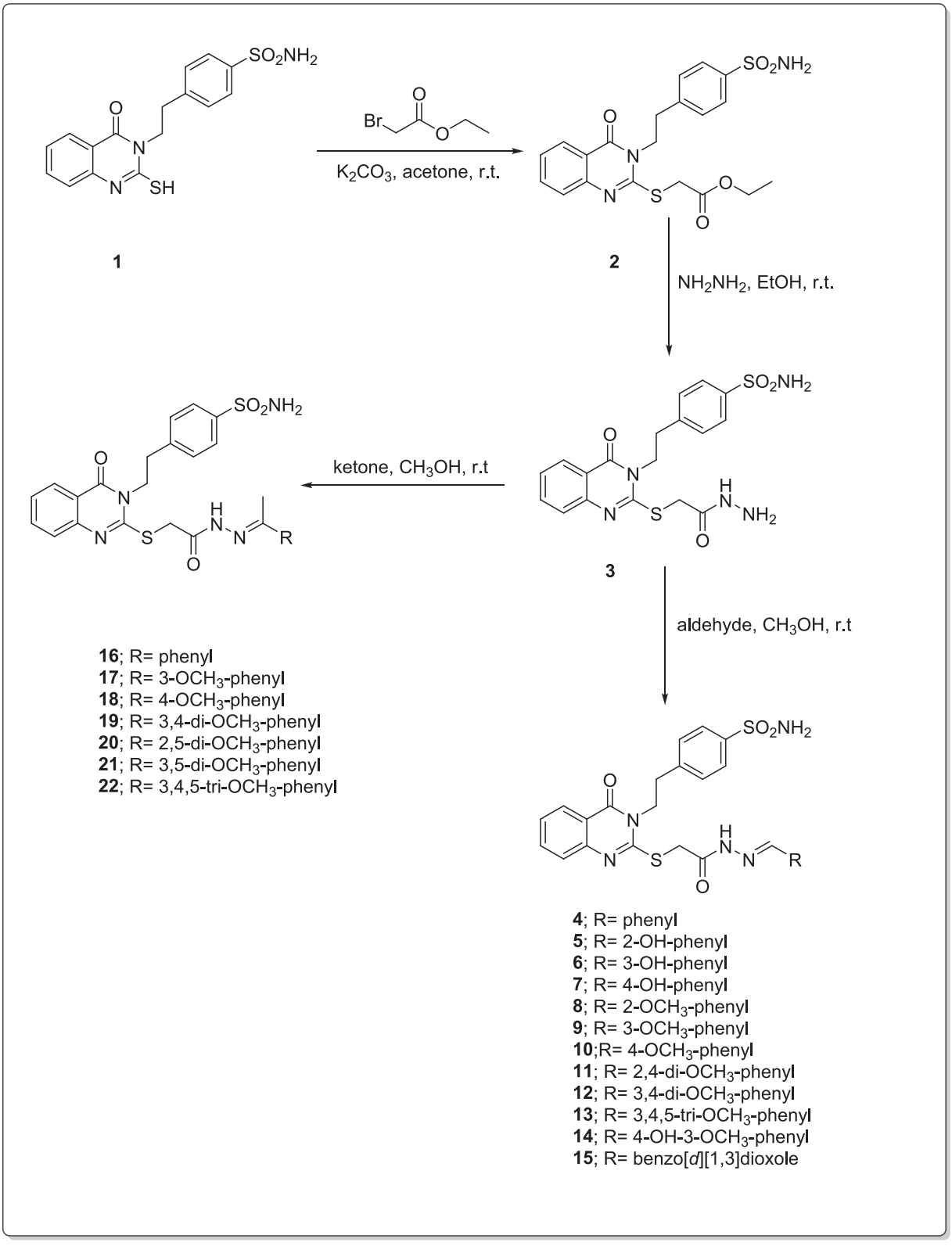
The reported method obtained compounds **1** (El-Azab et al., 2020, El-Azab et al., 2020), **2**–**5**, **8**, **9**, and **16**–**18**. (El-Azab et al., 2023) A series of benzylidenethioacetohydrazide and phenylethylidenethioacetohydrazide derivatives incorporating 2-mercaptoquinazolines **4**–**22** in isomeric mixture *E* and *Z* (Scheme 1) was produced synthetically by stirring derivative **3** with an aldehyde or acetophenone derivative in methanol, that including acetic acid, thus resulting in an 85–91 % yield.

