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Original article

Synthesis and *in vitro* anticancer evaluation of some novel hexahydroquinoline derivatives having a benzenesulfonamide moiety

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ABSTRACT

Inhibition of carbonic anhydrase isozymes has been found to have a role in the treatment of cancer. Several sulfonamide compounds bearing an aromatic or a heteroaromatic ring were found to posses potent carbonic anhydrase inhibitory activity and so can be used in the treatment of several types of cancer. In this paper, we present the synthesis of some novel quinoline **7**–**13**, **21**–**26**, **28** and pyrimidoquinoline **14**–**18**, **20**, **27** derivatives having a sulfonamide moiety. All the newly synthesized compounds were evaluated for their *in vitro* anticancer activity. Several compounds showed interesting cytotoxic activities when compared with the used 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.

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1. Introduction

Sulfonamide compounds have shown to posses several types of biological activities [1–4] including anticancer activity [5–10]. The anticancer activity of sulfonamides was found to take place 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 NF-Y, and carbonic anhydrase inhibition [11–14] which is reported to be the most prominent mechanism [15].

Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to give bicarbonate and a proton ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). CAs are involved in pH regulation, secretion of electrolytes, respiration [16,17], biosynthetic reactions which require CO_2/bicarbonate as substrate such as gluconeogenesis, lipogenesis, ureagenesis, and pyrimidines synthesis [18]. The catalytic domain of CAs contains an active site Zn^{2+}. This metal cation is a strong Lewis acid that binds to and activates a substrate H_2O molecule to catalyzes the reversible hydration reaction of carbon dioxide. The hydration of CO_2 does not proceed at an appreciable rate under physiological conditions in the absence of CA enzymes [19]. CA inhibition has been found to have an important role in cancer treatment through reducing the provision of bicarbonate for the synthesis of nucleotides and other cell components such as membrane lipids [20]. Actazolamide **I** and E7070 **II** are examples of carbonic anhydrase inhibitors.

Furthermore, among the compounds that have shown significant anticancer activity are the quinolines and reduced quinoline derivatives [21–24]. Also, combination of several quinoline derivatives with sulfonamide moiety has been reported to have significant anticancer activity [7,25,26]. In the light of these facts, and in a hope to obtain some novel compounds with significant anticancer activity, this work reports the synthesis of a novel series of quinoline and pyrimidoquinoline derivatives bearing a sulfonamide moiety and the testing of these compounds for their *in vitro* anticancer activity.

2. Results and discussion

2.1. Chemistry

During the course of our continued interest in the development of new general synthetic routes for the synthesis of biologically active quinolines and pyrimidoquinolines we decided to synthesize a new series of pyrimidoquinoline derivatives **14**–**18**, **20** and **27** starting from enaminone **3** [27]. The designed target compounds are depicted in Schemes 1–5. Enaminone **3** was obtained via reaction of 1,3-cyclohexandione **1** with sulfanilamide **2**. Treatment

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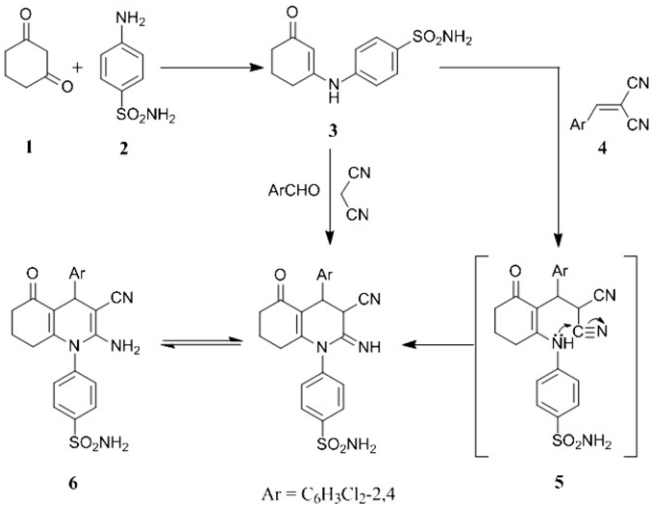
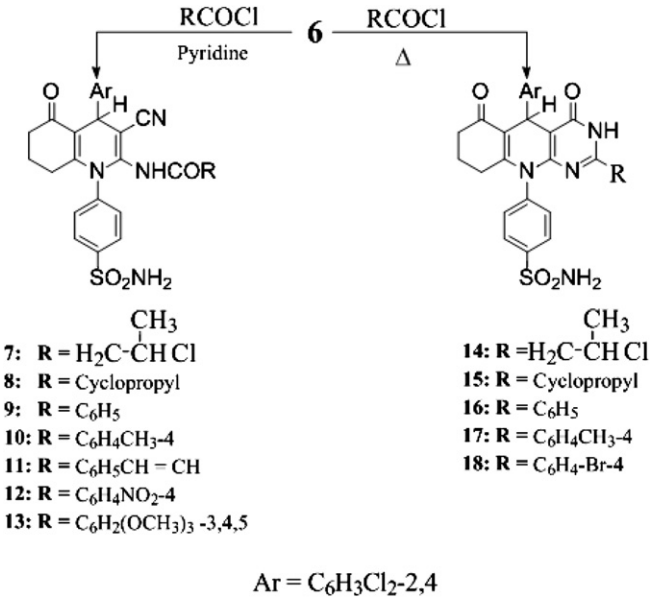


Scheme 1.

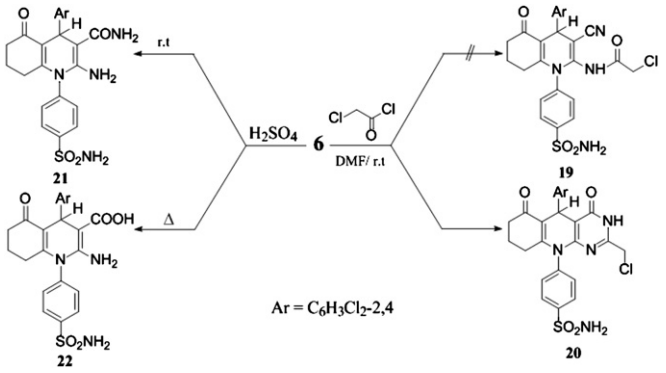
of enaminone **3** with 2,4-dichlorobenzylidenemalononitrile **4** in ethanol containing triethylamine as catalyst resulted in cycloaddition affording quinoline-*o*-aminocarbonitrile **6** [27], via the formation of the intermediate Michael type product **5**, followed by intramolecular cyclization (Scheme 1).

