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Original article

Synthesis and *in vitro* anticancer screening of some novel 4-[2-amino-3-cyano-4-substituted-5,6,7,8-tetrahydroquinolin-1-(4*H*)-yl] benzenesulfonamides

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ABSTRACT

It has been reported that aryl/heteroaryl sulfonamide compounds may act as anticancer agents through a variety of mechanisms and the most prominent of these mechanisms is through the inhibition of carbonic anhydrase isozymes. The present work reports the possible utility of 4-(cyclohexenylamino) benzenesulfonamide in the synthesis of some novel 4-(quinolin-1-yl)benzenesulfonamide derivatives **6a**–**u**. The structures of these compounds were confirmed by elemental analyses, IR, ^{1}H NMR and mass spectral data. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity. Some compounds showed interesting *in vitro* anticancer activities when compared with doxorubicin as a reference drug. In addition, docking of the synthesized compounds into carbonic anhydrase isozyme II (CA II) active site was performed in order to give a suggestion about the proposed mechanism of action.

1. Introduction

Sulfonamides posses many types of biological activities and representatives of this class of pharmacological agents are widely used in clinic as antibacterial [1], hypoglycemic [2], diuretic [3,4], anti-carbonic anhydrase [3,5] and antithyroid activity [6] among others. Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity *in vitro* and/or *in vivo* [7–11].

From literature survey, it was found that aryl/heteroaryl sulfonamides may act as antitumor agents through a variety of mechanisms such as cell cycle perturbation in the G1 phase, disruption of microtubule assembly, angiogenesis inhibition, and functional suppression of the transcriptional activator nuclear factor Y (NF-Y). Moreover, following an extensive evaluation, numerous sulfonamides were found to act as carbonic anhydrase (CA) inhibitors [12–17]. The most prominent mechanism was the inhibition of carbonic anhydrase isozymes (CA) [18].

Also, quinoline derivatives are important biologically active compounds showing anticancer activity [19–21]. In the light of these facts, and as a continuation of our work, the present work reports the possible utility of 4-(cyclohexenylamino)-benzenesulfonamide in the synthesis of some novel 4-(quinolin-1-yl)-benzenesulfonamide derivatives, in order to study their structure activity relationship and hoping that the new compounds might show significant anticancer activity.

2. Results and discussion

A general pharmacophore (Fig. 1) for the compounds acting as carbonic anhydrase inhibitors has been reported by Thiry et al. [22] from the analysis of the CA active site and from the structure of inhibitors described in the literature [15].

This pharmacophore includes the structural elements that are required to be present in the compounds in order to act as CA inhibitors. This includes the presence of a sulfonamide moiety which coordinates with the zinc ion of the active site of the CA and the sulfonamide is attached to a scaffold which is usually a benzene ring. The side chain might posses a hydrophilic link able to interact with the hydrophilic part of the active site and a hydrophobic moiety which can interact with the hydrophobic part of the CA active site. Fig. 2 includes a representative example of the synthesized compounds showing compliance to the above-mentioned

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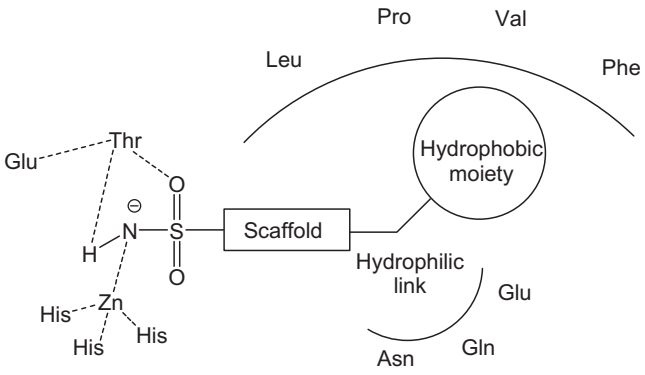


Fig. 1. Structural elements of CA inhibitors in the CA enzymatic active site.

pharmacophore and the compounds were synthesized according to Scheme 1.

2.1. Chemistry

The compounds were designed with the aim of exploring their antitumor activity. Thus, the present work reports the possible utility of 4-(cyclohexenylamino)benzenesulfonamide **3** in the synthesis of 4-(2-amino-3-cyano-4-substituted-5,6,7,8-tetrahydroquinolin-1(4*H*)-yl)benzenesulfonamide **6a**–**u**. Compound **3** was obtained via condensation of cyclohexanone 1 with sulfanilamide **2** in ethanol.

The structure of compound 3 was proved by elemental analysis, and spectral data. IR spectrum of **3** revealed the presence of bands for NH, NH_2 at 3380, 3340, 3210 cm^{-1} (SO_2), at 1379, 1186 cm^{-1}. ^1H NMR spectrum of **3** (in DMSO-d_6) indicated the presence of a singlet at 7.8 ppm which could be assigned to NH of the compound **3**. The corresponding quinoline derivatives **6a**–**u** were obtained via treatment of compound **3** with arylidenemalononitriles **4a**–**u** in ethanol containing triethylamine as catalyst. The formation of **6a**–**u** was proceed via the formation of intermediate Michael type products **5a**–**u**, followed by interamolecular cyclization (Scheme 1).

The *N*-aryl-substituted benzenesulfonamide decreases the nucleophilicity of the compound **3** towards arylidinemalononitriles **4a**–**u**. The base catalyst triethylamine was required to generate the anion of compound **3**, thus, facilitating the addition to the unsaturated nitriles **4a**–**u** (Scheme 2).

