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Synthesis, characterization, carbonic anhydrase inhibitor activity, and docking studies of phenylthiazol-2 (3h)-ylidene-isoquinoline-5-amine Derivatives

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ABSTRACT

Phenylthiazol-2(3h)-ylidene-isoquinoline-5-amine derivatives(5a-r) were easily prepared from 5-aminoisoquinoline, thioisocyanate, and phenacyl bromide derivatives in the presence of triethylamine in tetrahydrofuran (THF), and dimethyl formamide (DMF) in two steps. In the first step, thiourea was synthesized in THF reflux, 24 h and then in the second step, the formation of thiazole ring was ensured in EtOH-DMF (5:5 v/v), reflux, 24 h. The newly synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR, IR, and elemental analysis. The inhibitory effects of 18 newly synthesized compounds (5a-r) on hydratase and esterase activities of carbonic anhydrase isoenzymes (hCA I, II, IX, and X) were studied in vitro.

1. Introduction

Quinoline and its derivatives are well known to organic synthesizers because of their diverse chemical and biological properties [1–3]. Its derivatives are especially used with anti-bacterial [4], anti-malarial [5], anti-microbial [6,7], anti-cancer [8] and anti-tumor activities [9]. Also, the quinoline-based compounds show potent anti-tubercular activity [10–13]. Ciprofloxacin, Levofloxacin, and moxifloxacin are important agents used in the treatment of antitubercular activity with a quinoline moiety [14]. The chemical structures of some quinoline derivatives in clinical use are given in Fig. 1.

In addition to these structures, many heterocyclic compounds bearing a five-membered ring in their structure have a good spectrum of biological activities. Thiazole, a unique heterocycle containing sulfur and nitrogen atoms, is an essential core scaffold found in many natural (Vitamin B1-Thiamine) and synthetic medically important compounds [15–18]. Some of its derivatives have commonly used as anti-microbial (sulfazole) [19], anti-retroviral (ritonavir) [20], anti-fungal (abafungin) [21], anti-histamine and anti-thyroid activities [22–26].

Carbonic anhydrase was first purified from erythrocytes in 1933 [27], and different numbers of isoenzymes were characterized in later studies. Of these, those of animal and plant origin are grouped into α and β classes, respectively. The point common to all isoenzymes is that they contain a zinc (Zn^{+2}) ion in their active site. The main enzymatic event catalyzed by carbonic anhydrase is the hydration of carbon dioxide (CO_2). Kinetic studies have shown that there is a two-step mechanism in all carbonic anhydrase isoenzymes. In the first step, the nucleophilic attack of the zinc-bound hydroxide ion on CO_2 takes place. In the second step, the ionization of the water molecule bound to the zinc and the re-establishment of the active site by removing the proton take place. In the catalytic mechanism of carbonic anhydrases, His-64 unit plays an intermediary role in transferring the proton from the active site to the external solvent environment. The proton is transferred to His-64 via water molecules between it and the zinc atom. Studies show that Lys-91 and Tyr-131 units also play a role in the proton transfer mechanism [28, 29].

It was reported that the isoquinoline moiety established the numerous van der Waals interactions involving residues in both of the hydrophilic and hydrophobic areas of the hCA II (Gln92, Val121 and Phe131) [30]. In another study, it was suggested that the presence of C-1 small substituents on isoquinoline scaffold controls both CA inhibitory potency and selectivity [31]. Gitto et al. reported that CA inhibitors containing the isoquinoline scaffold, several of which proved to be very efficient (Ki <10 nM) inhibitors exhibiting good selectivity towards the

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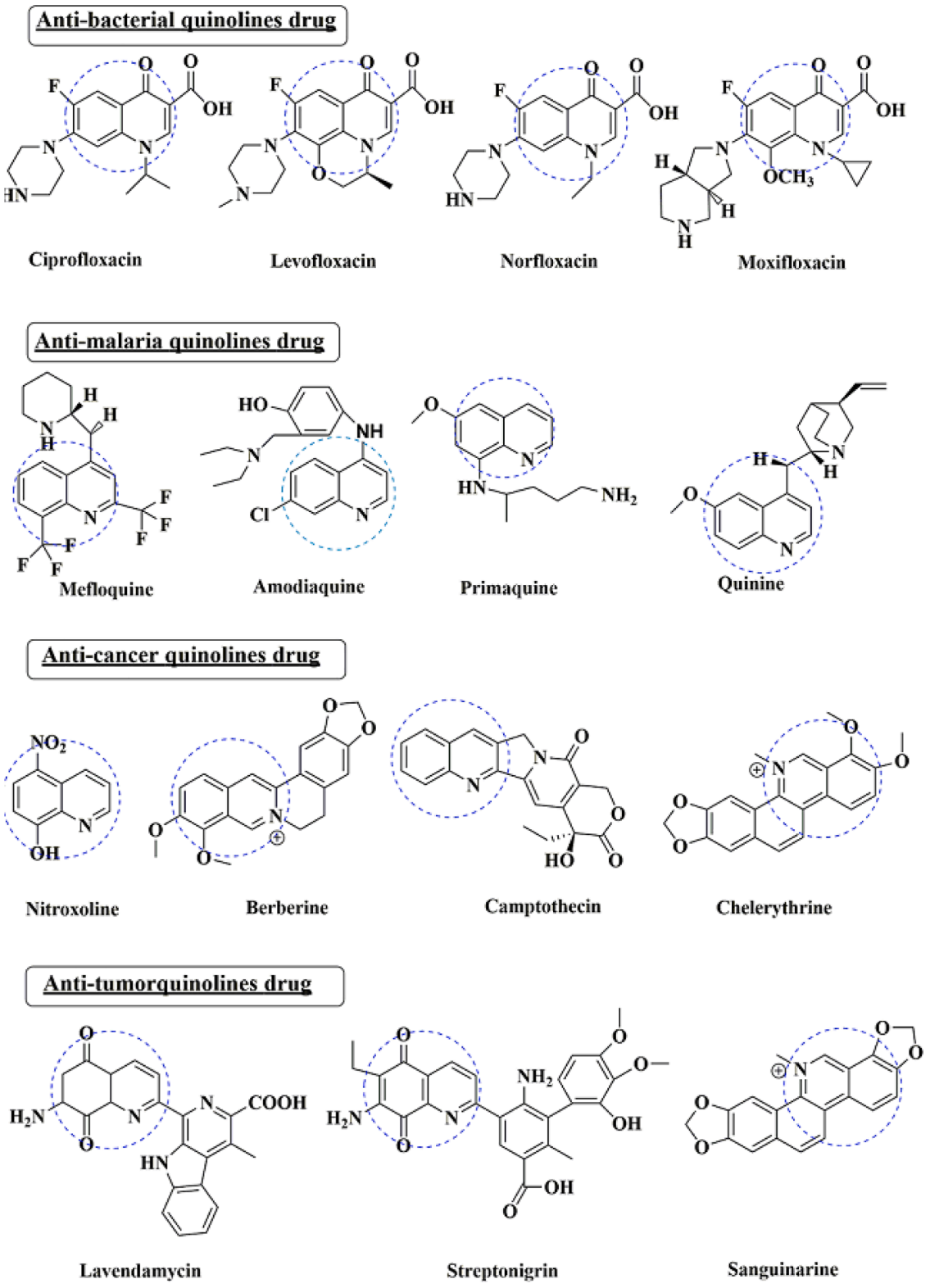
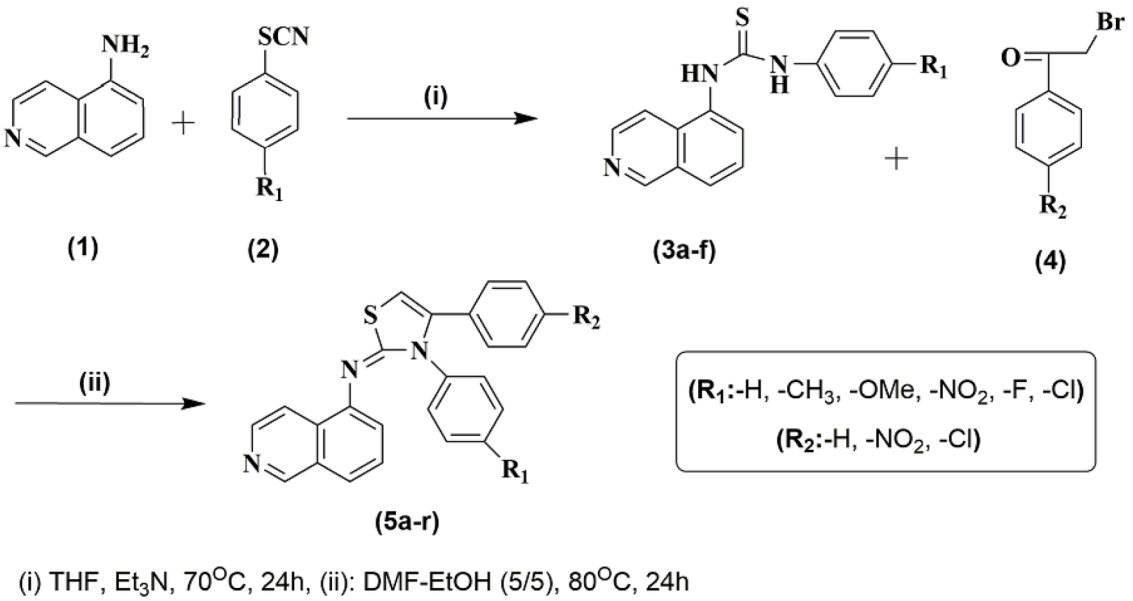


Fig. 1. Chemical structures of quinoline and a few quinoline derivatives in clinical use.