3.2. CA inhibitory activity

The synthesized quinazolines **2**–**22** were tested against CAI isoforms, such as hCA I, II, IX, XII and AAZ (Table 1). Substituted hydroxylbenzylidenethioacetohydrazides **6**–**15** (SI; 10.68–186.29) and hydroxyphenylethylidenethioacetohydrazides **17**–**22** (SI; 12.52–57.65) show significant selectivity for suppressing tumour-associated isoform hCA IX over hCAI compared to AAz (SI; 10.0). Schiff bases **6**, **7**, **8, 11**, and **22** exhibited an acceptable degree of selectivity (SI, 25.58–34.08) in comparison to AAZ (SI, 43.86). In a similar vein, 4-(2-(2-(2-(2-(4-hydroxy-3-methoxybenzylidene)hydrazineyl)-2-oxoethyl)thio)-4-oxoquinazolin-3 (4H)-yl)ethyl)benzenesulfonamide (**14**) demonstrated excellent selectivity for inhibiting hCA XII over hCA I (SI, 60.35). In comparison to AAz (SI; 0.48), the sulfonamide derivatives **4**–**22** (SI; 0.50–20.77) they have exhibited a selectivity that was one of a kind when it came to the concurrent tumour-associated isoform inhibition of hCA IX over hCA II. whereas compounds **5**–**8**, **12**–**14** and **16**–**22** expressed an impressively high selectivity for hCA XII inhibitory action over hCA II (SI; 2.09–7.27) as compared to AAZ (SI; 2.10). The quinazolines **2**–**5**, **7**, **10**, **15**, and **18** reduced the hCA I isoforms inhibition with a Ki value between 87.6 and 692.3 nM. This value is almost identical to the value found for AAZ, which was 250.0 nM. Compounds **6**, **9**, **10**, **12**, **14**, **16**, **19**, and **21** conveyed a moderately low inhibitory action against hCA I (Ki, 756.4–1157.0 nM). Derivatives **8**, **11**, **13**, **17**, **20**, and **22** exhibited only a small amount of inhibition with a Ki value ranging from 1634 to 2308 nM. The inhibiting effects of the hCA II isoform were decreased by molecules **2**, **3**, **5**, and **14** to a Ki value that was between 16.9 and 29.7 nM, which was close to the value for AAZ (Ki, 12.0 nM). Compounds **4**, **6**, **7**, **9**, **10**, **11**, **16**, and **17** only had a somewhat inhibitive effect on hCA II (Ki values ranged from 52.6 to 90.1 nM). Differentials **8**, **12**, **13**, **15**, and **18**–**22** demonstrated only slightly inhibitory effects (Ki values ranging from 100.7 to 665.1 nM). The activity of the hCA IX isoform was strongly inhibited by molecules **2**–**12** and **14**–**21** (Ki, 8.9–88.3 nM). Derivatives **13** and **22** showed only slightly inhibitory action (Ki, 123.5 and 184.3 nM) against hCA IX in compared to AAZ (Ki, 25.0 nM). The activity of the hCA XII isoform was significantly inhibited by the quinazolines **3**, **5**, **7**, **14**, and **16** (Ki, 5.4–19.5 nM) in comparison to the activity inhibited by AAZ (Ki, 5.7 nM). The compounds **2**, **4**, **6**, and **10** demonstrated a moderate inhibiting capacity (Ki, 34.7–46.5 nM) directed to hCA XII. There was just a hint of an inhibitory impact seen for **8**, **9**, **11**, **12**, **13**, **15**, and **17**–**22** (Ki values ranged from 52.8 to 91.5 nM).