The reaction of compound **6** with acid chloride derivatives in pyridine was carried out in a trial for obtaining pyrimidoquinoline derivatives **14**–**18**, but instead the quinoline derivatives **7**–**13** were obtained. While the reaction of compound **6** with acid chloride derivatives only affected cyclization to furnish pyrimidoquinoline derivatives **14**–**18**, based on the elemental analyses, IR, ^{1}H NMR and mass spectral data. The structure of compounds **7**–**13** was proved by elemental analyses, IR, ^{1}H NMR and mass spectral data. The IR spectra of compounds **7**–**13** revealed the presence of C≡N band. The IR spectra of compounds **14**–**18** exhibited the absence of C≡N band (Scheme 2).

Also, interaction of compound **6** with chloroacetyl chloride in dimethylformamide at room temperature effected cyclization to



Scheme 2.



Scheme 3.

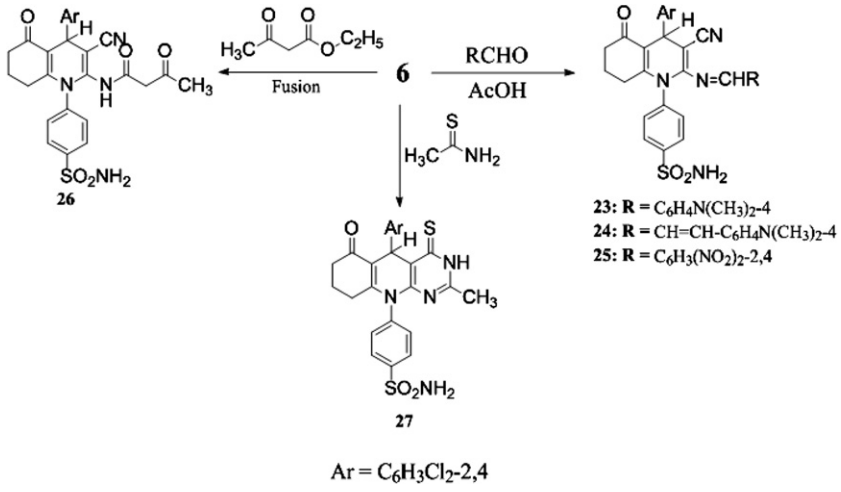
furnish pyrimidoquinoline derivative **20**, rather than the quinoline derivative **19**, based on the elemental analysis and IR spectrum, which showed the absence of C≡N band and presence of bands at 1697, 1652 cm^{-1} (2C=O). ^{1}H NMR spectrum of compound **20** (in DMSO-d_6) revealed signals at 3.5 ppm corresponding to CH_2Cl group and 10.9 ppm due to NH group. When compound **6** was stirred with concentrated H_2SO_4 at room temperature [28] for 2 h, afforded carboxamide derivative **21**, while refluxing of compound **6** with H_2SO_4 caused complete hydrolysis to afford the corresponding quinoline derivative **22**. The formation of amide **21** was confirmed by IR spectrum, which showed the disappearance of the cyano C≡N group and exhibited a carbonyl stretch at 1697 cm^{-1} along with characteristic (NH_2) and carboxamido ($CONH_2$) bands at 3364, 3272 cm^{-1}. IR spectrum of compound **22** exhibited the absence of C≡N band and presence of OH band at 3478 cm^{-1} (Scheme 3).

Condensation of compound **6** with aromatic aldehydes in glacial acetic acid afforded Schiff's bases **23**–**25**. The structure of compounds **23**–**25** was confirmed by elemental analysis, IR spectra which showed the presence of C≡N group at 2210–2215 cm^{-1}. This work was extended to cover the reactivity of compound **6** toward carbonyl compounds. Thus, condensation of **6** with ethyl acetoacetate under condition of fusion gave a product which was formulated as quinoline derivative **26**. The IR spectrum of **26** exhibited bands at 1719, 1680, 1641 cm^{-1} (3C=O). ^{1}H NMR spectrum of compound **26** (in DMSO-d_6) exhibited signals at 2.2 ppm due to $COCH_3$ group (Scheme 4).

Treatment of compound **6** with triethyl orthoformate yielded compound **28**. The formation of compound **28** was supported by microanalytical and spectral data, its IR spectrum showed the presence of band at 2200 cm^{-1} corresponding to the cyano group. Also ^{1}H NMR spectrum of compound **28** (in DMSO-d_6) exhibited the presence of a 3 protons triplet at 1.1 ppm and a 2 protons quartet at 4.19 ppm for the ethyl group. Finally, when compound **28** was treated with hydrazine hydrate at room temperature, the starting material **6** was obtained instead of the expected *N*-amino derivative **29**. This can explained on the basis of formation of an intermediate **30**, which upon elimination of ethoxymethylene-hydrazone [29] furnished compound **6**. This was confirmed by IR, mixed melting point and similar R_f value on the TLC (Scheme 5).