established antitumor target hCA IX and the potentially druggable one hCA XIV [32]. In addition, it was reported that isoquinoline sulfonamides that showed inhibitory effect against some hCA isozymes at nanomolar concentrations. It was suggested that these isoquinolines particularly useful for the design of new CAIs with minor side effects [33]. Distinto et al. synthesized new sulfonamide having thiazole ring in order to evaluate the effect of substituents in positions 3 and 4 of the dihydrothiazole ring on the inhibitory potency and selectivity toward human carbonic anhydrase isoforms I, II, IX, and XII. These compounds showed good activity against hCA II isoform with Ki values of 5.3, 4.5, 3.8, and 5.3 nM, respectively being more potent than reference drug AAZ (Ki = 12 nM).It seems that the introduction of halogen with a small atomic radius at the 4 position of the phenyl ring at the 4 position of thiazole moiety is beneficial. The introduction of halogen with a larger



Scheme 1. General synthesis procedure for target compounds.

atomic radius led to a decrease of activity [34]. Meleddu et .al. was synthesized sulfonamide having thiazole ring and the activity of the new compounds as inhibitors of hCA I, II, IX, and XII was evaluated. It was expressed that the nature of the substituents in the position 3 and 4 of the dihydro-thiazole ring was relevant in determining both activity and selectivity profiles. It was suggest that the introduction of a five member heterocycle in 4-position on the dihydrothiazole might be relevant to achieve a high inhibition of hCA II. The binding mode in a way proves the importance of substitution at position 3 and 4 of the thiazolidinone moiety because it allows better accommodation in the binding pocket. The nature of the substituent at position 4 of the dihydrothiazole ring was found to play an important role for determining the activity and selectivity of these compounds [35]. Kocyigit et al. synthesized a number of novel thiazole derivatives and evaluated their inhibitory characteristics against the human CA isoenzymes I and II. From the obtained results, it seems that these compounds can be lead compounds for the development of more active CA inhibitors [36].

This work concentrated on the design and synthesis of new quinoline analogs containing the thiazole ring. For this, we proposed the use of 5-aminoisoquinoline, thioisocyanate, and phenacyl bromide derivatives in the preparation of the phenyl thiazol-2(3 h)-ylidene-isoquinoline-5-amine derivatives. These newly synthesized compounds were investigated in vitro for their inhibitory activity towards the hCA isoforms I II, IX, and X using both hydratase and esterase assay. Also, in this study, elevated the intermolecular interactions of these compounds, and docking scores.

2. Experimental

2.1. Instruments

All starting materials and reagents were purchased from commercial suppliers. Reactions were monitored by TLC; the plates were visualized with short wave UV fluorescence (k=254 nm). Melting points were taken on a Yanagimoto micro melting point apparatus and were uncorrected. IR spectra (ν, cm^{-1}) were measured on a SHIMADZU Prestige21 (200 VCE) spectrometer. ^1H NMR and ^{13}C NMR spectra were taken on Bruker AV-400 MHz instrument (JEOL LNM-GSX 400a, JEOL JMN-ECP 400,) in DMSO-*d6* using TMS as the internal standard. The elemental analysis was carried out with a Leco CHNS-932 (St. Joseph, MI) instrument. All chemicals were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Taufkirchen, Germany).

2.2. Synthesis

2.2.1. General synthesis of thiourea derivatives (3a-f)

A mixture of 5-aminoisoquinoline (**1**) (1.0 mmol) and thioisocyanate derivatives (**2**) (1.0 mmol) with the addition of three drops of triethyl amine was stirred and refluxed at 70 °C for 24 hours in tetrahydrofuran (THF) (7 mL). After completion of the reaction, the mixture was cooled to room temperature and then poured into cold water (50 mL). Product (**3**) was filtered, washed with water, and dried.

2.2.2. General synthesis of thiazole derivatives (5a-r)

Thiourea derivatives (3a-f) (1 mmol) and phenacyl bromide derivatives (**4**) (1 mmol) in EtOH/DMF (5:5 v/v) were stirred and refluxed in 80 °C, for 24 h. After completion of the reaction, the mixture was allowed to cool to room temperature and poured into cold water (50 ml). The product (5a-r) was filtered, washed with water, and dried. Compounds **(3a-f)** and (5a-r) were synthesized as shown in Scheme 1.

2.2.2.1. (E)-N-(3,4-diphenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5a). Yellow solid; yield 98%; m.p.124–126 °C; IR (KBr, cm^{-1}): 3027, 1613, 1577, 1545, 1490, 1389; ^1H NMR (DMSO-d6): δ 9.73(1H, s, H30-isoquinoline), 8.51–7.41(5H, m, H29,31,32,33,34-isoquinoline), 7.31–7.15(10H, d, phenyl protons), 6.65(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 99.01, 120.45, 122.96, 123.89, 124.17, 128.54, 128.78, 128.86, 128.97, 129.03, 129.23, 129.47, 129.61, 129.67, 131.18, 131.64, 131.84, 133.69, 138.16, 147.05, 148.08, 162.04, 162.85, 166.67; Anal. Calcd for $C_{24}H_{17}N_3S$: C, 68.24; H, 4.52; N, 11.07; S, 8.45; Found: C, 68.27; H, 4.58; N, 11.00; S, 8.47%.

2.2.2.2. (E)-N-(4-(4-chlorophenyl)−3-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5b). Yellow solid; yield: 75%; m.p. 176–178 °C; IR (KBr, cm^{-1}): 3056, 1604, 1583, 1566, 1538, 1489; ^1H NMR (DMSO-d6): δ 9.77(1H, d, H30-isoquinoline), 8.53–7.87(5H, m, H29,31,32,33,34-isoquinoline), 7.46–7.19(9H, m, phenyl protons), 6.72(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 99.39, 120.64, 123.15, 123.89, 124.25, 128.54, 128.78, 128.86, 128.95, 129.03, 129.47, 129.65, 129.71, 130.02, 130.77, 131.76, 133.15, 133.75, 134.00, 137.96, 138.77, 147.08, 148.54, 161.83; Anal. Calcd for $C_{24}H_{16}ClN_3S$: C, 69.64; H, 3.10; Cl, 8.56; N, 10.15; S, 7.75; Found: C, 69.67; H, 3.09; Cl, 8.60; N,

10.12; S, 7.73%.

2.2.2.3. (E)-N-(4-(4-nitrophenyl)−3-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5c). Yellow solid; yield: 75%; m.p. 278–280 °C; IR (KBr, cm^{-1}): 3027, 1598, 1547, 1515, 1491, 1343; ^{1}H NMR (DMSO-d6): δ 9.57(1H, d, H30-isoquinoline), 8.46–7.93(5H, m, H29,31,32,33,34-isoquinoline), 7.87–7.36(9H, m, phenyl protons), 6.89(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 102.05, 121.78, 123.93, 124.08, 124.74, 128.54, 128.78, 128.86, 128.95, 129.01, 129.35, 129.59, 129.86, 129.97, 130.38, 130.77, 132.75, 136.30, 137.86, 137.98, 146.06, 147.64, 149.77, 161.34; Anal. Calcd for $C_{24}H_{16}N_4O_2S$: C, 67.91; H, 3.80; N, 13.20; O, 7.54; S 7.55; Found: C, 67.93; H, 3.89; N, 13.12; O, 7.61; S, 7.51%.

2.2.2.4. (E)-N-(4-phenyl-3-p-tolylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5d). Orange solid; yield: 97%; m.p. 140–142 °C; IR (KBr, cm^{-1}): 3029, 1594, 1544, 1508, 1485; ^{1}H NMR (DMSO-d6): δ 9.44(1H, m, H30-isoquinoline), 8.44–7.68(5H, m, H29,31,32,33,34-isoquinoline), 7.53–7.00(9H, m, phenyl protons), 6.54(1H, s, H35-thiazole ring), 2.26 (3H, m, CH_3); ^{13}C NMR (DMSO-d6): 21.24, 98.31, 120.97, 122.24, 123.27, 128.84, 128.92, 129.23, 129.46, 130.21, 130.44, 130.53, 130.67, 131.16, 131.40, 132.40, 134.88, 135.72, 137.62, 138.00, 138.17, 140.01, 147.16, 150.20, 161.67; Anal. Calcd for $C_{25}H_{19}N_3S$: C, 76.31; H, 4.87; N, 10.68; S 8.15; Found: C, 76.93; H, 4.89; N, 10.12; S, 8.13%.

2.2.2.5. (E)-N-(4-(4-chlorophenyl)−3-p-tolylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5e). Orange solid; yield: 78%; m.p. 250–252 °C; IR (KBr, cm^{-1}): 3060, 1637, 1601, 1585, 1540, 1477; ^{1}H NMR (DMSO-d6): δ 9.75(1H, m, H30-isoquinoline), 8.56–7.56(5H, m, H29,31,32,33,34-isoquinoline), 7.43–7.16(8H, m, phenyl protons), 6.66(1H, s, H35-thiazole ring), 2.27 (3H, m, CH_3); ^{13}C NMR (DMSO-d6): 21.50, 99.48, 120.73, 122.87, 123.35, 124.36, 128.92, 128.97, 129.34, 130.22, 130.77, 130.53, 130.67, 131.74, 133.20, 133.61, 133.94, 135.36, 137.62, 138.32, 138.87, 146.95, 148.35, 150.20, 162.10; Anal. Calcd for $C_{24}H_{18}ClN_3S$: C, 70.17; H, 4.24; Cl, 8.28; N, 9.82; S 7.49; Found: C, 70.13; H, 4.29; Cl, 8.26; N, 9.81; S, 7.51%.

2.2.2.6. (E)-N-(4-(4-nitrophenyl)−3-p-tolylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5f). Orange solid; yield: 85%; m.p. 271–273 °C; IR (KBr, cm^{-1}): 3063, 1597, 1576, 1555, 1553, 1538, 1476; ^{1}H NMR (DMSO-d6): δ 9.73(1H, m, H30-isoquinoline), 8.57–7.69(5H, m, H29,31,32,33,34-isoquinoline), 7.51–7.16(8H, m, phenyl protons), 6.90 (1H, s, H35-thiazole ring), 2.28 (3H, m, CH_3); ^{13}C NMR (DMSO-d6): 21.27, 102.24, 120.45, 123.09, 124.05, 124.38, 124.76, 128.97, 129.00, 130.33, 130.82, 130.92, 131.52, 133.46, 133.67, 133.94, 135.23, 137.42, 138.46, 138.87, 147.08, 147.53, 148.58, 150.20, 161.75; Anal. Calcd for $C_{25}H_{18}N_4O_2S$: C, 68.41; H, 4.15; N, 12.78; O, 7.33; S 7.55; Found: C, 68.93; H, 4.19; N, 12.72; O, 7.31; S, 7.50%.