Compounds **6a**–**u** were unambiguously synthesized by another route involving one-pot condensation of the aldehyde, malononitrile and compound **3**, in a molar ratio of (1:1:1), in refluxing ethanol containing triethylamine as catalyst. In this case, formation of compounds **6a**–**u** are illustrated in terms of initial condensation

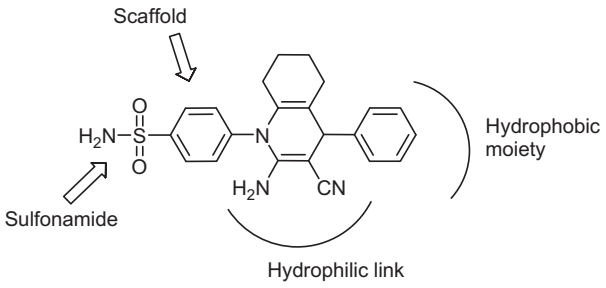


Fig. 2. Representative example of the synthesized compounds showing compliance to the general pharmacophore of sulfonamide compounds acting as CA inhibitors.

of the appropriate aldehyde with malononitrile affording the activiated arylidenemalononitriles **4a**–**u**, followed by addition of compound **3** to arylidenemalononitrile **4a**–**u**. IR spectra of compounds **6a**–**u** exhibited bands at 3478–3200 cm^{-1} due to (NH_2), 2228–2162 cm^{-1} (C≡N), 1397–1134 cm^{-1} (SO_2).

2.2. In vitro anticancer screening

Doxorubicin (CAS-23214–92–8), the reference drug used in this study, is one of the most effective antitumor agents used to produce regressions in acute leukemias, Hodgkin's desease and other lymphomas. The relationship between survival ratio and compound concentration was plotted to obtain the survival curve of Ehrlich Ascites Carcinoma (EAC) cells. The response parameter calculated was IC_{50} value (Table 1), which corresponds to the compound concentration causing 50% mortality in net cells.

The parent target molecule, having both hydroquinoline and sulfonamide moieties, was synthesized with free amino group at 2-position, cyano group at 3-position in addition to several substituents at 4-position, in order to study the structure activity relationship of the newly synthesized compounds **6a**–**u**. From the obtained results, it was found that quinoline derivative **6h** carrying benzo[*d*][1,3]dioxol at 4-position with (IC_{50} value < 22.2 μM), quinoline derivative 6g having 4-methoxyphenyl at 4-position with (IC_{50} value = 22.9 μM) and quinoline derivative 6e having styryl group at 4-position with (IC_{50} value = 23.1 μM) showed higher significant cytotoxic activity which was even higher activity than that of the reference drug doxorubicin with (IC_{50} value = 69.9 μM). On the other hand, compound **6b** bearing 4-methylphenyl at 4-position with (IC_{50} value = 85.7 μM) and compound **6d** having 4-hydrxyphenyl at 4-position with (IC_{50} value = 82.9 μM) are nearly as active as doxorubicin as positive control. Also, compounds **6c**, **6f**, **6l** and **6p** exhibited moderate cytotoxic activities, while compounds **6a**, **6i**–**k**, **6m**-**o** and **6q**–**u** showed no activity.

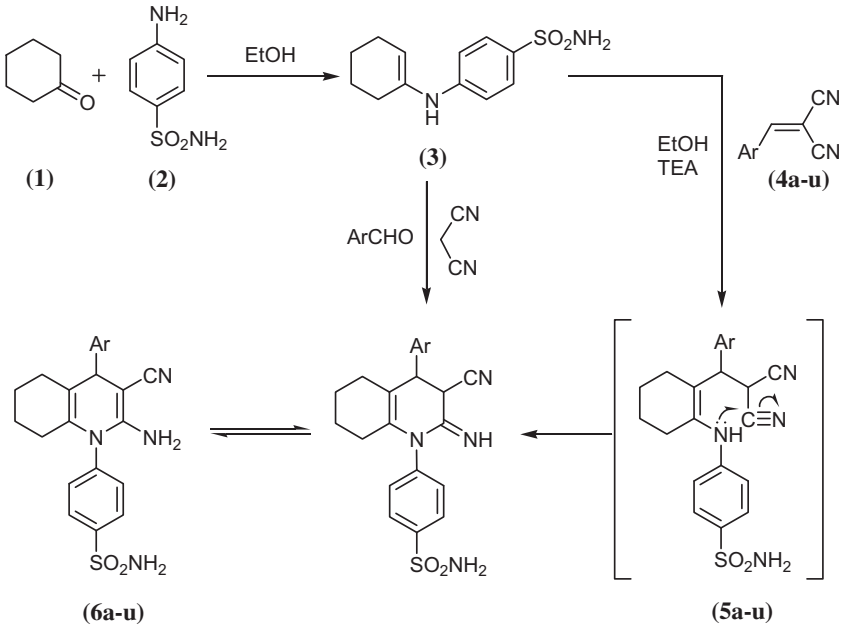
2.3. Docking studies

Previous literature shows that carbonic anhydrase inhibition is one of the anticancer mechanisms of sulfonamides, and this was clearly reported by Abbate et al. [7], who stated that the potent anticancer sulfonamide drug (E7070), currently undergoing clinical development for the treatment of several types of cancer, also acts as a strong carbonic anhydrase inhibitor, and this may contribute at least in part, to its *in vivo* efficacy (Fig. 3).