2.2. Biological testing

Doxorubicin, the reference drug used in this study is one of the most effective antitumor agents used to produce regressions in acute leukemias, Hodgkin's disease and other lymphomas. Doxorubicin, which has a significant anticancer activity, was chosen to be our reference drug in this phase so as to just compare its activity with the newly synthesized compounds in order to put a hand on



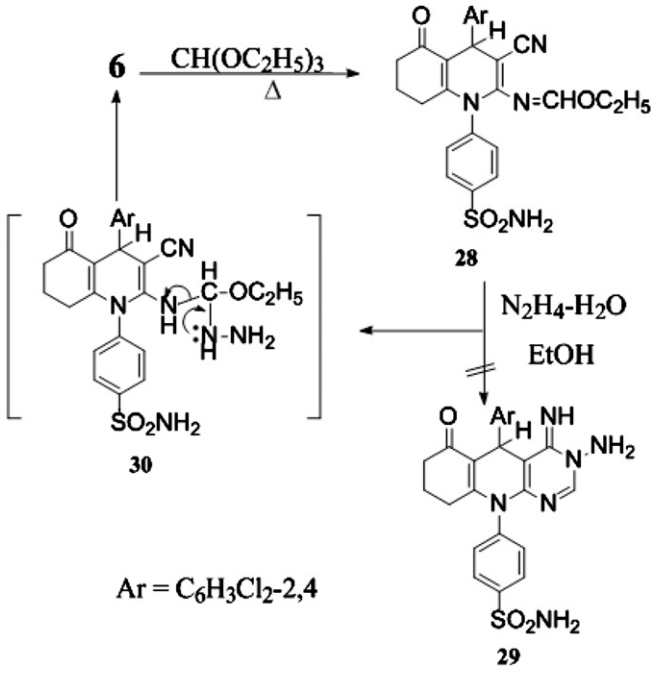
Scheme 4.

the most active compounds and to help use in optimizing the synthesis of the second phase compounds. The relationship between surviving ratio and drug concentration was plotted to obtain the survival curve of Ehrlich Ascites Carcinoma (EAC) cells. The response parameter calculated was IC_{50} value, which corresponds to the compound concentration causing 50% mortality in net cells.

All the synthesized compounds **6**–**18**, **20**–**28** were evaluated for their *in vitro* cytotoxic activity. Compounds **6**, **7**, **9**, **17** and **18** showed significant activity compared to the reference drug doxorubicin (CAS-23214-92-8) (Table 1).

The parent target molecule, containing both sulfonamide and quinoline or sulfonamide and pyrimidoquinoline nucleus, was synthesized with several substitutions in the 2-position, in order to study the structure activity relationship of the synthesized compounds **6**–**18**, **20**–**28**. From the obtained results (Table 1), it was found that several compounds showed significant cytotoxic activity such as compound **9** having benzamide moiety at 2-position and cyano group at the 3-position (IC_{50} value = 17 μM), the pyrimidoquinoline **18** carrying 4-bromophenyl at the 2-position (IC_{50} value = 37 μM), compound **7** containing butanamide at the 2-position (IC_{50} value = 45 μM), the pyrimidoquinoline **17** having *p*-tolyl at the 2-position (IC_{50} value = 46 μM) and compound **6** bearing a free amino group at the 2-position with cyano group at 3-position (IC_{50} value = 51 μM). These compounds showed cytotoxic activity which was even higher than that of reference drug doxorubicin (IC_{50} value = 70 μM) with the benzamide quinoline derivative **9** being the most active compound.

On the other hand, compounds **8**, **12**, **13**, **15** and **26** exhibited a moderate activity but less active than the reference drug



Scheme 5.

Table 1
In vitro cytotoxic activity of the synthesized compounds **6**–**18** and **20**–**28**.

Compd. No.	Non viable cells (%)[a]				IC_{50}[b] (μg/mL)	IC_{50} (μM)
	Concentration (μg/mL)					
	100	50	25	10		
Doxorubicin	100	68 ± 1.9	32 ± 1.6	19 ± 1.3	38	70
6	95 ± 1.5	65 ± 1.6	50 ± 2.1	40 ± 1.8	25	51
7	60 ± 1.9	55 ± 2.2	50 ± 2.6	20 ± 1.2	25	45
8	60 ± 2.1	40 ± 2.4	40 ± 2.0	20 ± 1.1	75	135
9	75 ± 1.8	70 ± 1.9	60 ± 1.8	50 ± 1.3	10	17
10	40 ± 3.1	40 ± 2.1	30 ± 1.5	20 ± 2.1	>100[c]	–
11	45 ± 1.7	40 ± 2.4	30 ± 1.7	30 ± 1.9	>100[c]	–
12	70 ± 2.2	50 ± 1.9	40 ± 2.4	40 ± 1.7	50	78
13	95 ± 2.1	95 ± 1.6	20 ± 2.8	0	60	88
14	60 ± 3.3	40 ± 1.2	40 ± 2.1	30 ± 2.2	>100[c]	–
15	60 ± 4.1	52 ± 1.6	44 ± 1.8	27 ± 1.8	56	101
16	60 ± 1.8	10 ± 2.1	0	0	>100[c]	–
17	60 ± 1.6	55 ± 3.2	55 ± 1.7	30 ± 1.4	28	46
18	100	60 ± 1.8	50 ± 1.5	20 ± 2.3	25	37
20	40 ± 2.1	35 ± 1.7	30 ± 2.1	30 ± 1.6	>100[c]	–
21	45 ± 3.1	40 ± 1.4	30 ± 1.9	30 ± 2.1	>100[c]	–
22	60 ± 2.2	10 ± 1.9	0	0	>100[c]	–
23	40 ± 2.3	30 ± 2.0	30 ± 2.2	10 ± 1.9	>100[c]	–
24	20 ± 3.1	20 ± 2.2	20 ± 3.1	10 ± 1.4	>100[c]	–
25	20 ± 4.2	20 ± 1.7	20 ± 2.6	10 ± 1.5	>100[c]	–
26	80 ± 1.5	60 ± 1.4	44 ± 1.7	40 ± 2.1	64	112
27	60 ± 2.1	40 ± 1.8	40 ± 1.4	30 ± 2.3	>100[c]	–
28	40 ± 1.6	35 ± 2.3	30 ± 1.9	30 ± 1.7	>100[c]	–

[a] Mean of non-viable percentage of three repeated experiments ± SD.
[b] IC_{50} value: corresponds to compound concentration causing 50% mortality in net cells.
[c] Compounds with IC_{50} > 100 μg/mL are considered to be inactive.

doxorubicin. Finally, compounds **10**, **11**, **14**, **16**, **20**–**25**, **27** and **28** showed no activity.