3.3. Molecular docking of compound 8 with hCAs IX and XII

Molecular modelling is an effective and valuable method for investigating bioactive drug's molecular structure, biological properties, and the binding mechanism of ligand molecules within probable receptor/enzyme binding sites (Goda et al., 2005, El-Ayaan et al., 2007, El-Azab et al., 2016). The designed molecules and co-crystallized bound inhibitor were docked into the interaction site of the target protein to achieve docking accuracy and demonstrate an optimal and acceptable binding orientation (El-Ayaan et al., 2007, Al-Suwaidan et al., 2015, Alkahtani et al., 2019, El-Azab et al., 2020). Compound **8** was used for molecular



Scheme 1. Quinazoline-based CA inhibitors synthesis (**4–22**).

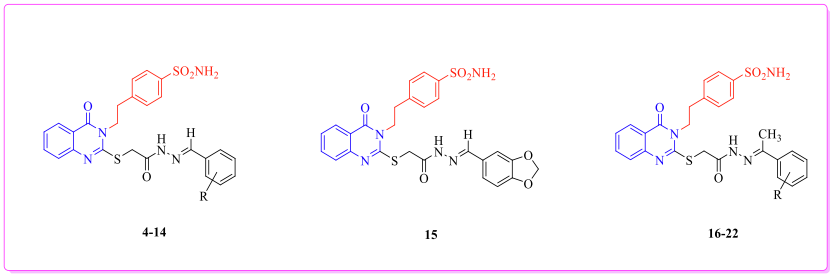
docking investigation in the binding sites of CA IX (PDB ID: 5FL4) and CA XII (PDB ID: 1JD0). These enzymes were co-crystallized with 5-(1-naphthalen-1-yl-1,2,3-triazol-4-yl)thiophene-2-sulfonamide (9FK) and 5-acetamido-1,3,4-thiadiazole-2-sulfonamide (AAZ), respectively. Molecular docking results showed compound **8** interacted favourably with the catalytic sites of hCAs IX and XII (Fig. 2,3 and Table S1).

4. Discussion

4.1. Chemistry

The obtained Schiff bases **4**–**22** were approved by the elimination of the NH_2 group singlet peak at 4.370 ppm and the appearance of

Table 1
Inhibition of hCAs I, II, IX, and XII, novel Schiff base quinazolines 2–22 and AAZ; [b]CAI data for 2–4 and 16 according to previous data (El-Azab et al., 2022), [a]Mean from 3 different assays, by a stopped-flow technique (errors range ± 5–10 % of the reported values).



Comps	R	Ki (nM)[a]				Selectivity analysis			
		hCA I	hCA II	hCA IX	hCA XII	hCA I/IX	hCA I/XII	hCA II/IX	hCA II/XII
2[b]	–	106.70	21.30	10.50	34.70	10.160	3.070	2.020	0.60
3[b]	–	87.60	16.90	52.10	5.40	1.68	16.22	0.31	3.11
4[b]	H	152.40	52.60	61.70	38.40	2.470	3.960	0.850	1.370
5	2-OH	132.4	27.5	14.7	10.2	9.00	12.98	1.87	2.70
6	3-OH	957.3	90.1	38.9	34.7	24.60	27.58	2.31	2.60
7	4-OH	664.7	76.4	62.2	19.5	10.68	34.08	1.23	3.92
8	2-OCH_3	1658	184.8	8.9	64.8	186.29	25.58	20.77	2.85
9	3-OCH_3	756.4	64.7	23.8	59.2	31.78	12.77	2.72	1.09
10	4-OCH_3	558.2	83.4	49.7	46.5	11.23	12.00	1.68	1.79
11	2,4-di-OCH_3	2298	50.9	52.7	74.3	43.60	30.928	2.68	0.68
12	3,4-di-OCH_3	1058	259.7	88.3	69.2	11.98	15.289	2.94	3.75
13	3,4,5-tri-OCH_3	1982	665.1	123.5	91.5	16.05	21.66	5.38	7.27
14	4-OH-3-OCH_3	857.0	29.7	60.1	14.2	14.25	60.35	0.50	2.09
15	–	692.3	100.7	16.5	52.8	41.95	13.11	6.10	1.91
16[b]	H	124.30	67.50	55.70	12.70	2.22	9.79	1.22	5.32
17	3-OCH_3	1634	55.8	54.6	80.1	29.92	20.40	1.02	0.70
18	4-OCH_3	627.30	126.00	43.90	57.50	14.28	10.909	2.87	2.19
19	3,4-di-OCH_3	824.6	188.5	40.3	68.1	20.46	12.108	4.70	2.77
20	2,5-di-OCH_3	1695	228.4	29.4	79.9	57.65	21.21	7.77	2.86
21	3,5-di-OCH_3	1157	130.8	52.7	58.0	21.95	19.95	2.48	2.25
22	3,4,5-tri-OCH_3	2308	332.7	184.3	85.2	12.52	27.09	1.80	3.90
AAZ	–	250	12	25	5.7	10	43.86	0.48	2.10