2.2.2.7. (E)-N-(3-(4-methoxyphenyl)−4-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5 g). Yellow solid; yield: 93%; m.p. 264–266 °C; IR (KBr, cm^{-1}): 3056, 1595, 1581, 1553, 1506, 1463; ^{1}H NMR (DMSO-d6): δ 9.23(1H, m, H30-isoquinoline), 8.43–7.51(5H, m, H29,31,32,33,34-isoquinoline), 7.42–6.79(9H, m, phenyl protons), 6.46(1H, s, H35-thiazole ring), 3.70 (3H, m, –OCH_3); ^{13}C NMR (DMSO-d6): 55.51, 98.56, 114.70, 115.32, 117.25, 119.17, 123.03, 128.79, 128.82, 128.93, 128.99, 130.70, 130.83, 131.03, 131.34, 131.52, 140.07, 140.34, 141.56, 147.13, 152.18, 156.12, 159.01, 161.43; Anal. Calcd for $C_{25}H_{19}N_3OS$: C, 73.33; H, 4.68; N, 10.26; O, 3.91; S 7.83; Found: C,73.32; H, 4.70; N, 10.30; O, 3.94; S, 7.89%.

2.2.2.8. (E)-N-(4-(4-chlorophenyl)−3-(4-methoxyphenyl)thiazol-2(3H)ylidene)isoquinoline-5-amine, (5 h). Orange solid; yield: 78%; m.p. 275–277 °C; IR (KBr, cm^{-1}): 3063, 1603, 1584, 1562, 1552, 1488, 1383; ^{1}H NMR (DMSO-d6): δ 9.78(1H, m, H30-isoquinoline), 8.55–7.77(5H, m, H29,31,32,33,34-isoquinoline), 7.38–6.96(8H, m, phenyl protons), 6.69(1H, s, H35-thiazole ring), 3.73 (3H, m, –OCH_3); ^{13}C NMR (DMSO-d6): 55.70, 99.30, 114.86, 120.92, 123.03, 129.33, 124.30, 128.96, 130.08, 131.88, 132.76, 133.86, 134.00, 139.03, 140.07, 146.95, 148.18, 155.35, 162.49; Anal. Calcd for $C_{25}H_{18}ClN_3OS$: C, 67.64; H, 4.09; Cl, 7.99; N, 9.47; O, 3.60; S 7.22; Found: C, 67.63; H, 4.12; Cl, 8.00; N, 9.41; O, 3.61; S, 7.24%.

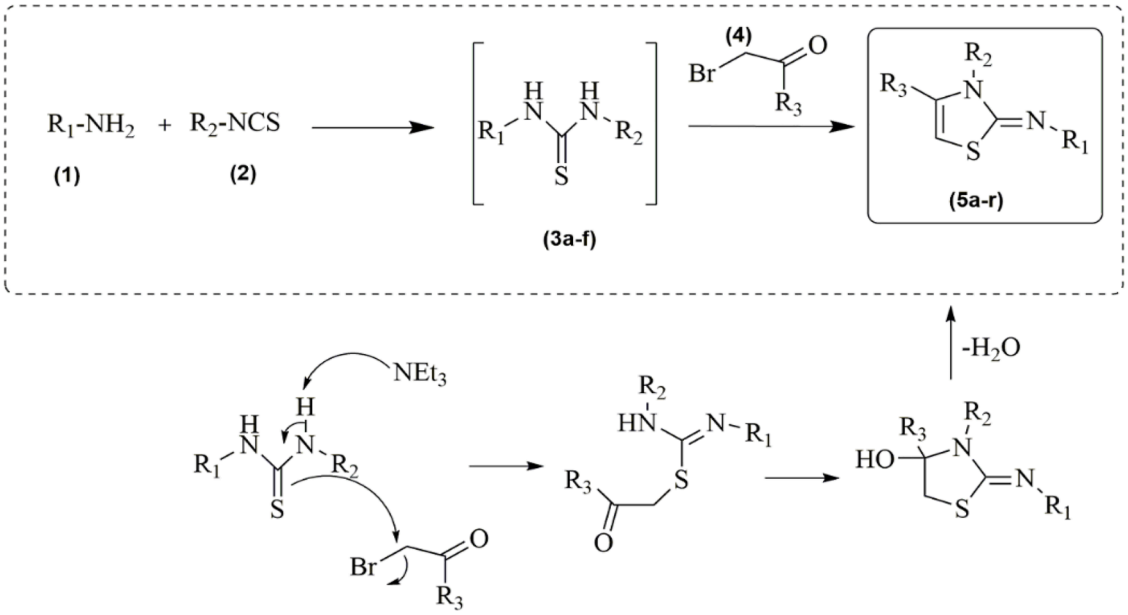
2.2.2.9. (E)-N-(3-(4-methoxyphenyl)−4-(4-nitrophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5ı). Yellow solid; yield: 72%; m.p. 230–232 °C; IR (KBr, cm^{-1}): 3065, 1696, 1574, 1553, 1505, 1385; ^{1}H NMR (DMSO-d6): δ 9.78(1H, m, H30-isoquinoline), 8.60–7.77(5H, m, H29,31,32,33,34-isoquinoline), 7.50–6.91(8H, m, phenyl protons), 6.63 (1H, s, H35-thiazole ring), 3.72 (3H, m, –OCH_3); ^{13}C NMR (DMSO-d6): 99.75, 100.13, 113.03, 119.21, 122.11, 123.00, 124.67, 125.10, 126.07, 127.85, 128.65, 128.83, 129.11, 129.74, 130.92, 131.30, 133.21, 133.62, 135.35, 136.40, 139.08, 140.26, 157.62, 168.42; Anal. Calcd for $C_{25}H_{18}N_4O_3S$: C, 66.07; H, 3.99; N, 12.33; O, 10.56; S 7.05; Found: C, 66.13; H, 3.97; N, 12.38; O, 10.57; S, 7.11%.

2.2.2.10. (E)-N-(3-(4-nitrophenyl)−4-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5i). Brown solid; yield: 93%; m.p. 264–266 °C; IR (KBr, cm^{-1}): 3076, 1693, 1590, 1547, 1489, 1330; ^{1}H NMR (DMSO-d6): δ 9.72(1H, m, H30-isoquinoline), 8.60–7.71(5H, m, H29,31,32,33,34-isoquinoline), 7.38–6.61(9H, m, phenyl protons), 6.63(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 101.02, 113.02, 117.99, 121.16, 121.90, 125.87, 126.05, 128.98, 130.51, 130.97, 131.77, 133.73, 134.21, 137.70, 139.62, 146.97, 148.18, 148.46, 151.77, 157.05, 165.98; Anal. Calcd for $C_{24}H_{16}N_4O_2S$: C, 67.91; H, 3.80; N, 13.20; O, 7.54; S 7.55; Found: C, 68.01; H, 3.87; N, 13.38; O, 7.57; S, 7.51%.

2.2.2.11. (E)-N-(4-(4-chlorophenyl)−3-(4-nitrophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5j). Brown solid; yield: 72%; m.p. 273–275 °C; IR (KBr, cm^{-1}): 3027, 1609, 1552, 1518, 1492, 1343; ^{1}H NMR (DMSO-d6): δ 9.75(1H, m, H30-isoquinoline), 8.60–7.71(5H, m, H29,31,32,33,34-isoquinoline), 7.38–6.61(8H, m, phenyl protons), 6.63 (1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 101.01, 113.02, 118.22, 120.91, 121.90, 122.14, 123.27, 124.85, 128.97, 129.88, 130.51, 130.73, 130.98, 131.62, 131.91, 133.44, 134.24, 137.88, 138.79, 143.54, 151.97, 157.13, 166.21; Anal. Calcd for $C_{24}H_{15}ClN_4O_2S$: C, 62.81; H, 3.29; Cl, 7.72; N, 12.21; O, 6.97; S 6.99; Found: C, 62.93; H, 3.27; Cl, 7.75; N, 12.28; O, 7.03; S, 7.01%.

2.2.2.12. (E)-N-(3,4-bis(4-nitrophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, ***(5k).*** Brown solid; yield: 83%; m.p. 290–292 °C; IR (KBr, cm^{-1}): 3056, 1736, 1603, 1570, 1553, 1504, 1386; ^{1}H NMR (DMSO-d6): δ 9.50(1H, m, H30-isoquinoline), 8.56–7.69(5H, m, H29,31,32,33,34-isoquinoline), 7.46–6.98(8H, m, phenyl protons), 6.64(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 103.21, 103.85, 112.89, 116.68, 121.51, 122.16, 124.32, 124.95, 124.77, 126.12, 128.32, 129.90, 130.73, 130.98, 131.91, 136.93, 138.19, 143.42, 146.94, 147.60, 151.33, 157.19, 162.16; Anal. Calcd for $C_{24}H_{15}N_5O_4S$: C, 61.40; H, 3.22; N, 14.92; O, 13.63; S 6.83; Found: C, 61.43; H, 3.27; N, 14.98; O, 13.57; S, 6.81%.

2.2.2.13. (E)-N-(3-(4-fluorophenyl)−4-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5l). Orange solid; yield: 95%; m.p. 299–301 °C; IR (KBr, cm^{-1}): 3057, 1737, 1603, 1565, 1553, 1547, 1386; ^{1}H NMR (DMSO-d6): δ 9.43(1H, m, H30-isoquinoline), 8.43–7.70(5H, m, H29,31,32,33,34-isoquinoline), 7.54–7.00(9H, m, phenyl protons), 6.58 (1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 98.71, 116.35, 116.57, 120.79, 123.29, 128.91, 129.03, 129.25, 130.11, 131.17, 131.86, 131.95, 132.32, 138.18, 139.78, 146.88, 150.57, 162.89; Anal. Calcd for $C_{24}H_{16}FN_3S$: C, 72.52; H, 4.06; F,4.78; N, 10.57; S, 8.07; Found: C,



Scheme 2. The synthesis reaction mechanism.