2.3. Molecular modeling

Previous literature showed that carbonic anhydrase inhibition is one of the anticancer mechanisms of sulfonamides, and this was clearly reported by Abbate et al. [5]. So we have performed a molecular modeling study to investigate the possible binding conformation for this group of compounds to the carbonic anhydrase binding site, which may give a suggestion about their proposed mechanism of action. The most active compounds **9** and **18** were docked in the (PDB entry: 1g54) using MOE 2007.09 (MOE) [30].

In order to validate our docking procedure, we docked the compound "*N*-[(2,3,4,5,6-pentaflourophenyl)methyl]-4-sulfamoyl-benzamide" which is the ligand already co-crystallized with the protein. The docking results showed that the best scored conformation exhibits a very similar fashion as the crystallized conformation where the essential interactions, between the sulfonamide moiety and the zinc ion in the active site, are maintained with also the benzenesulfonamide moieties bound to hCA II overlap each other completely while the tail adopts a slightly different conformation (Fig. 1).

We then docked the most active compounds, **9** and **18**, and in both cases, it was found that the sulfonamide moieties interact with the zinc ion in the active site like the co-crystallized ligand and also the benzenesulfonamide moieties significantly overlap each other with the tails adopting a slightly different conformation (Fig. 2).

Finally, it can be seen from our docking study that our synthesized compounds exhibit similar conformations and binding interactions with hCA II similar to the co-crystallized ligand. This suggests that our compounds might possess significant CA inhibitory activity, and this may contribute at least in part, to their anticancer activity.

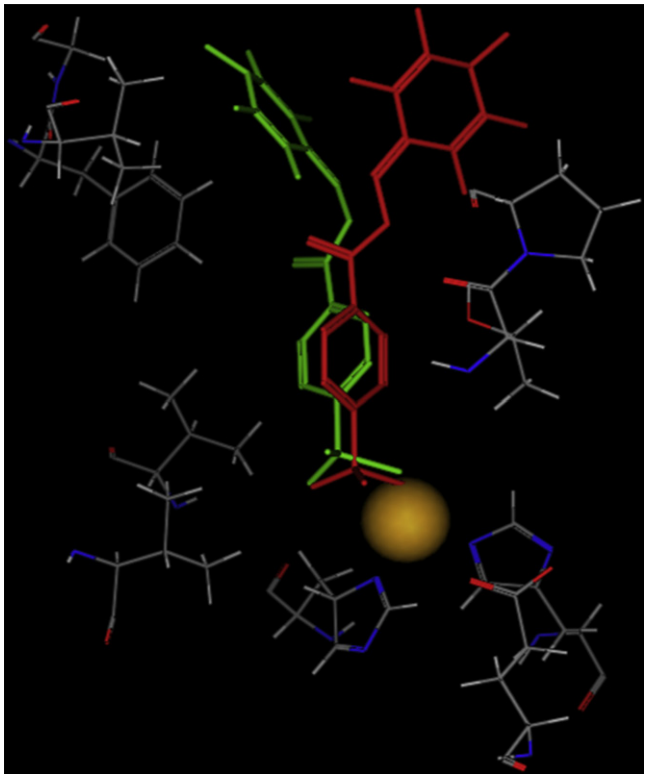


Fig. 1. Ligand (*N*-Pentafluorophenylmethyl-4-sulfamoyl-benzamide) co-crystallized with CAII (PDB code 1g54) "green" and its best scoring redocked conformer "red" in CAII active site (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

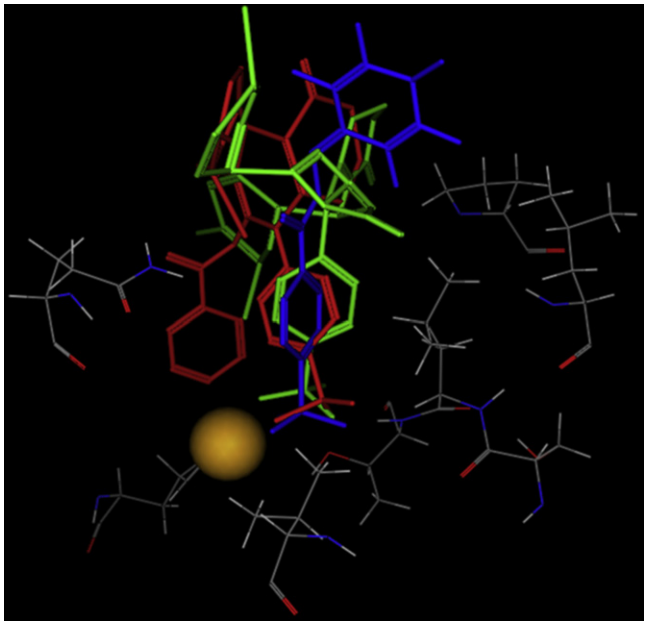


Fig. 2. Docking poses of compound 9 "red", compound 18 "green" and the ligand (*N*-Pentafluorophenylmethyl-4-sulfamoyl-benzamide) "blue" docked into the CAII active site (PDB code 1g54) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

3. Conclusion

The objective of the present study was to synthesize and investigate the anticancer activity of new quinoline and pyrimidoquinoline derivatives containing a free sulfonamide moiety. Several compounds have been shown to exhibit significant anticancer activity such as the quinoline derivatives having benzamide **9**, free amino group **6**, butanamide **7**, the pyrimidoquinoline derivatives having 4-bromophenyl **18** and *p*-tolyl **17**. Docking of the 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 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 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 $\pm$0.4% of the theoretical values.

*4.1.1. 4-(3-Oxocyclohex-1-enylamino)benzenesulfonamide (**3**) and 4-(2-amino-3-cyano-4-(2,4-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydro quinolin-1(4H)-yl)-benzenesulfonamide (**6**)*

Prepared according to the previously reported procedure [27].

*4.1.2. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl) substitutedamides (**7**–**13**)*

A mixture of **6** (4.89 g, 0.01 mol) and acid chloride derivatives (0.01 mol) in pyridine (20 mL) was refluxed for 5 h. The reaction mixture was cooled and poured onto cold water, then acidified by dilute HCl. The solid obtained was crystallized from the appropriate solvent to give **7**–**13**, respectively.