benzylidene moiety (-$SCH_2CONHN{=}CH$-R) or phenylethylidene moiety (-$SCH_2CONHN{=}C(CH_3)$-R) peaks. The singlet thiomethyl peak ($SCH_2CONHN{=}CH$-R) at 4.75–4.14 ppm in ^{1}H NMR and 33.64–33.59 ppm in ^{13}C NMR helped to identify the benzylidene moiety of Schiff bases **4**–**15**. The peak was further verified by benzylidene and aminocarbonyl protons ($SCH_2CONHN{=}CH$-R) at 9.68–7.99 and 12.25–11.39 ppm in ^{1}H NMR, whereas ^{13}C NMR confirmed the carbonyl and iminocarbon groups ($SCH_2CONHN{=}CH$-R) at 169.60–167.78 and 156.56–156.10 ppm, respectively. The ethylidene moiety of Schiff bases **16**–**22** was assigned by singlet thiomethyl peak ($SCH_2CONHN{=}C(CH_3)$-R) at 4.73–4.23 ppm in ^{1}H NMR and 33.63–33.60 ppm in ^{13}C NMR. The methyl peak ($SCH_2CONHN{=}C(CH_3)$-R) at 2.46–2.21 ppm in ^{1}H NMR and 18.64–12.49 in ^{13}C NMR, in addition to aminocarbonyl peak ($SCH_2CONHN{=}C(CH_3)$-R) at 11.11–10.71 ppm in ^{1}H NMR also verified the above result. The carbonyl and imino groups of the phenylethylidene moiety ($SCH_2CONHN{=}C(CH_3)$-R) were detected through ^{13}C NMR at 170.15–169.92 and 156.48–156.11, respectively (Scheme 1).

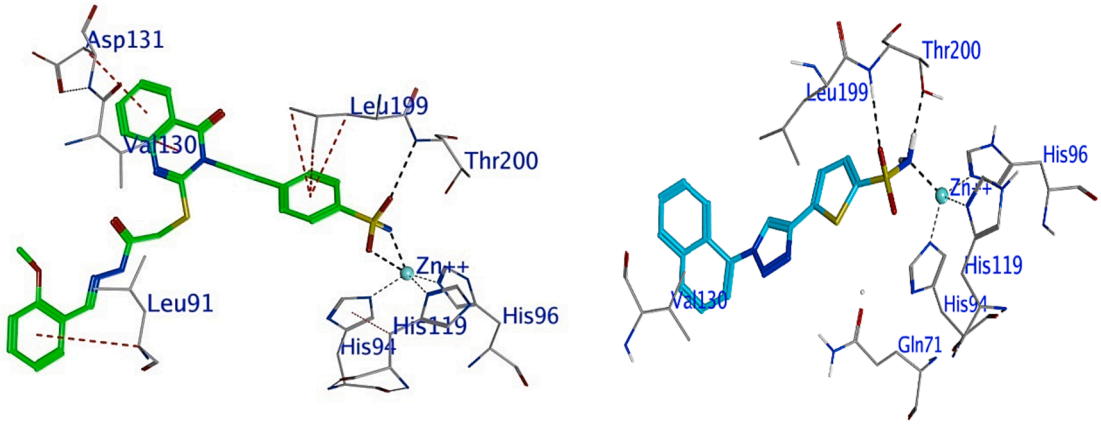


Fig. 2. The 3D of co-crystallized inhibitor (right panel) and docked compound **8** (left panel) into the binding site of human carbonic anhydrases IX (PDB code: 5FL4).