The X-ray crystal structure of the adduct of human carbonic anydrase II (hCA II) with E7070 revealed similar interactions between the inhibitor and the active site as those reported by Supuran et al. [14,22]. These interactions are found to be common for the sulfonamide compounds which are CA inhibitors and include: (i) binding of the compounds to the Zn(II) ion by the sulfonamide moiety in a tetrahedral geometry which is a stable geometry for the metal ion. (ii) the nitrogen atom of the sulfonamide is coordinated to the Zn(II) ion of the enzyme. (iii) the amino acid Thr 199 is participates in two hydrogen bonds, one with the NH moiety and the other with one of the oxygen atoms of the SO_2NH_2 (Fig. 4) [14,23]

Since, the synthesized compounds are sulfonamide derivatives and their design complies with the general pharmacophore of sulfonamide CA inhibitors,, it was interesting to perform docking studies on the synthesized compounds to hCA II and to compare their docking interactions with the previously reported interactions of E7070.

In order to validate our docking procedure, E7070 was docked into the active site of hCA II. The docking results clearly show that



a, Ar = C_6H_5
b, Ar = $C_6H_4CH_3$-4
c, Ar = C_6H_4OH-2
d, Ar = C_6H_4OH-4
e, Ar = $C_6H_5CH{=}CH$
f, Ar = $C_6H_5OCH_3$-2
g, Ar = $C_6H_4OCH_3$-4
h, Ar = (benzo[d][1,3]dioxol-5-yl)
i, Ar = $C_6H_4NO_2$-3
j, Ar = $C_6H_4NO_2$-4
k, Ar = C_6H_4Cl-2
l, Ar = $C_6H_3Cl_2$-2,4
m, Ar = $C_6H_3Cl_2$-3,4
n, Ar = $C_6H_3OCH_3$-3,OH-4
o, Ar = $C_6H_3OC_2H_5$-3,OCH_3-4
p, Ar = $C_6H_2(OCH_3)_3$-2,4,5
q, Ar = $C_{10}H_6OH$-2
r, Ar = $C_{10}H_6OCH_3$-2
s, Ar = $C_{10}H_6OCH_3$-4
t, Ar = 5-CH_3-2-furyl
u, Ar = 2-thienyl

Scheme 1.

indeed, the compound exhibits similar interactions as those previously reported in the literature and stated above (Fig. 5).

Docking of all the synthesized compounds was performed and it was found that the compounds exhibit similar interactions to that previously reported for E7070 and stated above. Fig. 6 shows an example of the interaction map of tetrahydroquinoline derivative **6h** docked pose with nearby binding site amino acids of hCA II.

To give a clearer comparison of the docked pose of E7070 and the most active compound **6h**. Fig. 7 shows a three-dimensional superimposition of docked poses of compound **6h** and E7070. It was found that the benzenesulfonamide moieties bound to hCA II overlap each other completely while the tail adopts a slightly different conformation.

Finally, it can be seen from our docking study that the synthesized compounds exhibit similar conformations and binding interactions with hCA II similar to those previously reported for other sulfonamide compounds that act as CA inhibitors. This suggests that synthesized compounds might possibly act as CA inhibitors, and this may contribute at least in part, to the anticancer activity.

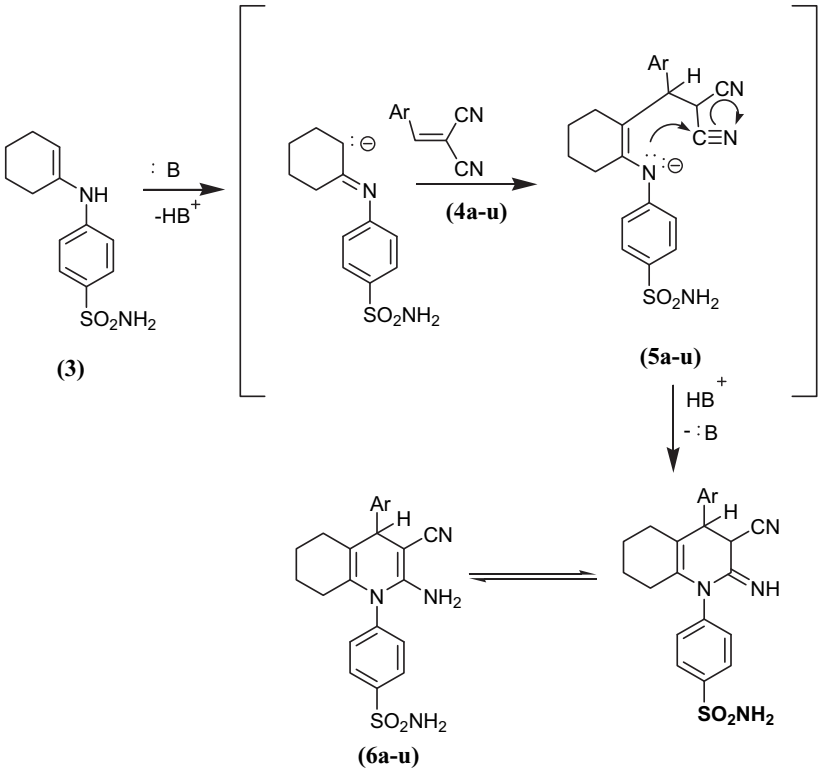
3. Conclusion

The present work showed that compounds combining hydroquinoline and free sulfonamide moieties revealed promising cytotoxic activity *in vitro* against EAC cells, specially compounds **6h**, **6g** and **6e** containing benzo[*d*][1,3]dioxol, 4-methoxyphenyl and styryl moieties at 4-position. On the other hand, compounds **6b** and **6d** are nearly as active as doxorubicin. Docking of the synthesized compounds in the carbonic anhydrase active site may give a suggestion that the synthesized compounds may act as carbonic anhydrase inhibitors and this may contribute in part to their anticancer activity.

4. Experimental

4.1. Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5 ml) mixture was used as a developing solvent system at room temperature and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra (in DMSO-d_6) were recorded on Bruker Ac-300 ultra shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz, using TMS as internal standard. Electron impact Mass Spectra were recorded on



Scheme 2. Postulated mechanism for the formation of compounds (**6a**–**u**)

a, Shimadzu Gc-Ms-Qp 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ±0.4% of the theoretical values.