*4.1.3. 3-Chloro-N-(3-cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)butanamide (**7**)*

Cryst. Solvent, Dioxane; Yield, 79%: m.p. >330 °C; IR (KBr, cm^{-1}): 3450, 3422-3380 (NH, NH_2), 3100 (CH arom.), 2929, 2880 (CH aliph.), 2188 (C≡N), 1680, 1627 (2C═O), 1369, 1188 (SO_2), 755 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.4–2.6 [m, 6H, $3CH_2$, cyclo], 1.85 [d, 3H, CH_3, J = 7.8 Hz], 2.3 [m, 1H, CH—Cl], 2.6 [d, 2H, CH_2CO, J = 7.4 Hz], 4.9 [s, 1H, CH, pyridine], 7.2–8.0 [m, 9H, Ar-H + SO_2NH_2], 9.5 [s, 1H, NH]. Anal. Calcd. for $C_{26}H_{23}Cl_3N_4O_4S$: C, 52.58; H, 3.90; N, 9.43. Found: C, 52.20; H, 3.60; N, 9.70.

*4.1.4. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)cyclopropane-carboxamide (**8**)*

Cryst. Solvent, Ethanol; Yield, 77%: m.p. 241–243 °C; IR (KBr, cm^{-1}): 3435, 3390, 3280 (NH, NH_2), 3093 (CH arom.), 2949, 2836 (CH aliph.), 2210 (C≡N), 1740, 1658 (2C═O), 1370, 1170 (SO_2), 730 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 0.3–0.9 [m, 4H, $2CH_2$, cyclopropyl], 1.3 [m, 1H, CH cyclopropyl], 1.6–2.6 [m, 6H, $3CH_2$, cyclo], 5.2 [s, 1H, CH pyridine], 7.3–8.0 [m, 9H, Ar-H + SO_2NH_2], 10.1 [s, 1H, NH]. Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_4S$: C, 56.02; H, 3.98; N, 10.05. Found: C, 55.76; H, 3.71; N, 10.35.

*4.1.5. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)benzamide (**9**)*

Cryst. Solvent, Ethanol; Yield, 87%: m.p. 173–175 °C; IR (KBr, cm^{-1}): 3390, 3310, 3260 (NH, NH_2), 3068 (CH arom.), 2950, 2860 (CH aliph.), 2210 (C≡N), 1382, 1170 (SO_2), 750 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.9–2.6 [m, 6H, $3CH_2$ cyclo], 4.9 [s, 1H, CH], 7.1–7.9 [m, 14H, Ar-H + SO_2NH_2], 9.8 [s, 1H, NH]. Anal. Calcd. for $C_{29}H_{22}Cl_2N_4O_4S$: C, 58.69; H, 3.74; N, 9.44. Found: C, 58.33; H, 3.48; N, 9.20.

*4.1.6. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)-4-methylbenzamide (**10**)*

Cryst. Solvent, Dioxane; Yield, 89%: m.p. 125–127 °C; IR (KBr, cm^{-1}): 3410, 3366, 3290 (NH, NH_2), 3067 (CH arom.), 2950, 2860 (CH aliph.), 2184 (C≡N), 1691, 1648 (2C═O), 1371, 1170 (SO_2), 747 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.6 [m, 6H, $3CH_2$ cyclo], 2.4 [s, 1H, CH_3], 5.0 [s, 1H, CH], 7.0–8.1 [m, 13H, Ar-H + SO_2NH_2], 12.8 [s, 1H, NH]. Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_4S$: C, 59.31; H, 3.98; N, 9.22. Found: C, 59.63; H, 3.64; N, 9.46.

*4.1.7. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)cinnamamide (**11**)*

Cryst. Solvent, Ethanol; Yield, 81%: m.p. 180–182 °C; IR (KBr, cm^{-1}): 3399, 3374, 3300 (NH, NH_2), 3062 (CH arom.), 2923, 2870 (CH aliph.), 2183 (C≡N), 1700, 1629 (2C═O), 1370, 1185 (SO_2), 765 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, $3CH_2$], 5.1 [s, 1H, CH], 6.5, 6.6 [2d, 2H, CH═CH, J = 7.5, 7.6 Hz], 7.2–8.1 [m, 14H, Ar-H + SO_2NH_2], 12.5 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6) δ: 21.4, 28.7, 35.6, 37.1, 60.2, 112.6, 117.0, 117.6, 118.7, 124.6, 125.8, 127.1, 127.8, 128.6, 129.5, 130.1, 130.7, 131.6, 133.1, 137.5, 141.3, 143.7, 145.2, 152.7, 153.6, 165.8, 197.4. Anal. Calcd. for $C_{31}H_{24}Cl_2N_4O_4S$: C, 60.10; H, 3.90; N, 9.04. Found: C, 60.43; H, 3.76; N, 9.37.

*4.1.8. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)-4-nitrobenzamide (**12**)*

Cryst. Solvent, Dioxane; Yield, 79%: m.p. 220–222 °C; IR (KBr, cm^{-1}): 3459, 3390, 3320 (NH, NH_2), 3071 (CH arom.), 2948, 2920 (CH aliph.), 2181 (C≡N), 1741, 1647 (2C═O), 1371, 1189 (SO_2), 724 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.9–2.4 [m, 6H, $3CH_2$], 5.1 [s, 1H, CH], 7.2–8.0 [m, 13H, Ar-H + SO_2NH_2], 8.9 [s, 1H, NH]. Anal. Calcd. for $C_{29}H_{21}Cl_2N_5O_6S$: C, 54.55; H, 3.32; N, 10.97. Found: C, 54.83; H, 3.61; N, 11.32.