72.53; H, 4.10; F,4.80; N, 10.55; S, 8.11%.

2.2.2.14. (E)-N-(4-(4-chlorophenyl)−3-(4-fluorophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5 m). Brown solid; yield: 72%; m.p. 295–297 °C; IR (KBr, cm^{-1}): 2970, 1738, 1600, 1583, 1557, 1519, 1352; ^{1}H NMR (DMSO-d6): δ 9.70(1H, m, H30-isoquinoline), 8.57–7.76(5H, m, H29,31,32,33,34-isoquinoline), 7.53–7.16(8H, m, phenyl protons), 6.71(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 99.65, 116.51, 116.74, 120.74, 123.07, 124.30, 128.98, 129.02, 129.89, 130.76, 131.68, 131.85, 131.94, 133.01, 133.29, 133.67, 134.10, 134.19, 138.65, 146.86, 148.37, 163.02; Anal. Calcd for $C_{24}H_{15}ClFN_3S$: C, 66.74; H, 3.50; Cl, 8.21; F, 4.40; N, 9.73; S 7.42; Found: C, 66.73; H, 3.57; Cl, 8.25; F, 4.43; N, 12.38; O, 10.57; S, 7.47%.

2.2.2.15. (E)-N-(3-(4-fluorophenyl)−4-(4-nitrophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5n). Yellow solid; yield: 81%; m.p. 233–235 °C; IR (KBr, cm^{-1}): 3027, 1738, 1599, 1554, 1535, 1489, 1355; ^{1}H NMR (DMSO-d6): δ 9.75(1H, m, H30-isoquinoline), 8.57–7.77(5H, m, H29,31,32,33,34-isoquinoline), 7.58–7.24(8H, m, phenyl protons), 6.95(1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 102.37, 116.65, 116.88, 120.88, 123.40, 124.11, 124.61, 128.93, 130.10, 131.75, 131.84, 132.91, 133.69, 134.01, 137.16, 137.88, 146.86, 147.60, 148.20, 163.08; Anal. Calcd for $C_{24}H_{15}FN_4O_2S$: C, 65.15; H, 3.42; F, 4.29; N, 12.66; O, 7.23; S 7.25; Found: C, 65.13; H, 3.47; F,4.32; N, 12.68; O, 7.27; S, 7.23%.

2.2.2.16. (E)-N-(3-(4-chlorophenyl)−4-phenylthiazol-2(3H)-ylidene)isoquinoline-5-amine, (5o). Orange solid; yield: 96%; m.p. 223–225 °C; IR (KBr, cm^{-1}): 2970, 1738, 1604, 1580, 1566, 1536, 1485, 1381; ^{1}H NMR (DMSO-d6): δ 9.57(1H, m, H30-isoquinoline), 8.48–7.63(5H, m, H29,31,32,33,34-isoquinoline), 7.49–7.18(9H, m, phenyl protons), 6.66 (1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 99.19, 121.83, 123.37, 123.56, 123.80, 128.98, 129.01, 129.06, 129.10, 129.25, 129.33, 129.46, 129.60, 130.02, 130.60, 131.04, 131.56, 131.64, 132.86, 133.09, 137.10, 139.60, 146.91, 149.63, 161.64; Anal. Calcd for $C_{24}H_{16}ClN_3S$: C, 69.64; H, 3.90; Cl, 8.56; N, 10.15; S 7.75; Found: C, 69.65; H, 3.97; Cl, 8.59; N, 10.17; S, 7.71%.

2.2.2.17. (E)-N-(3,4-bis(4-chlorophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5p). Orange solid; yield: 95%; m.p. 250–252 °C; IR (KBr, cm^{-1}): 3033, 2970, 1738, 1600, 1578, 1556, 1520, 1486, 1347; ^{1}H NMR (DMSO-d6): δ 9.57(1H, m, H30-isoquinoline), 8.48–7.63(5H, m, H29,31,32,33,34-isoquinoline), 7.49–7.18(8H, m, phenyl protons), 6.66 (1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 99.90, 120.32, 122.78, 124.33, 129.09, 129.72, 129.84, 130.07, 130.82, 131.42, 131.53, 133.28, 133.34, 134.13, 136.83, 138.42, 146.90, 148.85, 161.74; Anal. Calcd for $C_{24}H_{15}Cl_2N_3S$: C, 64.29; H, 3.37; Cl, 15.81; N, 9.37; S 7.15; Found: C, 64.33; H, 3.39; Cl, 15.85; N, 9.38; S, 7.11%.

2.2.2.18. (E)-N-(3-(4-chlorophenyl)−4-(4-nitrophenyl)thiazol-2(3H)-ylidene)isoquinoline-5-amine, (5r). Orange solid; yield: 75%; m.p. 225–227 °C; IR (KBr, cm^{-1}): 3027, 1613, 1577, 1545, 1490, 1388; ^{1}H NMR (DMSO-d6): δ 9.65(1H, m, H30-isoquinoline), 8.52–7.67(5H, m, H29,31,32,33,34-isoquinoline), 7.55–7.43(8H, m, phenyl protons), 6.95 (1H, s, H35-thiazole ring); ^{13}C NMR (DMSO-d6): 102.69, 119.88, 122.41, 124.17, 124.26, 129.15, 129.83, 130.01, 130.07, 131.09, 131.40, 132.95, 133.36, 135.32, 136.69, 137.15, 137.63, 146.85, 147.61, 149.33, 161.45; Anal. Calcd for $C_{24}H_{15}ClN_4O_2S$: C, 62.81; H, 3.29; Cl, 7.72; N, 12.21; O, 6.97; S, 6.99; Found: C, 62.87; H, 3.27; Cl, 7.75; N, 12.28; O, 7.01; S, 7.01%.

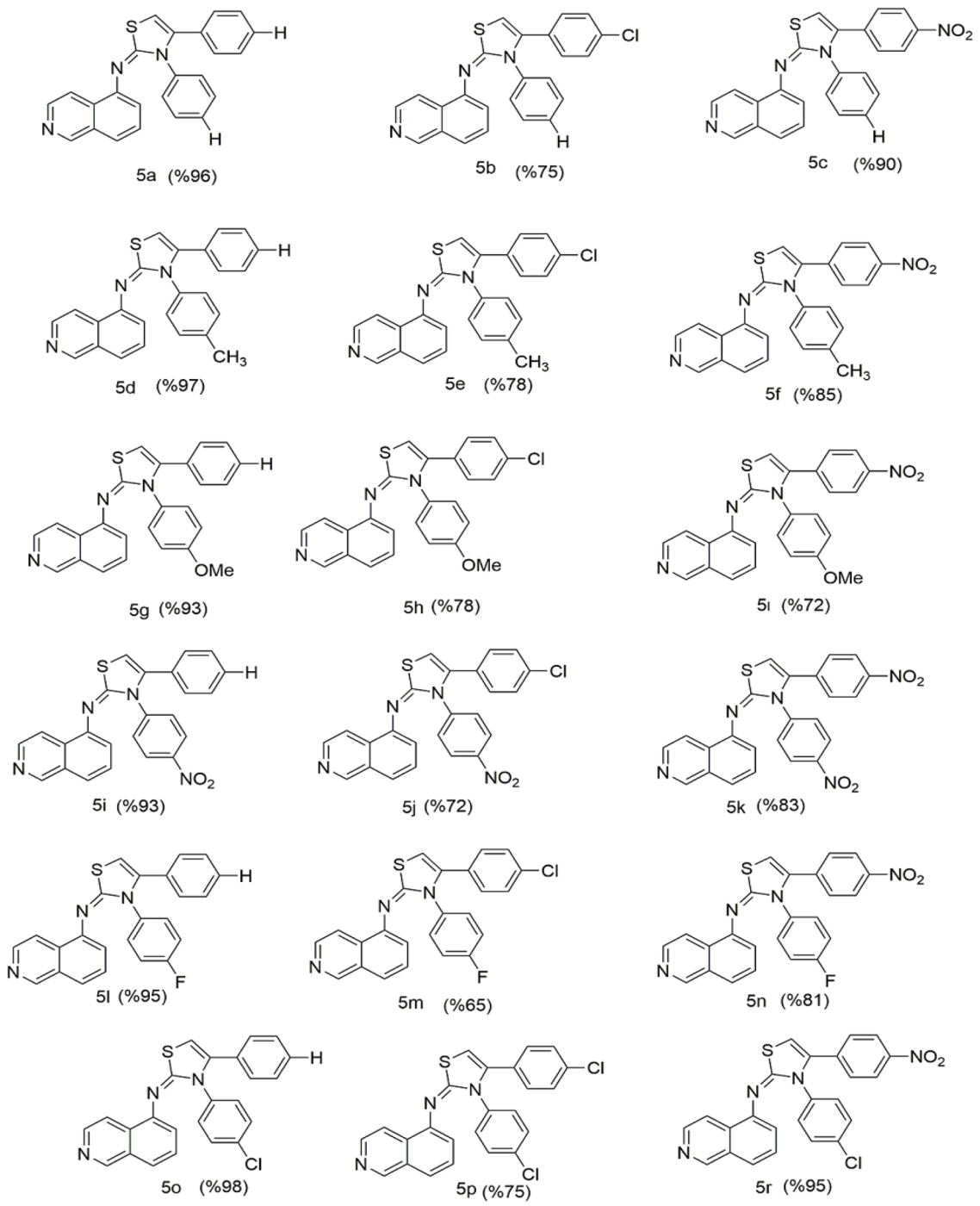
2.3. Biologic activity studies

2.3.1. Purification of carbonic anhydrase i and ii from human blood red cells

Blood samples (25 mL) from healthy human volunteers were collected. They were centrifuged at 1000x*g* for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then hemolyzed in cold water. The pH of the hemolysate was adjusted to 8.5 with a solid Tris-base. Twenty-five milliliters of hemolysate were applied to an affinity column containing Sepharose 4B-ethylene diamine-4-isothiocyanate-benzenesulfonamide [37]. CA isozymes were then eluted with 0.1 M NaCl / 25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa / 0.5 M $NaClO_4$ (pH 5.6), which recovered hCA I and II, respectively.