Table 1
In vitro cytotoxic activity of the newly synthesized compounds **6a**–**u.**

Compound No.	Non-viable cells (%)				IC_{50}[a] (μg/ml)	IC_{50} (μM)
	Concentration (μg/ml)					
	100	50	25	10		
6a	30	20	10	5	>100[b]	–
6b	90	80	30	20	36	85.7
6c	100	50	40	10	50	118.4
6d	100	60	42	20	35	82.9
6e	70	90	70	50	10	23.1
6f	100	50	40	30	50	114.6
6g	100	100	90	50	10	22.9
6h	100	100	100	100	<10	<22.2
6i	12	2	1	0	>100[b]	–
6j	20	10	5	0	>100[b]	–
6k	10	5	0	0	>100[b]	–
6l	70	50	50	0	50	105.2
6m	50	50	40	1	100	–
6n	50	30	2	0	100	–
6o	10	5	5	0	>100[b]	–
6p	100	50	0	0	50	100.8
6q	50	20	5	10	100	–
6r	50	10	10	5	100	–
6s	20	10	5	0	>100[b]	–
6t	30	10	10	0	>100[b]	–
6u	20	10	5	0	>100[b]	–
Doxorubicin	100	70	25	17	38	69.9

[a] IC_{50} value: corresponds to the compound concentration causing 50% mortality in net cells.
[b] Compounds with $IC_{50} \geq 100$ μg/ml are considered to be inactive.

*4.1.1. 4-(Cyclohexenylamino)benzenesulfonamide, **3***

A mixture of cyclohexanone 1 (0.98 g, 0.01 mol) and sulfanilamide 2 (1.72 g, 0.01 mol) in ethanol (30 ml) was refluxed for 5 h. The reaction mixture was cooled and poured onto ice-cold water. The obtained solid was crystallized from ethanol to give 3. Yield 79%; mp 129–131 °C. IR (KBr, cm^{-1}): 3380, 3340, 3210 (NH, NH_2), 3100 (CH arom.), 2940, 2836 (CH aliph.), 1379, 1186 (SO_2). ^{1}H NMR in (DMSO-d_6) δ: 1.0–1.4 [m, 8H, $4CH_2$ cyclo], 4.6 [s, 1H, CH], 6.9–8.0 [m, 7H, Ar–H + SO_2NH_2 + NH]. Anal. Calcd. For $C_{12}H_{18}N_2O_2S$: C, 56.67; H, 7.13; N, 11.01. Found: C, 56.31; H, 7.00; N, 11.32.

*4.1.2. 4-[2-Amino-3-cyano-4-substituted-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamides, **6a**–**u***

Method (A): a mixture of 3 (2.52 g, 0.01 mol) and arylidenemalononitriles **4a**–**u** (0.01 mol) in ethanol (30 ml) containing 3 drops of triethylamine was refluxed for 5 h. The reaction mixture was filtered while hot and the obtained solid was recrystallized from ethanol to give **6a**–**u**.

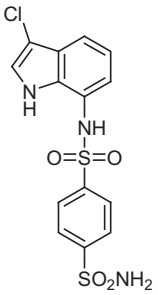


Fig. 3. E7070, a sulfonamide compound in advanced clinical trials as anticancer agent.

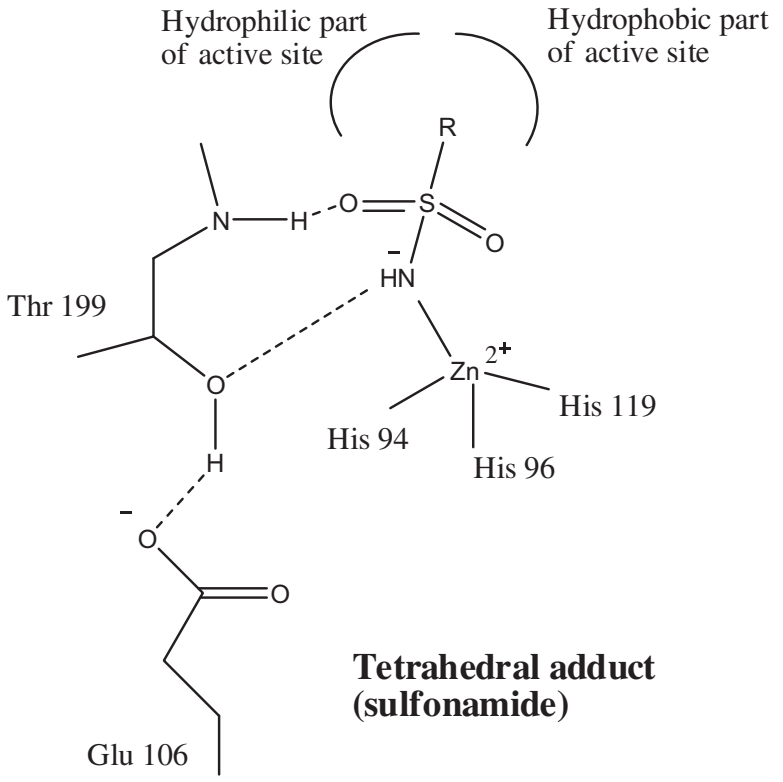


Fig. 4. CA inhibition mechanism by sulfonamides.

Method (B): a mixture of 3 (2.52 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and aldehyde (0.01 mol) in ethanol (20 ml) containing 3 drops of triethylamine was refluxed for 5 h. The reaction mixture was filtered while hot and the obtained solid was recrystallized from ethanol to give **6a**–**u** (m.p. and mixed m.p) and the same R_f.