*4.1.9. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)-3,4,5-trimethoxy-benzamide (**13**)*

Cryst. Solvent, Ethanol; Yield, 76%: m.p. 201–203 °C; IR (KBr, cm^{-1}): 3410, 3356, 3180 (NH, NH_2), 3089 (CH arom.), 2950, 2860 (CH aliph.), 2182 (C≡N), 1680, 1647 (2C═O), 1372, 1193 (SO_2), 708 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.8–2.4 [m, 6H, $3CH_2$], 3.7, 3.8, 3.9 [3s, 9H, $3OCH_3$], 5.2 [s, 1H, CH], 7.3–8.0 [m, 11H, Ar-H + SO_2NH_2], 8.7 [s, 1H, NH]. Anal. Calcd. for $C_{32}H_{28}Cl_2N_4O_7S$: C, 56.23; H, 4.13; N, 8.20. Found: C, 56.50; H, 4.37; N, 8.46.

*4.1.10. 4-(5-(2,4-Dichlorophenyl)-4,6-dioxo-2-substituted-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamides (**14**–**18**)*

To a solution of **6** (4.89 g, 0.01 mol), in acid chloride derivatives (10 mL) was refluxed for 4 h. The reaction mixture was evaporated under vacuum and the obtained product was crystallized from the appropriate solvent to give **14**–**18**, respectively.

*4.1.11. 4-(2-(2-Chloropropyl)-5-(2,4-dichlorophenyl)-4,6-dioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzene-sulfonamide (**14**)*

Cryst. Solvent, Dioxane; Yield, 68%: m.p. >330 °C; IR (KBr, cm^{-1}): 3435, 3390, 3360 (NH, NH_2), 3090 (CH arom.), 2927, 2883 (CH aliph.), 1734, 1654 (2C═O), 1378, 1188 (SO_2), 746 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 1.4 [d, 3H, CH_3, J = 7.4 Hz], 1.6–2.6 [m, 6H, $3CH_2$ cyclo], 3.5 [m, 1H, CH—Cl], 4.3 [s, 1H, CH pyridine], 4.6 [d, 2H, CH_2, J = 7.1 Hz], 7.4–8.0 [m, 9H, Ar-H + SO_2NH_2], 8.4 [s, 1H, NH]. Anal. Calcd. for $C_{26}H_{23}Cl_3N_4O_4S$: C, 55.58; H, 3.90; N, 9.43. Found: C, 55.22; H, 3.57; N, 9.12.

*4.1.12. 4-(2-Cyclopropyl-5-(2,4-dichlorophenyl)-4,6-dioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzene-sulfonamide (**15**)*

Cryst. Solvent, Dioxane; Yield, 77%: m.p. 174–176 °C; IR (KBr, cm^{-1}): 3422, 3390, 3320 (NH, NH_2), 3094 (CH arom.), 2926, 2860 (CH aliph.), 1714, 1655 (2C═O), 1371, 1186 (SO_2), 715 (C—Cl). ^{1}H NMR (DMSO-d_6) δ: 0.4–0.5 [m, 4H, $2CH_2$ cyclopropyl], 1.6–2.6 [m, 6H, $3CH_2$], 4.1 [s, 1H, CH], 5.4–5.6 [m, 1H, CH cyclopropyl], 7.3–8.0 [m, 10H, Ar-H + SO_2NH_2], 12.5 [s, 1H, NH]. Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_4S$: C, 56.02; H, 3.98; N, 10.05. Found: C, 56.36; H, 3.67; N, 9.78.

*4.1.13. 4-(5-(2,4-Dichlorophenyl)-4,6-dioxo-2-phenyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamide (**16**)*

Cryst. Solvent, Dioxane; Yield, 81%: m.p. >330 °C; IR (KBr, cm^{-1}): 3447, 3370, 3330 (NH, NH_2), 3064 (CH arom.), 2945, 2880 (CH aliph.), 1690, 1646 (2C═O), 1370, 1170 (SO_2), 706 (C—Cl).

^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2], 4.3 [s, 1H, CH], 7.4–8.0 [m, 14H, Ar-H + SO_2NH_2], 12.4 [s, 1H, NH]. Anal. Calcd. for $C_{29}H_{22}Cl_2N_4O_4S$: C, 58.69; H, 3.74; N, 9.44. Found: C, 58.46; H, 3.48; N, 9.76.

4.1.14. 4-(5-(2,4-Dichlorophenyl)-4,6-dioxo-2-p-tolyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamide (**17**)

Cryst. Solvent, Ethanol; Yield, 79%: m.p. >330 °C; IR (KBr, cm^{-1}): 3447, 3370, 3280 (NH, NH_2), 3068 (CH arom.), 2934, 2883 (CH aliph.), 1653, 1635 (2C=O), 1399, 1174 (SO_2), 755 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.2–2.4 [m, 6H, 3CH_2], 2.45 [s, 3H, CH_3], 4.3 [s, 1H, CH], 7.2–8.0 [m, 13H, Ar-H + SO_2NH_2], 8.3 [s, 1H, NH]. Anal. Calcd. for $C_{30}H_{24}Cl_2N_4O_4S$: C, 59.31; H, 3.98; N, 9.22. Found: C, 59.66; H, 3.71; N, 9.55.

4.1.15. 4-(2-(4-Bromophenyl)-5-(2,4-dichlorophenyl)-4,6-dioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamide (**18**)

Cryst. Solvent, Ethanol; Yield, 76%: m.p. 104–106 °C; IR (KBr, cm^{-1}): 3446, 3390, 3340 (NH, NH_2), 3068 (CH arom.), 1724, 1635 (2C=O), 1569 (C=N), 1381, 1165 (SO_2), 730 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.2–2.4 [m, 6H, 3CH_2], 4.3 [s, 1H, CH], 7.1–8.0 [m, 13H, Ar-H + SO_2NH_2], 13.2 [s, 1H, NH]. Anal. Calcd. for $C_{29}H_{21}Cl_2BrN_4O_4S$: C, 51.80; H, 3.15; N, 8.33. Found: C, 51.98; H, 3.50; N, 8.70.