2.3.2. Esterase and hydratase activity assay

hCA IX and hCA X (CA 9, human recombinant (SRP6483) and CA10, human recombinant (SRP6490)) was obtained from Sigma-Aldrich. hCA IX and hCA X vials were centrifuged at 5000x*g* for 3 min at 4 ºC before opening. They were reconstituted in sterile PBS, pH 7.4 at a



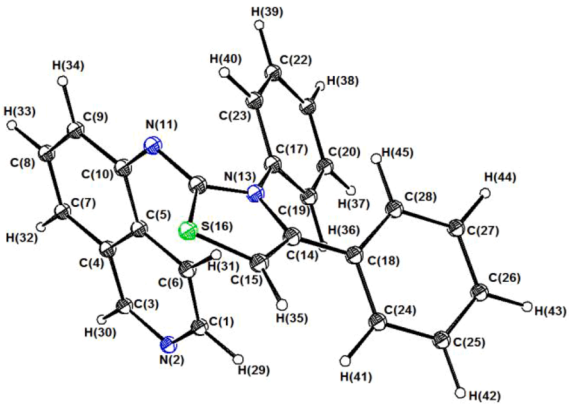
Scheme 3. Synthesis of derivatives (5a–r) from the compounds (3a–f) and the phenacyl bromide derivatives (4).

concentration of 50 μg/mL.

The determination of esterase activities of carbonic anhydrase enzymes is based on the hydrolysis of p-nitrophenyl acetate. CA activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenyl-acetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25 °C using a spectrophotometer (Biotek Power Wave XS) according to the method described in the literature [38]. The CO_2—Hydratase activity was calculated using equation (t_0- tc/tc) where t_0 and t_c are the pH change times of non-enzymatic and enzymatic reactions, respectively, and this was used to express the enzyme unit (EU) [39]. The clinically used isoform non-selective CA inhibitor acetazolamide (AAZ) was chosen as a standard inhibitor.

2.3.3. *In vitro inhibition studies*

The effects of increasing concentrations of 18 newly synthesized Phenylthiazol-2(3 h)-ylidene-isoquinoline-5-amine derivatives**(5a-r)** on hCA I, II, IX, and X isozymes activities were determined both hydratase and esterase activity assay. Carbonic anhydrase activity without new inhibitors was considered as 100% activity, and the graph was used to calculate the inhibitor concentration causing up to 50% inhibition. IC_{50} analysis graphs were drawn by plotting inhibitor concentrations against enzyme activity using Microsoft Excel Program. The inhibition impacts of thiazole derivatives **5a-5r** on the both hydratase and esterase activity of hCA I, II, IX, and X isoenzymes were investigated.



Scheme 4. ORTEP plot of the molecular structure of compound (5a).

Table 1
IC_{50} values of hCA I, hCA II, hCA IX and hCA X isoenzyme esterase and hydratase activities obtained after inhibition experiments.

Compound	IC_{50}(mM) (Hydratase)		IC_{50}(mM) (Esterase)		IC_{50}(mM) (Esterase)	
	hCA I	hCA II	hCA I	hCA II	hCA IX	hCA X
5a	14.90	29.94	20.10	16.32	4.60	4.09
5b	16.30	26.03	13.02	17.15	4.46	5.24
5c	20.51	18.10	19.70	16.46	5.15	5.04
5d	23.05	33	20.34	17.16	6.01	5.80
5e	29.16	33.5	18.85	17.06	5.43	6.60
5f	15.28	16.95	20.40	13.42	5.54	4.29
5 g	28.50	16.20	20.55	21.65	4.57	4.42
5h	27.80	27.05	19.95	18.40	4.18	6.19
5ı	20.45	21.21	21.75	18.50	4.67	5.99
5i	15.88	25.83	17.50	20.50	4.74	5.12
5j	13.33	15.90	18.50	20.68	5.54	5.05
5k	34.75	36.10	18.40	19.44	5.96	4.54
5l	18.70	30.21	19.74	22.01	5.87	5.24
5m	29.35	20.20	21.22	21.75	4.89	5.38
5n	20.10	15	21.11	17.62	4.87	4.93
5o	27.50	24.31	20.77	17.41	5.47	4.85
5p	26.55	27.96	19.96	16.43	5.27	4.83
5r	29.18	32.88	20.95	18.63	4	6.82
AAZ	0.19	0.108	0.233	0.354	1.32	1.96

2.4. Molecular docking studies

The molecular docking studies were carried out using SwissDock (http://www.swissdock.ch/) [40] molecular docking server based on docking software EADock DSS [41]. The ligand-bound crystal structures of human carbonic anhydrase enzymes I and II (PDB ID: 1BZM [42] for hCA I and PDB ID: 1AVN [43] for hCA II) were retrieved from RCSB PDB [44] for hCA I and hCA II respectively and prepared using UCSF-Chimera (http://www.rbvi.ucsf.edu/chimera) software developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco [45]. The three-dimensional structures of the novel **5a** derivate of phenylthiazol-2 (3 h)-ylidene-isoquinoline-5-amines were sketched and prepared by the MarvinSketch software version 21.1 of ChemAxon (http://www.chemaxon.com) software solutions and services for chemistry and biology. The ligand was suitably optimized for its ionization state at pH 7.4 prior to docking. The minimization, equilibration, and molecular dynamic simulations were performed using NAMD2 software. NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign [46].

3. Results and discussion

3.1. Chemistry

The detailed reaction mechanism and the products of (5a-r) are shown in Schemes 2 and 3. Firstly, the aromatic thiourea derivatives (3a-f) were synthesized with 5-aminoisoquinoline (1) and thioisocyanates (2) in THF at 70 °C for 24 h [47]. Afterward, these compounds (3a-f) were converted to compounds (5a-r) to give a thiazole ring in EtOH-DMF (5:5 v/v) at 80 °C for 24 h and were formed in a very high yield (98–65%). Phenacyl bromide derivatives (4) were used for this reaction step [48]. The prepared analogs were characterized by NMR (^{1}H and ^{13}C), IR, and elemental analysis. The resulting spectroscopic investigation agreed with the heteroaromatic structure (5a-r) shown in Scheme 3. Also, ORTEP plot of the molecular structure of compound (5a) showed in Scheme 4.

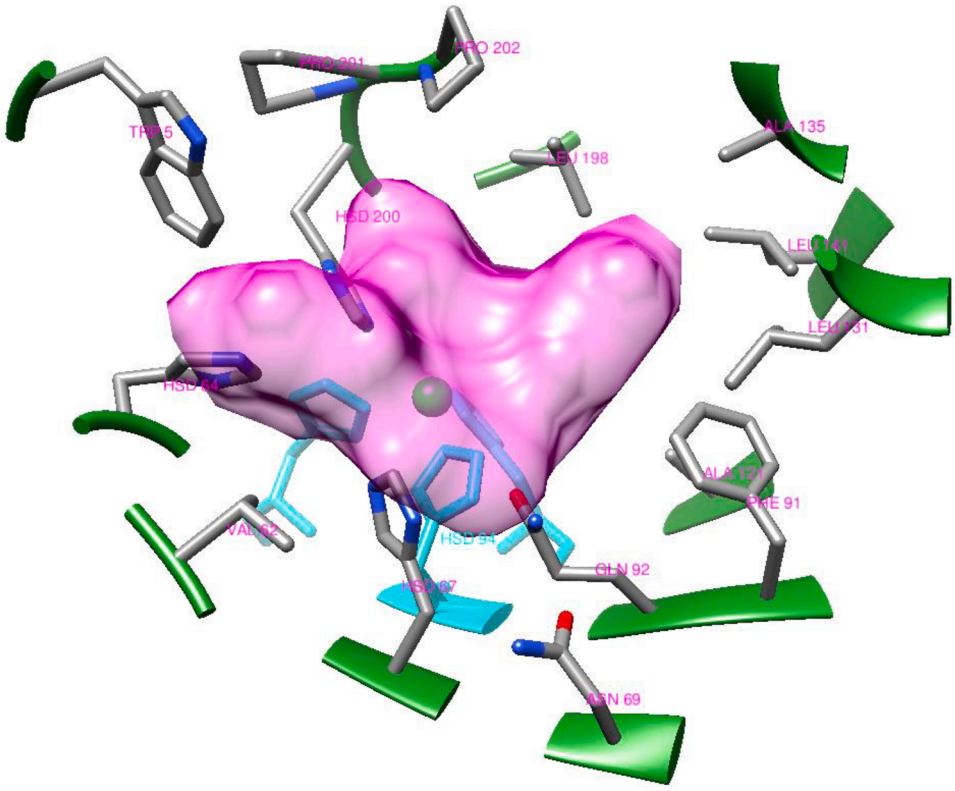
The product was identified from spectroscopic and analytical data. In the FT-IR spectra of the compounds, (5a-r) exhibited characteristic absorption bands of around 3000, 1700, 1650, and 1350 regions, confirming the presence of (=C–H), (C=N), and (C=C), respectively. It is known that aromatic systems generally exhibit vibrational bands at around 3000 cm^{-1} because of aromatic CH stretching vibrations [49]. In the FT-IR spectrum recorded for the molecules (5a-r), the band observed in the region of 3076–2920 cm^{-1} is assigned as aromatic CH stretching vibration. In the six-membered aromatic rings such as benzene and pyridine, the (C = C) stretching vibrations occur in the region 1625–1430 cm^{-1} [50].