4.1.2.1. 4-[2-Amino-3-cyano-4-phenyl-5,6,7,8-tetrahydroquinolin-1 (4H)-yl]benzenesulfonamide, ***6a****.* Yield 86%; mp 159–161 °C; IR (KBr, cm^{-1}): 3477, 3377, 3320, 3244 ($2NH_2$), 2224 (C≡N), 1311, 1186 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.3 [s, 1H, CH], 5.7 [s, 2H, NH_2], 7.0–7.8 [m, 11H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{22}H_{22}N_4O_2S$: C, 65.00; H, 5.46; N, 13.78. Found: C, 65.22; H, 5.36; N, 13.94.

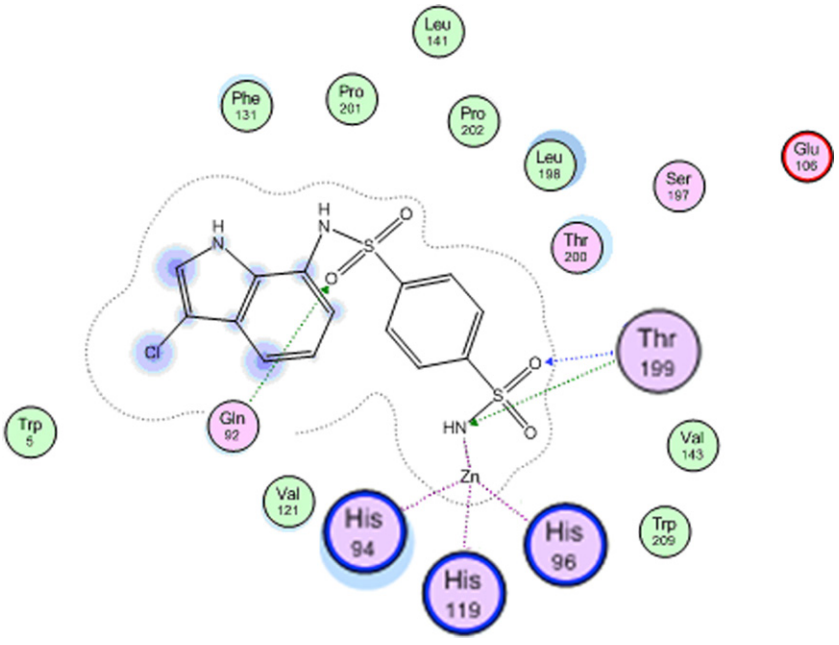


Fig. 5. Interaction map of E-7070 with the active site of hCA II showing similar interactions as those previously reported.

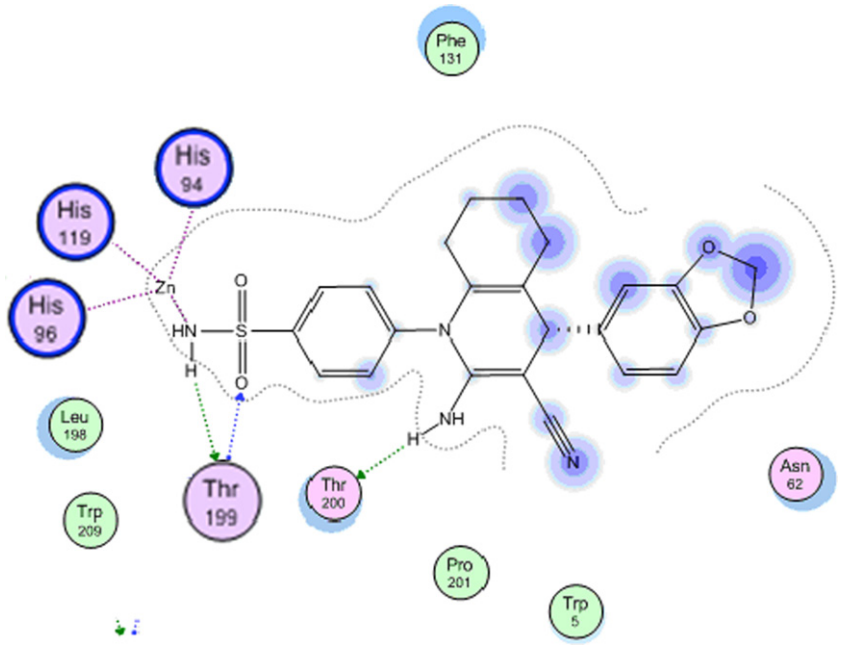


Fig. 6. Interaction map of compound 6 h with the active site of hCA II showing similar interactions as those previously reported.

4.1.2.2. 4-[2-Amino-3-cyano-4-(p-tolyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6b****.* Yield 81%; mp 147–149 °C; IR (KBr, cm^{-1}): 3462, 3375, 3246 ($2NH_2$), 3036 (CH arom.), 2960, 2880 (CH aliph.), 2224 (C≡N), 1310, 1191 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.4 [m, 8H, $4CH_2$ cyclo], 2.4 [s, 3H, CH_3], 4.3 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.5–8.4 [m, 10H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{23}H_{24}N_4O_2S$: C, 65.69; H, 5.75; N, 13.32. Found: C, 65.30; H, 5.80; N, 13.54.

4.1.2.3. 4-[2-Amino-3-cyano-4-(2-hydroxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6c****.* Yield 73%; mp 146–148 °C; IR (KBr, cm^{-1}): 3477 (OH), 3383, 3320, 3242 ($2NH_2$), 3068 (CH arom.), 2972, 2940 (CH aliph.), 2187 (C≡N), 1310, 1184 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.3 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.8–8.0 [m, 10H, Ar–H + SO_2NH_2], 8.6 [s, 1H, OH]. Anal. Calcd. For $C_{22}H_{22}N_4O_3S$: C, 62.54; H, 5.25; N, 13.26. Found: C, 62.62; H, 5.50; N, 13.11.