4.1.16. 4-(2-(Chloromethyl)-5-(2,4-dichlorophenyl)-4,6-dioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamide (**20**)

A mixture of **6** (4.89 g, 0.01 mol) and chloroacetyl chloride (1.13 g, 0.01 mol) in DMF (20 mL) was stirred at room temperature for 2 h. The reaction mixture was poured onto ice water and the obtained solid was crystallized from ethanol to give **20**. Yield, 91%: m.p. 324–326 °C; IR (KBr, cm^{-1}): 3364, 3272, 3240 (NH, NH_2), 3104 (CH arom.), 2958, 2929 (CH aliph.), 1697, 1652 (2C=O), 1627 (C=N), 1372, 1185 (SO_2), 796 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2], 5.2 [s, 1H, CH], 5.5 [s, 2H, CH_2–Cl], 7.3–8.0 [m, 9H, Ar-H + SO_2NH_2], 10.9 [s, 1H, NH]. ^{13}C NMR (DMSO-d_6) δ: 21.7, 28.2, 36.1, 36.2, 41.2, 99.9, 115.1, 116.3, 127.4, 128.8, 129.7, 129.9, 130.5, 133.8, 134.7, 139.3, 139.5, 145.1, 155.6, 162.7, 168.1, 194.6. Anal. Calcd. for $C_{24}H_{19}Cl_3N_4O_4S$: C, 50.94; H, 3.38; N, 9.90. Found: C, 50.61; H, 3.71; N, 9.63.

4.1.17. 2-Amino-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (**21**)

A solution of **6** (4.89 g, 0.01 mol), in conc. H_2SO_4 (20 mL) was stirred at room temperature for 2 h. The obtained solid was crystallized from ethanol to give **21**.Yield, 88%: m.p. 207–209 °C; IR (KBr, cm^{-1}): 3420, 3380, 3255 (NH_2), 3091 (CH arom.), 2953, 2860 (CH aliph.), 1683, 1629 (2C=O), 1373, 1164 (SO_2), 713 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2], 4.8 [s, 1H, CH], 6.2 [s, 2H, NH_2], 6.9 [s, 2H, $CONH_2$], 7.0–8.0 [m, 9H, Ar-H + SO_2NH_2]. Anal. Calcd. for $C_{22}H_{20}Cl_2N_4O_4S$: C, 52.08; H, 3.97; N, 11.04. Found: C, 52.28; H, 3.67; N, 10.86.

4.1.18. 2-Amino-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**22**)

A solution of **6** (4.89 g, 0.01 mol), in conc. H_2SO_4 (10 mL) was refluxed for 1 h. The reaction mixture was cooled and poured onto ice water and the obtained solid was crystallized from dioxane to give **22**. Yield, 69%: m.p. >330 °C; IR (KBr, cm^{-1}): 3478 (OH), 3390, 3260 (NH_2), 3100 (CH arom.), 2950, 2929 (CH aliph.), 1719, 1654 (2C=O), 1313, 1189 (SO_2), 669 (C–Cl). MS *m*/*z* (%): 508 [M$^+$] (3.2), 125 (100). Anal. Calcd. for $C_{22}H_{19}Cl_2N_3O_5S$: C, 51.98; H, 3.77; N, 8.27. Found: C, 51.60; H, 3.45; N, 8.56.

4.1.19. 4-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-2-substituted-5,6,7,8-tetrahydroquinolin-1(4H)-yl)benzenesulfonamides (**23**–**25**)

A mixture of **6** (4.89 g, 0.01 mol) and aromatic aldehydes (0.01 mol) in acetic acid (20 mL) was refluxed for 3 h. The reaction mixture was poured onto ice water and the obtained solid was crystallized from the appropriate solvent to give **23**–**25**, respectively.

4.1.20. 4-(3-Cyano-4-(2,4-dichlorophenyl)-2-(4-(dimethylamino) benzylideneamino)-5-oxo-5,6,7,8-tetrahydroquinolin-1(4H)-yl) benzenesulfonamide (**23**)

Cryst. Solvent, Ethanol; Yield, 65%: m.p. >330 °C; IR (KBr, cm^{-1}): 3380, 3310 (NH_2), 2940, 2836 (CH aliph.), 2210 (C≡N), 1651 (C=O), 1612 (C=N), 1389, 1168 (SO_2), 753 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2 cyclo], 3.0 [s, 6H, N(CH_3)$_2$] 7.0–8.0 [m, 13H, Ar-H + SO_2NH_2], 8.2 [s, 1H, N=CH]. MS *m*/*z* (%): 620 [M$^+$] (2.4), 63 (100). Anal. Calcd. for $C_{31}H_{27}Cl_2N_5O_3S$: C, 60.00; H, 4.39; N, 11.29. Found: C, 60.33; H, 4.71; N, 11.57.

4.1.21. 4-(3-Cyano-4-(2,4-dichlorophenyl)-2-((E)-3-(4-(dimethylamino)phenyl)allylideneamino)-5-oxo-5,6,7,8-tetrahydroquinolin-1(4H)-yl)benzenesulfonamide (**24**)

Cryst. Solvent, Dioxane; Yield, 62%: m.p. >330 °C; IR (KBr, cm^{-1}): 3446, 3360 (NH_2), 3094 (CH arom.), 2929, 2886 (CH aliph.), 2215 (C≡N), 1654 (C=O), 1617 (C=N), 1384, 1180 (SO_2), 740 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2 cyclo], 1.7 [d, 2H, CH=CH-Ph, J = 7.2 Hz], 3.0 [s, 6H, N(CH_3)$_2$], 5.3 [s, 1H, CH pyridine], 7.3–8.1 [m, 13H, Ar-H + SO_2NH_2], 9.8 [d, 1H, N=CH, J = 7.6 Hz]. MS *m*/*z* (%): 646 [M$^+$] (2.6), 47(100). Anal. Calcd. for $C_{35}H_{33}Cl_2N_5O_3S$: C, 62.31; H, 4.93; N, 10.38. Found: C, 62.68; H, 4.66; N, 10.73.