4.2. CA inhibitory activity

The structure–activity correlations for hCA I activities (Table 1) showed that mercapto-ethyl ester **2** and acetohydrazide **3** had significantly higher hCA I activities (Ki, 106.70 and 87.60 nM, respectively) than AZZ (Ki, 250.0 nM). Upon converting acetohydrazide **3** into Schiff bases **4**–**22** (Ki, 124.3–2308 nM), distinct hCA I activity was noted. All substituted benzylidenes, including hydrazones **5**–**15** (Ki, 132.4–2298 nM), were less effective than unsubstituted derivative **4** (Ki, 152.40 nM), excluding 2-hydroxybenzylidenes **5**, which had a Ki value of 132.4 nM, indicating considerable hCA I activity. Moreover, 4-hydroxybenzylidene **7** (Ki, 664.7) was more potent than 3-hydroxybenzylidene **6** (Ki, 957.3 nM); however, it was not as potent as 2-hydroxybenzylidene **5** (Ki, 132.4 nM). When the hydroxy-group of 2-hydroxybenzylidene **5** (Ki, 132.4 nM) was replaced with a methoxy group, 2-methoxybenzylidene **8** (Ki, 1658.0 nM) was produced, which had significantly less CA I activity. Furthermore, compared to 2-methoxybenzylidene **8** (Ki, 1658.0 nM), 3-methoxybenzylidene **9** (Ki, 756.4 nM) had greater potency, but it was less potent than 4-methoxybenzylidene **10** (Ki, 558.2 nM). The CAI activity of dimethoxybenzylidenes **11** and **12** (Ki, 2298 and 1058.0 nM) and trimethoxybenzylidene **13** (Ki, 1982 nM) was dramatically decreased by the addition of a methoxy group to 4-methoxybenzylidene **10** (Ki, 558.2 nM). Compared to substituted phenylethylidenehydrazinyl derivatives **18**–**22** (Ki, 627.3–2308 nM), phenylethylidenehydrazinyl **16** (Ki, 124.30 nM) exhibited greater potency. Moreover, among 3-methoxyphenylethylidenehydrazinyl derivative **17** (Ki, 1634 nM), dimethoxybenzylidenes **19**–**21** derivatives (Ki, 824.6–1695.0 nM), and trimethoxyphenylethylidenehydrazinyl derivative **22** (Ki, 2308 nM), 4-methoxyphenylethylidenehydrazinyl derivative **18** (Ki, 627.3) demonstrated considerable CA I activity. According to structure–activity correlations for hCA II activities (Table 1), mercaptoethylester **2** and acetohydrazide **3** exhibited considerable hCA II activities (Ki, 21.3 and 16.9 nM, respectively), compared with AZZ (Ki, 12.0 nM). When acid-hydrazide **3** was changed to the appropriate Schiff bases **4**–**22**, various hCA II activities were generated (Ki, 27.5–665.1 nM). Substituting the hydroxy group of 2-hydroxybenzylidene **5** (Ki, 27.5 nM) via a methoxy group yielded 2-methoxybenzylidene **8** (Ki, 184.8.0 nM) with significantly reduced CA II activity. However, substituting the hydroxyl groups of benzylidenes **6** and **7** (Ki, 90.1 or 76.4 nM) with methoxy groups yielded benzylidenes **9** and **10** (Ki, 64.7 and 83.4 nM) with moderate enhancement in CA II activity. In particular, 2-Hydroxybenzylidene **5** (Ki, 27.5 nM) was more potent than unsubstituted analouge **4** (Ki, 52.60, nM), 3-hydroxybenzylidene **6** (Ki, 90.1 nM), and 4-hydroxybenzylidene **7** (Ki, 76.4 nM). However, **6** and **7** (Ki, 90.1 and 76.4 nM, respectively) were less potent than the unsubstituted derivative **4** (Ki, 52.60 nM). Methoxybenzylidenes **8** and **10** (Ki, 184.8 and 83.4 nM) generated 2,4-dimethoxybenzylidene **11** (Ki, 50.9 nM) with a much higher CA II activity when additional methoxy groups were added. By contrast, adding another methoxy group to methoxybenzylidenes like **9** and **10** (64.7 and 83.4 nM) produced benzylidenes**12** and **13** (Ki; 259.7 and 665.1 nM) with dramatically reduced CA II activity. Introducing a hydroxy group to 3-methoxybenzylidene **9** (Ki, 64.7 nM) yielded benzylidene **14** (Ki, 29.7 nM) with significantly enhanced CA II activity. Phenylethylidenehydrazinyl