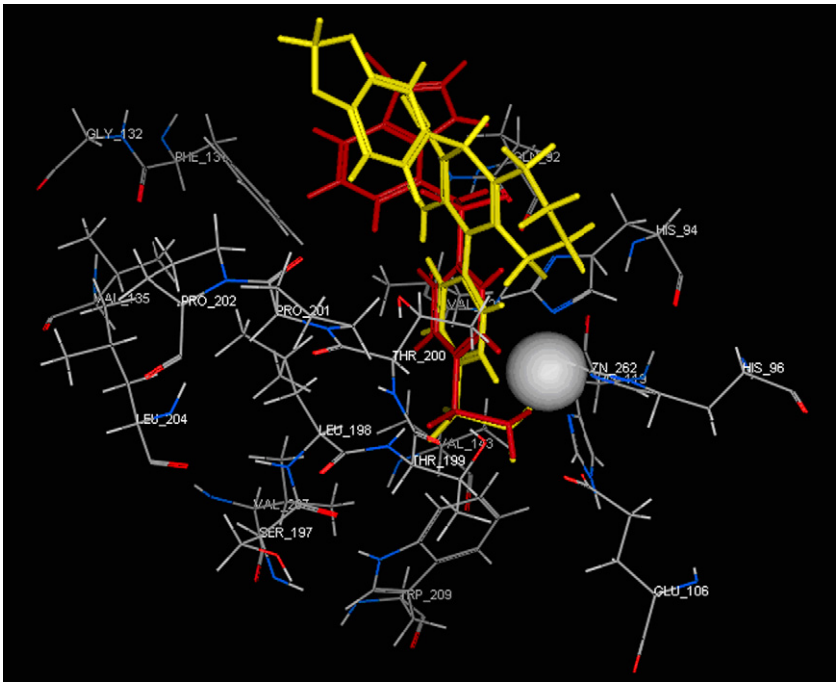


Fig. 7. Superimposition of compound 6 h (yellow) and E7070 (red) in the active site of hCA II shows that the benzenesulfonamide moieties bound to hCA II overlap each other completely while the tails adopts slightly different conformations.

4.1.2.4. 4-[2-Amino-3-cyano-4-(4-hydroxyphenyl)-5,6,7,8-tetrahydro-quinolin-1(4H)-yl]benzenesulfonamide, ***6d***. Yield 77%; mp 140–142 °C; IR (KBr, cm^{-1}): 3477 (OH), 3381, 3319, 3243 ($2NH_2$), 3100 (CH arom.), 2950, 2860 (CH aliph.), 2225 (C≡N), 1373, 1149 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.4 [m, 8H, $4CH_2$ cyclo], 4.2 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.7–8.0 [m, 10H, Ar–H + SO_2NH_2], 9.2 [s, 1H, OH]. Anal. Calcd. For $C_{22}H_{22}N_4O_3S$: C, 62.54; H, 5.25; N, 13.26. Found: C, 62.23; H, 5.59; N, 12.96.

4.1.2.5. 4-[2-Amino-3-cyano-4-styryl-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6e***. Yield 68%; mp 238–240 °C; IR (KBr, cm^{-1}): 3477, 3382, 3318 ($2NH_2$), 2217 (C≡N), 1311, 1184 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.6 [m, 8H, $4CH_2$ cyclo], 4.0 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.1, 6.4 [2d, 2H, CH=CH, $J = 7.3$, 7.4 Hz], 7.0–7.8 [m, 11H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{24}H_{24}N_4O_2S$: C, 66.64; H, 5.59; N, 12.95. Found: C, 66.32; H, 5.49; N, 13.13.

4.1.2.6. 4-[2-Amino-3-cyano-4-(2-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6f***. Yield 81%; mp 155–157 °C; IR (KBr, cm^{-1}): 3478, 3384, 3318, 3243 ($2NH_2$), 2228 (C≡N), 1310, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 3.6 [s, 3H, OCH_3], 4.1 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.8–7.9 [m, 10H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{23}H_{24}N_4O_3S$: C, 63.28; H, 5.54; N, 12.83. Found: C, 63.41; H, 5.77; N, 13.01.

4.1.2.7. 4-[2-Amino-3-cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6g***. Yield 86%; mp 159–161 °C; IR (KBr, cm^{-1}): 3478, 3383, 3319 ($2NH_2$), 2210 (C≡N), 1311, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 3.7 [s, 3H, OCH_3], 4.2 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.5–8.0 [m, 10H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{23}H_{24}N_4O_3S$: C, 63.28; H, 5.54; N, 12.83. Found: C, 63.29; H, 5.33; N, 12.94.

4.1.2.8. 4-[2-Amino-4-(benzo[d][1,3]dioxol-5-yl)–3–cyano-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6h***. Yield 79%; mp 178–180 °C; IR (KBr, cm^{-1}): 3350, 3310, 3293 ($2NH_2$), 2925, 2886 (CH aliph.), 2225 (C≡N), 1390, 1155 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.4 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.1 [s, 2H, O-CH_2-O], 7.0–8.5 [m, 9H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 450.1 [M^+] (2.62), 40 (100). Anal. Calcd. For $C_{23}H_{22}N_4O_4S$: C, 61.32; H, 4.92; N, 12.44. Found: C, 61.43; H, 5.24; N, 12.10.