^{1}H NMR and ^{13}C NMR spectra were taken on the Bruker AV-400 MHz instrument in DMSO-d6. The ^{1}H NMR spectra of all the compounds have shown that the H(35) proton peak on thiazole ring resonances around 6.50 ppm [51] and the H(36–45) proton peaks on aromatic benzene ring come between 6.61 and 7.87 ppm [52]. Also, the isoquinoline ring protons H(29–34) gave a peak around 9.77–8.00 ppm [53–55]. While the methoxy protons of compounds 5 g, 5 h, and 5ı give signals at 3.70, 3.73, and 3.72 ppm, the methyl protons of 5d, 5e, 5f give at 2.26, 2.27, and 2.28 ppm, respectively. From the ^{13}C NMR spectra, carbon double bonds the thiazole ring (C12=N11 and C14=C15) gave a peak around 160 and 100 ppm [52]. Also, the aromatic C atoms give NMR signals in the range of 150–110 ppm [48]. In our work, benzene ring C atoms give NMR signals at around 113–150 ppm. The methoxy group C atoms of compounds 5 g, 5 h, and 5ı have given signals at around 55.60 ppm, and also the methyl C atoms of 5d, 5e, and 5f have given signals at around 21.30 ppm. All spectra and elemental analyses support the structure of the synthesized compounds.

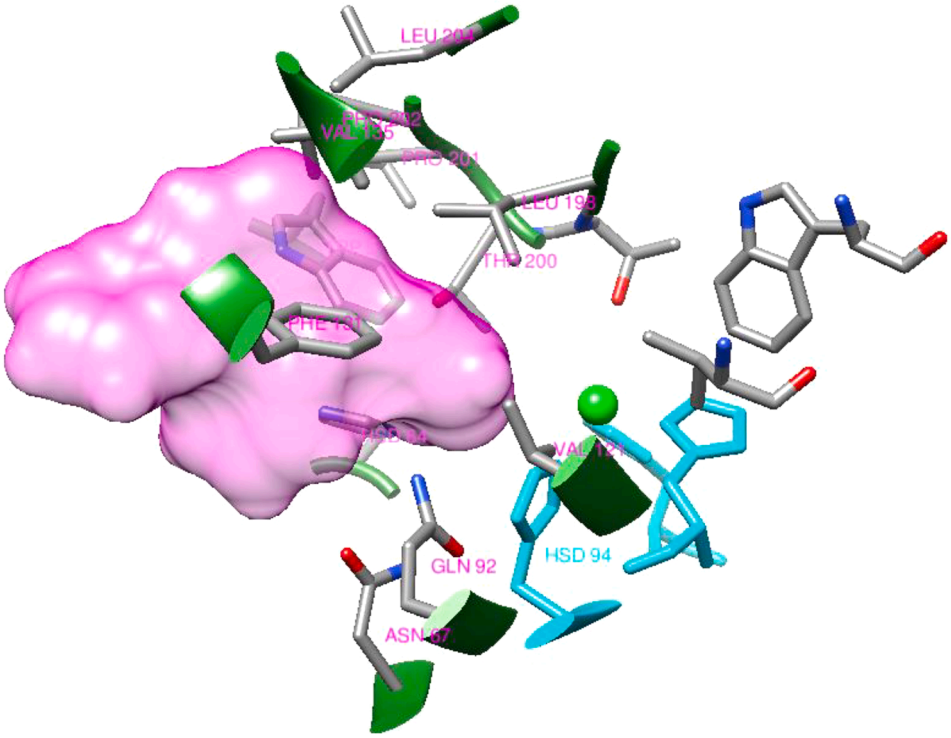
3.2. CA activity assay

The effects of these new inhibitors on human carbonic anhydrase I (hCA I), II (hCA II), IX (hCA IX), and X (hCA X) were investigated. While hCA I and hCA II isozymes have been purified from human erythrocytes by affinity chromatography, hCA IX and hCA X were purchased from sigma. The inhibitor concentrations causing up to 50% (IC_{50} values) were investigated from regression analysis graphs for these novel derivatives. The IC_{50} values found for these compounds are shown in Table 1.

The hCA I hydratase and esterase activities were inhibited by the novel-synthesized isoquinoline-5-amine derivatives, with IC_{50} values ranging from 13.33 to 34.75 and 13.02–21.22 mM, respectively. Also, all studied compounds showed inhibition activity against hCA II hydratase and esterase, with IC_{50} values ranging from 15 to 36.1 and 13.42–22.01 mM, respectively, close to hCA I. The hCA IX and X isoenzymes were inhibited by all derivatives, with IC_{50} values ranging from 4 to 6.01 and 4.09–6.82 mM, respectively. The strongest inhibitors among the studied compounds were found according to their esterase and hydratase activities for hCA I 5b (IC_{50}: 13.02 mM), hCA II 5f (IC_{50}:13.42 mM), hCA IX 5r (IC_{50}: 4 mM), hCA X 5a (IC_{50}:4.09 mM), and



Scheme 5. Ligand focused surface-focused binding pose (front) of 6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-sulfonamide to the hCA I. Zinc ion (green) with three coordinated histidine residues (cyan) were shown together with amino acid residues within 4.2 Å of the ligand molecule.



Scheme 6. Ligand surface-focused binding pose (side) of 6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-sulfonamide to the hCA II. Zinc ion (green) with three coordinated histidine residues (cyan) were shown together with amino acid residues within 4.2 Å of the ligand molecule.

for hCA I 5j (IC_{50}: 13.33 mM), hCA II 5n (IC_{50}:15 mM) respectively.

As seen in Table 1, the IC_{50} values of the new inhibitors, esterase activity hCA IX and hCA X, were lower than IC_{50} values of both hydratase and esterase activities hCA I and hCA II. All compounds inhibited hCA IX and hCA X more potently (from 4.0 to 6.82 mM). However, these inhibition values are weaker than AAZ. Carbonic anhydrase IX is known highly expressed in various human malignancies and tissues. CA IX is a significant biomarker for some cancers caused by overexpressed in hypoxic tumors.

For hCA I hydratase activity, the strongest inhibitor was the **5j** compound with an IC_{50} value of 13.33 mM, while the weakest inhibitor was the **5k** compound with an IC_{50} value of 34.75 mM. Hydratase activity is measured using the enzyme's specific substrate, carbon dioxide. Among the compounds studied according to their hydratase activity, the **5k** compound was determined as the weakest inhibitor for both hCA I and hCA II (Table 1).In our previous work, we explored thiazole derivatives hat showed inhibitory effects against some hCA isozymes at micromolar concentrations [55–59].

3.3. Docking studies

The novel 5a derivate of phenylthiazol-2(3 h)-ylidene-isoquinoline-5-amines synthesized in this study was docked against hCA I and hCA II active sites to better evaluate the molecular basis of the binding affinity of the enzymes. The docking results were compared to the crystal structure of the hCA II and 6,7-dimethoxy-3,4-dihydroisoquinoline-2 (1H)-sulfonamide ligand (DT7) complex, (PDB ID: 3IGP) [54], was derived from RCSB PDB. For the validation of the *in silico* molecular docking studies, the co-crystalized ligand DT7 was extracted and redocked into the binding site of the protein chain A of the 3IPG. To evaluate the quality of the docking results, the results with the best full-fitness score were superimposed with the DT7 ligand and root mean square deviation (RMSD) values were calculated. The RMSD scores were computed as less than 2 Å.

According to previous studies, the inhibitor binding does not cause any major structural changes to the hCA I and hCA II enzymes [56]. Hydrophobic interactions of the isoquinoline moiety are known to stabilize the inhibitor within the active site cavity which. The isoquinoline moiety is known to form numerous Van der Waals interactions (distance of <4.2 Å) with the bottom of the active site (residues Gln92, Val121, Val143, Trp209) and the loop formed by residues Ser197−Pro202. Also, the side chain of Phe131 from helix 4 contributes to interaction with the substituting methoxy-groups. Phenylthiazol-2(3 h)-ylidene-isoquinoline-5-amine derivatives synthesized in this study showed comparable inhibition activity to 3,4-dihydroisoquinoline-2(1H)-sulfonamides previously reported in the literature with 14,90 and 29,94 μM IC50 values respectively for hCA I and hCA II.

According to the molecular docking study results, in addition to the expected Van der Walls interactions, novel phenylthiazol-2(3 h)-ylidene-isoquinoline-5-amine derivatives synthesized in this study has the potential to form H bonds between the sulfur atom of the phenylthiazol group and His67 and conserved Gln92 residues of hCA I. The sulfur atom has the potential to act as two proton acceptors whereas His67 and Gln92 act as proton donors through their ND1-HD1 and NE2-HE22 atoms respectively. The average distance of the H bonds formed between the ligand and the active site residues were calculated as 2.62 Å and 3.31 Å respectively with His-and Gln, which corresponds to one strong and one weak hydrogen bond. In addition to the same hydrophobic interactions of the isoquinoline moiety stabilize the inhibitor within the active site cavity including Gln92, Ala121, Val143, Trp209, Ser197, Pro202, and Phe131 phenylthiazol-2(3 h)-ylidene group also forms many van der Waals interactions including Val62, His64, Trp5, Pro201, Pro202, Leu198, Ala135, Val131, Leu141, Phe91 and Asn69 (Scheme 5).

Similar to hCA I, phenylthiazol-2(3 h)-ylidene-isoquinoline-5-amine derivatives have the potential to form H bonds between the sulfur atom of the phenylthiazol group and Asn62 or Asn67 and conserved Gln92 residues of hCA III. The sulfur atom acts as a two proton acceptor whereas Asn62/67 and Gln92 act as proton donors through their ND2-HD21/22 and NE2-HE22 atoms. The average distance of the H bonds formed between the ligand and the active site residues were calculated as 2.70 Å and 3.06 Å respectively which corresponds to one strong and one weak hydrogen bond. In addition to the same hydrophobic interactions of the isoquinoline moiety stabilize the inhibitor within the active site cavity including Gln92, Val121, Val143, Trp209, Ser197, Pro202, and Phe131 phenylthiazol-2(3 h)-ylidene group also forms many van der Waals interactions including Asn62, His64, Trp5, Pro201, Pro202, Leu204, Leu198, Val135, Phe131, Val121, Leu141, Ile91 and Glu69 (Scheme 6).