derivative **16** (Ki, 67.5 nM) was more potent than substituted phenylethylidenehydrazinyl derivatives **18**–**22** (Ki, 126.0–332.7 nM). However, none of the abovementioned compounds were more potent than the 3-methoxyphenylethylidenehydrazinyl derivative **17** (Ki, 55.8 nM). Comparing monomethoxyphenylethylidenehydrazinyl derivatives **17** and **18** (Ki, 55.8 and 126.0 nM) with dimethoxybenzylidenes **19**–**21** derivatives (Ki, 130.8–228.4 nM) and trimethoxyphenylethylidenehydrazinyl derivative **22** (Ki, 332.7 nM), the 3-methoxyphenylethylidenehydrazinyl derivative **17** exhibited significant CA II activity among these. As evidenced by the structure–activity correlations for hCA IX activities (Table 1), compared to AAZ (K_I, 25.00 nM). The hCA IX activity of acetohydrazide**3** and its related hydrazones **4**–**22** are varied (K_I, 8.90–184.30 nM). Except for **7** (K_I, 62.20 nM), an hCA IX inhibitor that is equivalent to unsubstituted hydrazone **4**, mono-substituted benzylidenes **5**–**10** (K_I, 8.90–49.70 nM) were more powerful inhibitors than **4** (K_I, 61.70 nM). When hydroxybenzylidenes **5**, **6**, and **7** (K_I, 14.70, 38.90, and 62.60 nM, respectively) were converted into methoxybenzylidenes **8**, **9**, and **10** (K_I, 8.90, 23.80, and 49.70 nM, respectively), CA IX activity increased significantly, indicating that the 2-substituent is powerful than the 3- and 4-substituents of benzylidenes **5**–**10**. Adding more methoxy groups to benzylidenes **8**, **9**, **10**, as well as **6** (K_I, 8.90, 23.80, 49.70, and 38.90 nM, respectively) lowered their hCA IX inhibiting action when compared to benzylidenes **11**, **12**, **13**, additionally **14** (K_I, 52.70, 88.30, 123.50, and 60.10 nM, respectively). Substituting the benzene ring of **4** (K_I, 61.70 nM) to benzo[d][1,3]dioxole ring of benzylidene **15** increased CA IX activity (K_I, 16.50 nM). Even though methoxylated phenylethylidenes **17**–**22** (K_I, 29.40–54.60 nM) exhibited greater efficacy than unsubstituted phenylethylidene **16** (K_I, 55.70 nM), nevertheless 3,4,5-trimethoxyphenylethylidene **22** (K_I, 184.30 nM) had weak hCA IX inhibiting effectiveness. The results of the structure–activity relationship between hCA XII activity and K_I values were as follows (Table 1). Merqaptoethylester **2** exhibited a moderate level of hCA XII activity (KI, 34.70 nM) in comparison to which had a KI value of 5.70 nM). Hydrazinolysis of merqaptoethyl ester **2** (K_I, 34.70 nM) afforded acetohydrazide **3** (K_I, 5.40 nM) with enhanced hCA XII inhibitory effect. Unsubstituted derivative **4** (K_I, 38.40 nM) exhibited a lower hCA XII inhibition compared with hydroxylbenzylidenes **5**–**7** (K_I, 10.2–34.7 nM). In comparison, 2-hydroxylbenzylidene **5** (K_I, 10.2 nM) showed a higher hCA XII inhibitory activity related to other hydroxylbenzylidenes **6** and **7** (K_I, 34.7 and 19.5 nM, respectively). The CA XII activity was substantially reduced when a hydroxyl group in benzylidenes **5**–**7** (Ki, 10.2–34.7 nM) was substituted with a methoxy group in benzylidenes **8**–**10** (Ki, 46.5–64.8 nM). Incorporating other methoxy groups to benzylidenes **8**–**10** (Ki, 46.5–64.8 nM) yielded benzylidenes **11**–**13**, with lower CA XII inhibitor activity (K_I, 69.2–91.5 nM), whereas benzylidene **14** (Ki, 14.2 nM) enhanced the activity of benzylidene **7** (Ki, 19.5 nM). Benzylidene **15** with lower hCA XII activity (Ki, 52.80 nM) was obtained by substituting the phenyl ring of **4** (K_I, 38.40 nM) with a benzo[d][1,3]dioxole ring. Compared with phenylethylidene **16** (K_I, 12.70 nM), substituted phenylethylidenes **17**–**22** (K_I, 57.5–85.2 nM) have exhibited lower hCA XII inhibition.