4.1.2.9. 4-[2-Amino-3-cyano-4-(3-nitrophenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6i***. Yield 78%; mp 264–266 °C; IR (KBr, cm^{-1}): 3417, 3348, 3252 ($2NH_2$), 3091 (CH arom.), 2960, 2910 (CH aliph.), 2223 (C≡N), 1352, 1153 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.3 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.8–7.6 [m, 10H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 451.05 [M^+] (6.3), 43 (100). Anal. Calcd. For $C_{22}H_{21}N_5O_4S$: C, 58.52; H, 4.69; N, 15.51. Found: C, 58.70; H, 4.79; N, 15.21.

4.1.2.10. 4-[2-Amino-3-cyano-4-(4-nitrophenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6j***. Yield 83%; mp 157–159 °C; IR (KBr, cm^{-1}): 3470, 3384, 3315, 3243 ($2NH_2$), 2219 (C≡N), 1311, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.2 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.7–7.8 [m, 10H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{22}H_{21}N_5O_4S$: C, 58.52; H, 4.69; N, 15.51. Found: C, 58.40; H, 4.55; N, 15.30.

4.1.2.11. 4-[2-Amino-4-(2-chlorophenyl)–3–cyano-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6k***. Yield 68%; mp 163–165 °C; IR (KBr, cm^{-1}): 3463, 3376, 3272 ($2NH_2$), 2212 (C≡N), 1335, 1186 (SO_2), 721 (C-Cl). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.3 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.9–7.9 [m, 10H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 440.1 [M^+] (4.1), 56 (100). Anal. Calcd. For $C_{22}H_{21}ClN_4O_2S$: C, 59.92; H, 4.80; N, 12.71. Found: C, 60.11; H, 4.70; N, 12.92.

4.1.2.12. 4-[2-Amino-3-cyano-4-(2,4-dichlorophenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6l***. Yield 91%; mp 238–240 °C; IR (KBr, cm^{-1}): 3418, 3354, 3256 ($2NH_2$), 3090 (CH arom.), 2960, 2910 (CH aliph.), 2221 (C≡N), 1377, 1134 (SO_2), 765 (C-Cl). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.1 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.8–7.9 [m, 9H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 475.1 [M^+] (7.6), 44 (100). Anal. Calcd. For $C_{22}H_{20}Cl_2N_4O_2S$: C, 55.58; H, 4.24; N, 11.79. Found: C, 55.46; H, 4.11; N, 11.55.

4.1.2.13. 4-[2-Amino-3-cyano-4-(3,4-dichlorophenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6m***. Yield 88%; mp 208–210 °C; IR (KBr, cm^{-1}): 3419, 3384, 3321, 3254 ($2NH_2$), 3100 (CH arom.), 2970, 2940 (CH aliph.), 2222 (C≡N), 1378, 1184 (SO_2), 741 (C-Cl). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.2 [s, 1H, CH], 5.8 [s, 2H, NH_2], 7.1–7.9 [m, 9H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 475.05 [M^+] (9.5), 55 (100). Anal. Calcd. For $C_{22}H_{20}Cl_2N_4O_2S$: C, 55.58; H, 4.24; N, 11.79. Found: C, 55.46; H, 4.41; N, 11.60.

4.1.2.14. 4-[2-Amino-3-cyano-4-(4-hydroxy-3-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6n***. Yield 79%; mp 161–163 °C; IR (KBr, cm^{-1}): 3478 (OH), 3384, 3319, 3243 ($2NH_2$), 3078 (CH arom.), 2940, 2880 (CH aliph.), 2210 (C≡N), 1311, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 0.9–1.3 [m, 8H, $4CH_2$ cyclo], 3.8 [s, 3H, OCH_3], 4.3 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.5–7.4 [m, 10H, Ar–H + SO_2NH_2 + OH]. Anal. Calcd. For $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38. Found: C, 61.24; H, 5.47; N, 12.02.

4.1.2.15. 4-[2-Amino-3-cyano-4-(3-ethoxy-4-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6o***. Yield 71%; mp 135–137 °C; IR (KBr, cm^{-1}): 3310, 3280, 3210 ($2NH_2$), 2978, 2890 (CH aliph.), 2222 (C≡N), 1397, 1178 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 1.3 [t, 3H, CH_3], 3.8 [s, 3H, OCH_3], 4.2 [q, 2H, CH_2], 6.5 [s, 1H, CH], 6.9 [s, 2H, NH_2], 7.0–8.5 [m, 9H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{25}H_{28}N_4O_4S$: C, 62.48; H, 5.87; N, 11.66. Found: C, 62.69; H, 5.71; N, 11.49.

4.1.2.16. 4-[2-Amino-3-cyano-4-(2,4,5-trimethoxyphenyl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6p***. Yield 78%; mp 141–143 °C; IR (KBr, cm^{-1}): 3478, 3384, 3319, 3243 ($2NH_2$), 2951, 2837 (CH aliph.), 2216 (C≡N), 1342, 1191 (SO_2). ^1H NMR (DMSO-d_6) δ: 0.9–1.4 [m, 8H, $4CH_2$ cyclo], 3.7, 3.92, 3.94 [3s, 9H, 3 CH_3], 4.1 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.5–8.1 [m, 8H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{25}H_{28}N_4O_5S$: C, 60.47; H, 5.68; N, 11.28. Found: C, 60.16; H, 5.48; N, 11.00.

4.1.2.17. 4-[2-Amino-3-cyano-4-(2-hydroxynaphthalen-1-yl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6q***. Yield 89%; mp 264–266 °C; IR (KBr, cm^{-1}): 3490 (OH), 3335, 3233, 3210 ($2NH_2$), 3093 (CH arom.), 2970, 2920 (CH aliph.), 2208 (C≡N), 1343, 1176 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.6 [m, 8H, $4CH_2$ cyclo], 4.2 [s, 1H, CH], 5.8 [s, 2H, NH_2], 6.7–7.9 [m, 12H, Ar–H + SO_2NH_2]., 8.6 [s, 1H, OH]. MS (*m*/*z*): 472.1 [M^+] (5.6), 73 (100). Anal. Calcd. For $C_{26}H_{24}N_4O_3S$: C, 66.08; H, 5.12; N, 11.86. Found: C, 66.22; H, 5.23; N, 11.60.