4.1.22. 4-(3-Cyano-4-(2,4-dichlorophenyl)-2-(2,4-dinitrobenzylideneamino)-5-oxo-5,6,7,8-tetrahydroquinolin-1(4H)-yl)benzenesulfonamide (**25**)

Cryst. Solvent, Dioxane; Yield, 74%: m.p. 317–319 °C; IR (KBr, cm^{-1}): 3364, 3272 (NH_2), 3106 (CH arom.), 2965, 2865 (CH aliph.), 2210 (C≡N), 1651 (C=O), 1626 (C=N), 1372, 1185 (SO_2), 796 (C–Cl). ^{1}H NMR (DMSO-d_6).δ: 1.6–2.4 [m, 6H, 3CH_2], 5.1 [s, 1H, CH], 7.0–7.9 [m, 12H, Ar-H + SO_2NH_2], 10.4 [s, 1H, N=CH]. MS *m*/*z* (%): 667 [M$^+$] (3.2), 45 (100). Anal. Calcd. for $C_{29}H_{20}Cl_2N_6O_7S$: C, 52.18; H, 3.02; N, 12.59. Found: C, 52.53; H, 3.29; N, 12.84.

4.1.23. N-(3-Cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)-3-oxo-butanamide (**26**)

A solution of **6** (4.89 g, 0.01 mol), in ethyl acetoacetate (10 mL) was refluxed for 5 h. The reaction mixture was removed under vacuum and the obtained solid was crystallized from ethanol to give **26**. Yield, 79%: m.p. 113–115 °C; IR (KBr, cm^{-1}): 3421, 3380, 3360 (NH, NH_2), 3086 (CH arom.), 2929, 2870 (CH aliph.), 2164 (C≡N), 1719, 1680, 1641 (3C=O), 1373, 1170 (SO_2), 778 (C–Cl) ^{1}H NMR (DMSO-d_6) δ: 1.6–2.4 [m, 6H, 3CH_2], 2.2 [s, 3H, $COCH_3$], 3.3 [s, 2H, CH_2CO], 4.4 [s, 1H, CH], 7.0–7.6 [m, 9H, Ar-H + SO_2NH_2], 8.4 [s, 1H, NH]. Anal. Calcd. for $C_{26}H_{22}Cl_2N_4O_5S$: C, 54.46; H, 3.87; N, 9.77. Found: C, 54.81; H, 3.56; N, 9.44.

4.1.24. 4-(5-(2,4-Dichlorophenyl)-2-methyl-6-oxo-4-thioxo-3,4,6,7,8,9-hexahydropyrimido[4,5-b]quinolin-10(5H)-yl)benzenesulfonamide (**27**)

A mixture of **6** (4.89 g, 0.01 mol), and thioacetamide (0.75 g, 0.01 mol) in trifluoroacetic acid (10 mL) was refluxed for 5 h. The reaction mixture was cooled and poured onto ice water. The obtained solid was crystallized from dioxane to give **27**. Yield, 80%: m.p. 141–143 °C; IR (KBr, cm^{-1}): 3370, 3251, 3190 (NH, NH_2), 3090 (CH arom.), 2951, 2860 (CH aliph.), 1624 (C=O), 1286 (C=S), 1370,

1164 (SO_2), 713 (C–Cl). MS *m/z* (%): 547 [M^+] (3.6), 62 (100). Anal. Calcd. for $C_{24}H_{20}Cl_2N_4O_3S_2$: C, 52.65; H, 3.68; N, 10.23. Found: C, 52.29; H, 3.41; N, 10.56.

*4.1.25. Ethyl N-(3-cyano-4-(2,4-dichlorophenyl)-5-oxo-1-(4-sulfamoylphenyl)-1,4,5,6,7,8-hexahydroquinolin-2-yl)formimidate (**28**)*

A solution of 6 (4.89 g, 0.01 mol), in triethyl orthoformate (10 mL) was refluxed for 4 h. The reaction mixture was removed under vacuum and the obtained solid was crystallized from ethanol to give **28**. Yield, 88%: m.p. 117–119 °C IR (KBr, cm^{-1}): 3365, 3290 (NH_2), 3067 (CH arom.), 2978, 2880 (CH aliph.), 2200 (C≡N), 1643 (C=O), 1372, 1188 (SO_2), 732 (C–Cl). ^{1}H NMR (DMSO-d_6) δ: 1.1 [t, 3H, CH_3 ethyl]. 1.6–2.4 [m, 6H, $3CH_2$], 4.3 [q, 2H, CH_2 ethyl], 5.2 [s, 1H, CH], 7.4–8.0 [m, 9H, Ar-H + SO_2NH_2], 8.8 [s, 1H, N=CH]. Anal. Calcd. for $C_{25}H_{22}Cl_2N_4O_4S$: C, 55.05; H, 4.07; N, 10.27. Found: C, 55.42; H, 4.37; N, 10.58.

4.2. Biological testing

4.2.1. Animals, chemicals and facilities

Ehrlich Ascites Carcinoma cells (EAC) were maintained in female Swiss albino mice weighing 25–30 g (the holding company for biological products and vaccines, VACSERA, Cairo, Egypt). The animals 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. Facilities including animal house, biochemical equipments have been made 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 health organization, Geneva, Switzerland and approved from the committee for animal care at NCRRT, AEA.

4.2.2. Anticancer activity

Ehrlich Ascites Carcinoma cells (EAC) were obtained by needle aspiration of ascitic fluid from preinoculated mice under aseptic conditions. Tumor cell suspension (2.5×10^6 per mL) was prepared in RPMI-1640 media. Tested compounds were 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 (2.5×10^5) 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 [31]. Trypan blue dye was prepared as follows; A stoke solution was prepared by dissolving 1 g of the dye in distilled water (100 mL). The working solution was prepared by diluting 1 mL of the stock solution with 9 mL of distilled water. The stain was used for staining the dead EAC cells. The results of *in vitro* cytotoxic activity experiments are presented in Table 1.

4.3. Molecular modeling

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

The carbonic anhydrase structure was downloaded from the PDB data bank (http://www.rcsb.org/ – PDB code: 1g54). The protein was prepared for the docking where: (i) Hydrogen atoms were added to the protein with MOE and minimized keeping all the heavy atoms fixed until an RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ was reached (ii) Partial charges were computed using Amber99 forcefield.

The ligand was drawn on ChemDraw and imported in MOE. The structure was subjected to energy minimization using MMFF94x forcefield and the partial charges were computed using the same forcefield.

Docking calculations were done using Alpha triangle placement method and poses were prioritized by London dG scoring method.

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