The docking scores of **5a** derivate was found to be higher for hCA I than hCA II with −1274.64 full fitness score, 57.26 kJ/moL energy and −7.42 kJ/moL ΔG instead of −1336.14 full fitness score, 60.29 kJ/moL energy and −7.19 kJ/moL ΔG respectively which also supports the lower IC_{50} value observed for hCA I.

CRediT author statement

Nurcan Berber: Synthesis and characterization of isoquinoline compounds, Writing- Reviewing. **Arif Sercan Şahutoğlu:** Molecular docking studies. **Basak Gökçe:** Enzyme purification and inhibition studies Kubra Çıkrıkçı: Enzyme purification and inhibition studies. **Nahit Gencer:** Design the work, Evaluation of results, Writing-Reviewing and Editing.

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.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2023.136061.

References

[1] K.S.P. Mc Naught, P.A. Carrupt, C. Altomare, S. Cellamare, A. Carotti, B. Testa, P. Jenner, C.D. Marsden, Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease, Biochem. Pharmacol. 56 (8) (1998) 921–933, https://doi.org/10.1016/S0006-2952(98)00142-7.

[2] S. Kumar, S. Bawa, H. Gupta, Biological activities of quinoline derivatives, Mini Rev. Med. Chem. 9 (14) (2009) 1648–1654.

[3] R. Musiol, J. Jampilek, V. Buchta, L. Silva, H. Niedbala, B. Podeszwa, A. Palka, K. Majerz-Maniecka, B. Oleksyn, J. Polanski, Antifungal properties of new series of quinoline derivatives. Bioorganic and Medicinal Chemistry. 2006, 14(10):3592–8, 10.1016/j.bmc.2006.01.016.

[4] R. Anderson, P.W. Groundwater, A. Todd, A. Worsley, Antibacterial agents: chemistry, Mode of action, Mechanisms of Resistance and Clinical Applications, John Wiley and Sons, 2012, pp. 38–48.

[5] P.B. Bloland, E.M. Lackritz, P.N. Kazembe, J.B. Were, R. Steketee, C.C. Campbell, Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa, J. Infect. Dis. 167 (4) (1993) 932–937, https://doi.org/10.1093/infdis/167.4.932.

[6] L. Strekowski, J.L. Mokrosz, V.A. Honkan, A. Czarny, M.T. Cegla, R.L. Wydra, S. E. Patterson, R.F. Schinazi, Synthesis and quantitative structure-activity relationship analysis of 2-(aryl or heteroaryl) quinolin-4-amines, a new class of anti-HIV-1 agents, J. Med. Chem. 34 (5) (1991) 1739–1746.

[7] M.A. Abdelgawad, A.M. Mohamed, A. Musa, E.M. Mostafa, H.M. Awad, Synthesis, chromatographic separation and antimicrobial evolution of new azoquinoline-8-ol, J. Pharm. Sci. Res. 10 (6) (2018) 1314–1318.

[8] O. Afzal, S. Kumar, M.R. Haider, M.R. Ali, R. Kumar, M. Jaggi, S. Bawa, A review on anticancer potential of bioactive heterocycle quinoline, Eur. J. Med. Chem. 97 (2015) (2015) 871–910, https://doi.org/10.1016/j.ejmech.2014.07.044.

[9] A.E. Rashad, W.A. El-Sayed, A.M. Mohamed, M.M. Ali, Synthesis of new quinoline derivatives as inhibitors of human tumor cells growth, Arch. Pharm. 343 (8) (2010) 440–448, https://doi.org/10.1002/ardp.201000002.

[10] S. Rasoul-Amini, A. Khalaj, A. Shafiee, M. Daneshtalab, A. Madadkar-Sobhani, S. Fouladdel, E. Azizi, Anti-tumor activity of new quinoline derivatives in human breast cancer T47D cells, Int. J. Cancer Res. 2 (2) (2006) 102–108.

[11] D. De, F.M. Krogstad, L.D. Byers, D.J. Krogstad, Structure activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines, J. Med. Chem. 41 (25) (1998) 4918–4926, https://doi.org/10.1021/jm980146x.

[12] R.S. Keri, S.A. Patil, Quinoline: a promising antitubercular target, Biomed. Pharmacother. 68 (8) (2014) 1161–1175, https://doi.org/10.1016/j.biopha.2014.10.007.

[13] L.E. Bermudez, P. Kolonoski, M. Petrofsky, M. Wu, C.B. Inderlied, L.S. Young, Mefloquine, moxifloxacin, and ethambutol are a triple-drug alternative to macrolide-containing regimens for treatment of Mycobacterium avium disease, J. Infect. Dis. 187 (12) (2003) 1977–1980, https://doi.org/10.1086/375352.

[14] A.S. Ginsburg, J.H. Grosset, W.R. Bishai, Fluoroquinolones, tuberculosis, and resistance, Lancet Infect. Dis. 3 (7) (2003) 432–442, https://doi.org/10.1016/S1473-3099(03)00671-6.

[15] M.K. Kumawat, Thiazole containing heterocycles with antimalarial activity, Curr. Drug Discov. Technol. 15 (3) (2018) 196–200, https://doi.org/10.2174/1570163814666170725114159.

[16] B.B. Ma, X.B. Lan, D.S. Shen, F.S. Liu, C. Xu, Direct CH bond (Hetero) arylation of thiazole derivatives at 5-position catalyzed by N-heterocyclic carbene palladium complexes at low catalyst loadings under aerobic conditions, J. Organomet. Chem. 897 (2019) (2019) 13–22, https://doi.org/10.1016/j.jorganchem.2019.06.016.

[17] V.V. Dabholkar, D.R. Tripathi, Synthesis and antibacterial activity of isochromene and isoquinoline derivative, J. Heterocycl. Chem. 48 (3) (2011) 529–532, https://doi.org/10.1002/jhet.245.

[18] A.R.S. Butt, M.A. Abbasi, S.Z. Siddiqui, H. Raza, M. Hassan, S.A.A. Shah, S.Y. Seo, Synthesis, kinetics, binding conformations and structure-activity relationship of potent tyrosinase inhibitors: aralkylated 2-aminothiazole-ethyltriazole hybrids, Iran. J. Pharm. Res. 20 (2) (2021) 206, https://doi.org/10.22037/ijpr.2020.15521.13145.

[19] T.N. An, P.T. Phuong, N.M. Quang, N.V. Son, N.V. Cuong, L.V. Tan, M.D. Tri, M. Alam, P.V. Tat, Synthesis, docking study, cytotoxicity, antioxidant, and anti-microbial activities of novel 2, 4-disubstituted thiazoles based on phenothiazine, Curr. Org. Synth. 17 (2) (2020) 151–159, https://doi.org/10.2174/1570179417666191220100614.

[20] D.J. Kempf, H.L. Sham, K.C. Marsh, C.A. Flentge, D. Betebenner, B.E. Green, E. McDonald, S. Vasavanonda, A. Saldivar, N.E. Wideburg, W.M. Kati, L. Ruiz, C. Zhao, L.M. Fino, J. Patterson, A. Molla, J.J. Plattner, D.W. Norbeck, Discovery of ritonavir, a potent inhibitor of HIV protease with high oral bioavailability and clinical efficacy, J. Med. Chem. 41 (4) (1998) 602–617, https://doi.org/10.1021/jm970636+.

[21] S.R. Pattan, N.S. Dighe, S.A. Nirmal, A.N. Merekar, R.B. Laware, H.V. Shinde, D. S. Musmade, Synthesis and biological evaluation of some substituted amino thiazole derivatives, Asian J. Res. Chem. 2 (2) (2009) 196–201.

[22] C. Borelli, M. Schaller, M. Niewerth, K. Nocker, B. Baasner, D. Berg, R. Tiemann, B. Fugmann K.Tietjen, S. Lang-Fugmann, H.C. Korting, Modes of action of the new arylguanidine abafungin beyond interference with ergosterol biosynthesis and in vitro activity against medically important fungi, Chemotherapy 54 (4) (2008) 245–259, https://doi.org/10.1159/000142334.

[23] M.T. Chhabria, S. Patel, P. Modi, S.P. Brahmkshatriya, Thiazole: a review on chemistry, synthesis and therapeutic importance of its derivatives, Curr. Top. Med. Chem. 16 (26) (2016) 2841–2862.

[24] A. Rouf, C. Tanyeli, Bioactive thiazole and benzothiazole derivatives, Eur. J. Med. Chem. 97 (2015) (2015) 911–927, https://doi.org/10.1016/j.ejmech.2014.10.058.

[25] N. Siddiqui, M.F. Arshad, W. Ahsan, M.S. Alam, Thiazoles: a valuable insight into the recent advances and biological activities, Int. J. Pharm. Sci. Drug Res. 1 (3) (2009) 136–143, https://doi.org/10.2174/1570163814666170915134018.

[26] G.L. Khatik, A.K. Datusalia, W. Ahsan, P. Kaur, M. Vyas, A. Mittal, S.K. Nayak, A retrospect study on thiazole derivatives as the potential antidiabetic agents in drug discovery and developments, Curr. Drug Discov. Technol. 15 (3) (2018) 163–177, https://doi.org/10.2174/1570163814666170915134018.

[27] D. Keilin, T. Mann, Carbonic anhydrase, Purification and nature of the enzyme, Biochem. J. 34 (8–9) (1940) 1163–1176, https://doi.org/10.1042/bj0341163.

[28] B.C. Tripp, K. Smith, J.G. Ferry, Minireview: carbonic anhydrase: new insights for an ancient enzyme, J. Biol. Chem. 276 (52) (2001) 48615–48618, https://doi.org/10.1074/jbc.R100045200.

[29] C.T. Supuran, Structure and function of carbonic anhydrases, Biochem. J. 473 (14) (2016) 2023–2032, https://doi.org/10.1042/BCJ20160115.