4.1.2.18. 4-[2-Amino-3-cyano-4-(2-methoxynaphthalen-1-yl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6r***. Yield 77%; mp 146–148 °C; IR (KBr, cm^{-1}): 3410, 3375, 3280 ($2NH_2$), 2970, 2910 (CH aliph.), 2227 (C≡N), 1381, 1192 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 3.9 [s, 3H, OCH_3], 4.2 [s, 1H, CH], 5.8 [s, 2H, NH_2], 7.0–8.8 [m, 12H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{27}H_{26}N_4O_3S$: C, 66.65; H, 5.39; N, 11.51. Found: C, 66.51; H, 5.30; N, 11.22.

4.1.2.19. 4-[2-Amino-3-cyano-4-(4-methoxynaphthalen-1-yl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6s****.* Yield 79%; mp 159–161 °C; IR (KBr, cm^{-1}): 3447, 3380, 3220 ($2NH_2$), 2992, 2950 (CH aliph.), 2218 (C≡N), 1372, 1167 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.1–1.5 [m, 8H, $4CH_2$ cyclo], 3.8 [s, 3H, OCH_3], 4.3 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.8–8.1 [m, 12H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{27}H_{26}N_4O_3S$: C, 66.65; H, 5.39; N, 11.51. Found: C, 66.55; H, 5.61; N, 11.44.

4.1.2.20. 4-[2-Amino-3-cyano-4-(5-methylfuran-2-yl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6t****.* Yield 66%; mp 163–165 °C; IR (KBr, cm^{-1}): 3420, 3384, 3200 ($2NH_2$), 2950, 2920 (CH aliph.), 2211 (C≡N), 1334, 1157 (SO_2). ^1H NMR (DMSO-d_6) δ: 0.9–1.4 [m, 8H, $4CH_2$ cyclo], 2.4 [s, 3H, CH_3], 4.2 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.5–8.1 [m, 8H, Ar–H + SO_2NH_2]. Anal. Calcd. For $C_{21}H_{22}N_4O_3S$: C, 61.44; H, 5.40; N, 13.65. Found: C, 61.46; H, 5.26; N, 13.41.

4.1.2.21. 4-[2-Amino-3-cyano-4-(thiophen-2-yl)-5,6,7,8-tetrahydroquinolin-1(4H)-yl]benzenesulfonamide, ***6u****.* Yield 63%; mp 151–153 °C; IR (KBr, cm^{-1}): 3384, 3318, 3242 ($2NH_2$), 3094 (CH arom.), 2970, 2886 (CH aliph.), 2162 (C≡N), 1310, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.0–1.5 [m, 8H, $4CH_2$ cyclo], 4.2 [s, 1H, CH], 5.7 [s, 2H, NH_2], 6.5–8.0 [m, 9H, Ar–H + SO_2NH_2]. MS (*m*/*z*): 412.05 [M^+] (5.1), 40 (100). Anal. Calcd. For $C_{20}H_{20}N_4O_2S_2$: C, 58.23; H, 4.89; N, 13.58. Found: C, 58.44; H, 4.99; N, 13.60.

4.2. In vitro anticancer screening

Ehrlich ascites carcinoma (EAC) cells were maintained in female Swiss albino mice weighing 25–30 g (the holding company for biological products and vaccines. VACSERA, Cairo, Egypt) were housed at a constant temperature (24 ± 2 °C) with alternating 12-h light and dark cycles and fed standard laboratory food (Milad Co, Cairo, Egypt) and water *ad libitum*. All chemicals and reagents were of the highest grade commercially available by the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt. Animal care and handling was done according to the guidelines set by the world heath organization, Geneva, Switzerland and approved from the committee for animals care at NCRRT, AEA.

Ehrlich Ascites Carcinoma (EAC) cells were obtained by needle aspiration of ascetic fluid from preinoculated mice; under aseptic conditions. Tumor cells suspension (2.5×10^6 per ml) was prepared with various dilutions by dissolving; 100, 50, 25 and 10 μg of the tested compounds in DMSO (1 ml).

In a set of sterile test tubes 0.8 ml RPMI-1640 media containing (glutamine, fetal calf serum as nutrient, streptomycin and penicillin), 0.1 ml of each of the tested compounds (corresponding to 100, 50, 25, 10 μg) were mixed then 0.1 ml of tumor cell suspension (2×10^6) was added. The test tubes were incubated at 37 °C for 2 h. Trypan blue exclusion test was carried out to calculate the percentage of non-viable cells after 2 h of incubation [24]. The results of *in vitro* antitumor screening are presented in Table 1.

4.3. Docking studies

All molecular modeling calculations and docking studies were performed using "Molecular Operating Environment (MOE) version 2007.09".

The ligand was drawn on ChemDraw and imported in MOE. The structure was subjected to energy minimization using MMFF94× forcefield and the partial charges were computed using the same forcefield.

The X-ray crystallographic structure of hCA II complexed with *N*-(2,3,4,5,6-pentafluoro-benzyl)-4-sulfamoyl-benzamide (1g54) was obtained from the Protein Data Bank. The enzyme was prepared for the docking studies where: (i) the ligand molecule was removed from the enzyme active site. (ii) Hydrogen atoms were added to the structure with their standard geometry. (iii) Partial charges were computed using Amber99 forcefield.

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