4.3. Molecular docking of compound 8 with hCAs IX and XII

The benzenesulfonamide group of **8** penetrated deep into the active areas of both targets, thus forming critical interactions with the zinc ions present at the catalytic sites (El-Azab et al., 2019). In the case of hCA IX, the negatively charged SO_2NH- group of compound **8** coordinated with the zinc ions (ZN 264) is a critical component of the catalytic site (Figs. 2, 3 and Table S1). This interaction could hint at the compound's potential inhibitory mechanism, which might involve obstructing or chelating the metal ion vital for enzyme activity. Additionally, the SO_2NH- group formed a hydrogen bond with THR 200, located near the zinc ion via its O17 atom, suggesting its potential to stabilize within the active site. Also, compound **8** formed pi-H interactions with several amino acid residues, including LEU 91, VAL 130, ASP 131, and LEU 199. These pi-H interactions emphasize the compound's ability to form extensive binding interactions within the active site, which could result in potent inhibition. Similarly, in the case of hCA XII, the benzenesulfonamide moiety of compound **8** showed interaction with the zinc ion (ZN 901) at the catalytic site through the SO_2NH- group (Figs. 2, 3 and Table S1). This interaction between benzenesulfonamide and zinc ions was particularly significant for stabilizing the ligand-receptor complex. Moreover, the strong ionic interaction between ZN 901 and HIS 94, in particular, might be pivotal in its inhibitory action. Also, compound **8** established hydrogen bonds with GLN 92, THR 199, and THR 200, stabilizing the complex inside the hCA XII active site. Evidently, the orientation of the benzenesulfonamide group in **8** was similar to that of