[30] E. Bruno, M.R. Buemi, L.D. Luca, S. Ferro, A.M. Monforte, C.T. Supuran, D. Vullo, G.D. Sarro, E. Russo, R. Gitto, *In vivo* evaluation of selective carbonic anhydrase inhibitors as potential anticonvulsant agents, ChemMedChem 11 (2016) 1812–1818.

[31] R. Gitto, S. Agnello, S. Ferro, L. De Luca, D. Vullo, J. Brynda, P. Mader, C. T. Supuran, A. Chimirri, Identification of 3,4-dihydroisoquinoline-2(1H)-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme—Ligand X-ray studies, J. Med. Chem. 53 (2010) 2401–2408, https://doi.org/10.1021/jm9014026.

[32] R. Gitto, F.M. Damiano, L.D. Luca, S. Ferro, D. Vullo, C.T. Supuran, Alba Chimirri, Synthesis and biological profile of new 1,2,3,4-tetrahydroisoquinolines as selective carbonic anhydrase inhibitors, Bioorg. Med. Chem. 19 (23) (2011) 7003–7007.

[33 R.S. Gitto, Agnello, S. Ferro, D. Vullo, C.T. Supuran, A. Chimirri, Identification of potent and selective human carbonic anhydrase VII (hCA VII) inhibitors, ChemMedChem 5 (2010) 823–826, https://doi.org/10.1002/cmdc.201000044.

[34] S. Distinto, R. Meleddu, F. Ortuso, F. Cottiglia, S. Deplano, L. Sequeira, C. Melis, B. Fois, A. Angeli, C. Capasso, et al., Exploring new structural features of the 4-[(3-methyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino] benzene sulphon amide scaffold for the inhibition of human carbonic anhydrases, J. Enzym. Inhib. Med. Chem. 34 (2019) 1526–1533.

[35 R. Meleddu, E. Maccioni, S. Distinto, et al., New 4-[(3-*cyclo*-*hexyl*-4-*aryl*-2,3-*dihydro*-1,3-*thiazol*-2-*ylidene*)*amino*]benzene-1-sulphonamides, synthesis and inhibitory activity towardcarbonic anhydrase I, II, IX, XII, Bioorg. Med. Chem. Lett. (2015).

[36 U.M. Kocyigit, O.N. Aslan, I. Gulcin, Y. Temel, M. Ceylan, Synthesis and carbonic anhydrase inhibition of novel 2-(4-(Aryl)-thiazole-2-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione Derivatives, Arch. Pharm. Chem. Life Sci. 349 (2016) 955–963.

[37 M. Bozdag, S. Isik, S. Beyaztas, O. Arslan, C.T. Supuran, Synthesis of a novel affinity gel for the purification of carbonic anhydrases, J. Enzyme Inhib. Med. Chem. 30 (2) (2015) 240–244, https://doi.org/10.3109/14756366.2014.912216.

[38 J.A. Verpoorte, M. Suchinta, T.E. John, Esterase activities of human carbonic anhydrases B and C, J. Biol. Chem. 242 (18) (1967) 4221–4229, https://doi.org/10.1016/S0021-9258(18)95800-X.

[39 K.M. Wilbur, N.G. Anderson, Electrometric and colorimetric determination of carbonic anhydrase, J. Biol. Chem. 176 (1948) 147–154, https://doi.org/10.1016/S0021-9258(18)51011-5.

[40] A. Grosdidier, V. Zoete, O. Micheilin, SwissDock, a protein-small molecule docking web service based on EADock DSS, Nucleic Acid Res. (2011), https://doi.org/10.1093/nar/gkr366.

[41] A. Grosdidier, V. Zoete, O. Michielin, Fast docking using the CHARMM force field with EADock DSS, J. Comput. Chem. 32 (10) (2011) 2149–2159, https://doi.org/10.1002/jcc.21797.

[42] S. Chakravarty, K. Kannan, Drug-protein interactions. refined structures of three sulfonamide drug complexes of human carbonic anhydrase I enzyme, J. Mol. Biol. 243 (2) (1994) 294–309, https://doi.org/10.1006/jmbi.1994.1655.

[43] F. Briganti, S. Mangani, P. Orioli, A. Scozzafava, G. Vernaglione, C.T. Supuran, Carbonic anhydrase activators: x-ray crystallographic and spectroscopic investigations for the interaction of isozymes I and II with histamine, Biochemistry 36 (34) (1997) 10384–10392, https://doi.org/10.1021/bi970760v.

[44] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I. N. Shindyalov, P.E. Bourne, The Protein Data Bank, Nucleic Acids Res. 28 (1) (2000) 235–242, https://doi.org/10.1093/nar/28.1.235.

[45] I. Petrescu, J. Lamotte-Brasseur, J.P. Chessa, P. Ntarima, M. Claeyssens, B. Devreese, G. Marino, C. Gerday, UCSF Chimera - A visualization system for exploratory research and analysis, J. Comput. Chem. 25 (13) (2004) 1605–1612, https://doi.org/10.1002/jcc.20084.

[46] J.C. Hardy, D.J. Maia, J.D.C. Stone, E.S. Ribeiro, J.V. Bernardi RC, et al., Scalable molecular dynamics on CPU and GPU architectures with NAMD, J. Chem. Phys. 153 (4) (2020), 044130, https://doi.org/10.1063/5.0014475.

[47] N. Berber, M. Arslan, E. Yavuz, C. Bilen, N. Gencer, Synthesis and evaluation of new phthalazine urea and thiourea derivatives as carbonic anhydrase inhibitors. Hindawi, J. Chem. 2013 (2013) 1–9, https://doi.org/10.1155/2013/742178.

[48] N. Berber, Synthesis of certain new morpholine derivatives bearing a thiazole moiety, Sak. Univ. J. Sci. Technol. 23 (4) (2019) 554–558, https://doi.org/10.16984/saufenbilder.414310.

[49] A. Pekparlak, Ö. Tamer, S.D. Kanmazalp, N. Berber, M. Arslan, D. Avcı, N. Dege, E. Tarcan, Y. Atalay, Crystal structure, spectroscopic (FT-IR, 1H and 13C NMR) characterization and density functional theory calculations on Ethyl 2-(dichloromethyl)-4-methyl-1-phenyl-6-thioxo-1, 6-dihydropyrimidine-5-carboxylate, J. Mol. Struct. 1171 (2018) (2018) 762–770, https://doi.org/10.1016/j.molstruc.2018.06.059.

[50] A. Teimouri, A.N. Chermahini, K. Taban, H.A. Dabbagh, Experimental and CIS, TD-DFT, ab initio calculations of visible spectra and the vibrational frequencies of sulfonyl azide-azoic dyes, Spectrochim. Acta A 72 (2) (2009) 369–377, https://doi.org/10.1016/j.saa.2008.10.006.

[51] N. Berber, M. Arslan, F. Vural, A. Ergun, N. Gençer, O. Arslan, Synthesis of new series of thiazol-(2(3H)-ylideneamino) benzenesulfonamide derivatives as carbonic anhydrase inhibitors, J. Biochem. Mol. Toxicol. 34 (12) (2020) e22596, https://doi.org/10.1002/jbt.22596.

[52] B.Z. Kurt, Synthesis and anticholinesterase activity of novel non-hepatotoxic naphthyridine-11-amine derivatives, Mol. Divers. 23 (3) (2019) 625–638, https://doi.org/10.1007/s11030-018-9897-1.

[53] M.C. Al-Matarneh, R.M. Amărandi, I.I. Mangalagiu, R. Danac, Synthesis and biological screening of new cyano-substituted pyrrole fused (iso) quinoline derivatives, Molecules 26 (7) (2021) 2066, https://doi.org/10.3390/molecules26072066.

[54] J. Fiorito, F. Saeed, H. Zhang, A. Staniszewski, Y. Feng, Y.I. Francis, S. Rao, D. M. Thakkar, S.X. Deng, D.W. Landry, O. Arancio, Synthesis of quinoline derivatives: discovery of a potent and selective phosphodiesterase 5 inhibitor for the treatment of Alzheimer's disease, Eur. J. Med. Chem. 60 (2013) (2013) 285–294, https://doi.org/10.1016/j.ejmech.2012.12.009.

[55] X. Fang, J. Mao, E.M. Levin, K. Schmidt-Rohr, Nonaromatic core-shell structure of nanodiamond from solid-state NMR spectroscopy, J. Am. Chem. Soc. 131 (4) (2009) 1426–1435, https://doi.org/10.1021/ja8054063.
[56] Kurt B.Z., Sonmez F., Gokce B., Ergun A., Gencer N., Demir T., Arslan O., Kucukislamoglu M. In vitro inhibition effects on erythrocyte carbonic anhydrase I and II and structure-activity relationships of cumarylthiazole derivatives. 42, 506–511, (2016), 10.1134/S1068162016050046.
[57 S. Kılıcaslan, A. Mustafa, R. Zeynep, B. Cigdem, A. Ergun, G. Nahit, Arslan O. Synthesis and evaluation of sulfonamide-bearing thiazole as carbonic anhydrase isoforms hCA I and hCA II, J. Enzyme Inhib. Med. Chem. 31 (6) (2016) 1300–1305, https://doi.org/10.3109/14756366.2015.1128426.
[58 B.Z. Kurt, F. Sonmez, C. Bilen, A. Ergun, N. Gencer, O. Arslan, M. Kucukislamoglu, Synthesis, antioxidant and carbonic anhydrase I and II inhibitory activities of novel sulphonamide-substituted coumarylthiazole derivatives, J. Enzyme Inhib. Med. Chem. 31 (6) (2016) 991–998, https://doi.org/10.3109/14756366.2015.1077823.
[59 A.B. Musatat, A. Atahan, A. Ergun, K. Cikrikci, N. Gencer, O. Arslan, M. Zengin, Synthesis, enzyme inhibition and molecular docking studies of a novel chalcone series bearing benzothiazole scaffold, Biotechnol. Appl. Biochem. (2023), https://doi.org/10.1002/bab.2445. Early online.