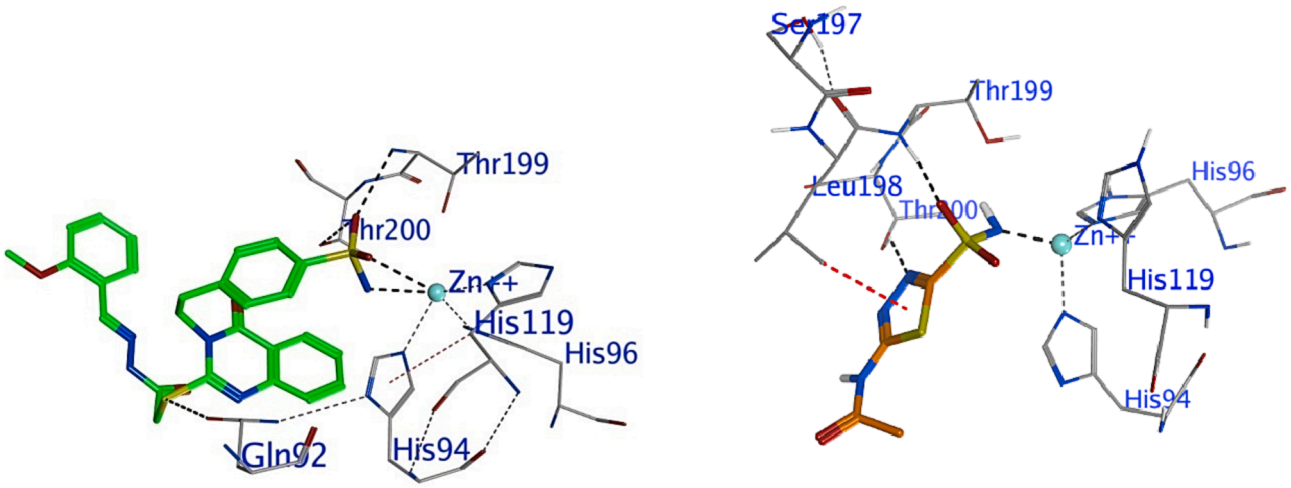


Fig. 3. The 3D of co-crystallized inhibitor (right panel) and docked compound **8** (left panel) into the binding site of human carbonic anhydrases XII (PDB code: 1JD0).

the corresponding groups in the co-crystallized bound inhibitors of both hCA IX and hCA XII enzymes, thus indicating that benzylidene **8** might interact similarly with these targets as co-crystallized ligands. This observation is consistent with the binding scores of **8** with both targets, thus suggesting that it can effectively interact with the receptor and stabilize the complex. Therefore, benzylidene **8** might be a promising lead compound for developing new inhibitors targeting these enzymes.

5. Conclusion

This study aimed to evaluate the inhibitory effects of a range of newly substituted hydroxyl-Schiff bases, which were dependent on the ethylbenzensulfonamide **4**–**22** scaffold, against the I, II, IX, and XII isoforms (hCA, EC 4.2.1.1). In comparison to AAZ (Ki, 250.0 nM), the hydrazones **2**, **3**, **4**, **5**, **7**, **10**, **15**, and **18** demonstrated significant efficacy in reducing the activity of the hCA I isoforms (K_I, 87.6–692.3 nM). When compared to AAZ (Ki, 12.0 nM), the inhibitory effects of quinazolines **2**, **3**, **5**, and **14** on the hCA II isoform (K_I, 16.9–29.7 nM) were dramatically reduced. When compared to AAZ (Ki, 25.0 nM), the activity of the hCA IX isoform was significantly lowered by quinazolines **2**–**12** and **14**–**21** (K_I, 8.9–88.3 nM). The activity of the hCAXII isoform was severely decreased by quinazolines **3**, **5**, **7**, **14**, and **16**, exhibiting KI values ranging from 5.4 to 19.5 nM. When compared to AAZ (SI, 10.0), the substituted hydroxyl-benzylidenethioacetohydrazides **6**–**15** and phenylethylidenethioacetohydrazides **17**–**22** exhibited significant levels of selective inhibitory action against hCA IX, with selectivity indexes ranging from 10.68 to 168.29 and 12.52 to 57.65, respectively, over hCA I. The compound known as benzenesulfonamide **14** had a higher degree of selectivity in inhibiting the enzyme hCA XII over hCA I, achieving a selectivity index (SI) of 60.35. This selectivity level was superior than that of AAZ, which had an SI of 43.86. Compounds **5**, **6**, **7**, **8**, **12**, **13**, **14**, **16**, **18**, **19**, **20**, **21**, and **22** had a higher selectivity index for inhibiting the hCA XII enzyme over the hCA II enzyme, with selectivity index values ranging from 2.09 to 7.27, in contrast, AAZ demonstrated selectivity index values of 2.10 for the abovementioned enzyme. Compounds **4**–**22** exhibited notable inhibition of hCA IX over hCA II, with a substantial selectivity index (SI, 0.50–20.77); this level of selectivity was better than AAZ, which had a SI of.0.48.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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