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Original article

Design, synthesis and anticancer evaluation of novel tetrahydroquinoline derivatives containing sulfonamide moiety

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ABSTRACT

Sulfonamides posses many types of biological activities and have recently been reported to show substantial antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity and the most prominent of these is through the inhibition of carbonic anhydrase isozymes. The present work reports the synthesis of some novel quinoline and pyrimido[4,5-*b*]quinoline derivatives bearing a substituted or unsubstituted sulfonamide moiety. The design of the structures of these compounds complies with the general pharmacophore of the sulfonamide compounds that act as carbonic anhydrase (CA) inhibitors as this may play a role in their anticancer activity. All the newly synthesized compounds were evaluated for their in vitro anticancer activity against breast cancer cell line (MCF7). Most of the screened compounds showed interesting cytotoxic activities compared to a reference drug.

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 [6]. Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity in vitro and/or in vivo [7–11].

It has been known 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 NF-Y. Moreover, following an extensive evaluation, numerous sulfonamides were found to act as carbonic anhydrase (CA) inhibitors [12–15]. The most prominent mechanism was the inhibition of carbonic anhydrase isozymes [16].

In brief, the α-CA is a family of metalloenzymes involved in the catalysis of an important physiological reaction: the hydration of CO_2 to bicarbonate and a proton ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$). At least 13 enzymatically active isoforms have been discovered in higher vertebrates [12–15]. CAs are involved in pH regulation, secretion of electrolytes, respiration [17,18], biosynthetic reactions which require CO_2/bicarbonate as substrate such as gluconeogenesis, lipogenesis, ureagenesis, and pyrimidine synthesis [19]. The mechanism of tumor inhibition by these sulfonamide CA inhibitors was suggested by Chegwidden and Spencer [20], that these compounds may reduce the provision of bicarbonate for the synthesis of nucleotides and other cell components such as membrane lipids.

In addition, quinoline and fused quinoline derivatives are known to possess several biological activities including anticancer activity [21–23]. Also, several reduced quinoline derivatives have shown significant anticancer activity [24]. In the light of these facts, and as a continuation of reported work [25,26], we report here the synthesis of a novel series of quinoline and pyrimidoquinoline derivatives having a substituted or unsubstituted sulfonamide moiety where their design complies with the general pharmacophore of the sulfonamide CA inhibitors. We also aim to test the influence of the substitution of the sulfonamide moiety on the anticancer activity and to study their structure–activity relationship. The anticancer activity is tested against a breast cancer cell line (MCF7).

2. Results and discussion

A general pharmacophore (Fig. 1) for the compounds acting as carbonic anhydrase inhibitors has been reported by Thiry et al. [27] from the analysis of the CA active site and from the structure of inhibitors described in the literature [15].

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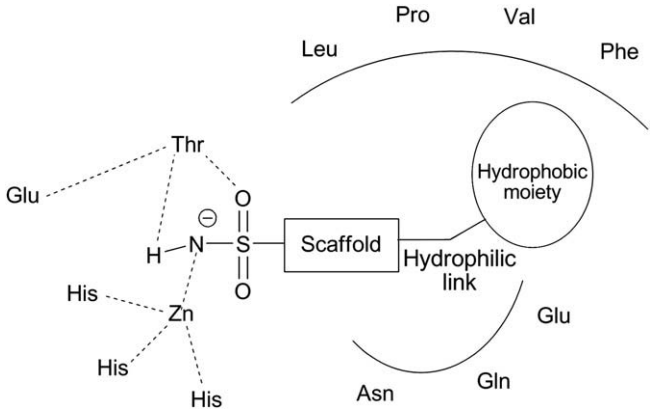


Fig. 1. Structural elements of CA inhibitors in the CA enzymatic active site.

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 representative examples of the synthesized compounds showing compliance to the above-mentioned pharmacophore and the compounds were synthesized according to Schemes 1–4.

2.1. Chemistry

Reaction of cyclohexanone with acetaldehyde and malononitrile in the presence of ammonium acetate yielded the corresponding tetrahydroquinoline derivative **1** (Scheme 1). The reaction proceeds first when the acetaldehyde reacts with malononitrile to give an α,β-unsaturated nitrile intermediate, which reacts with the carbonyl compound cyclohexanone in the presence of ammonium acetate giving an intermediate which upon intramolecular cyclization and finally oxidation yielded compound **1**. Compound **2** was prepared in a similar way as compound **1** but 5,5-dimethylcyclohexane-1,3-dione was used instead of cyclohexanone (Scheme 2).

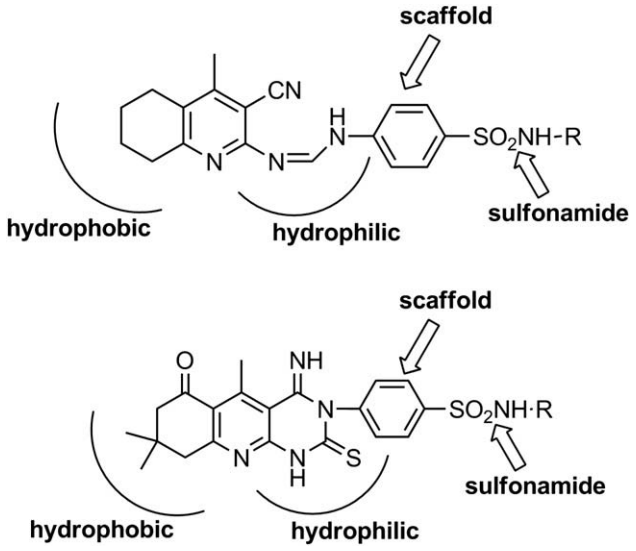


Fig. 2. Representative examples of the synthesized compounds showing compliance to the general pharmacophore of sulfonamide compounds acting as carbonic anhydrase inhibitors.

Compounds **1** and **2** were confirmed from their microanalytical and spectral data. IR spectra showed bands at 3403, 3322 cm^{-1} and 3324, 3216 cm^{-1} (NH_2), (C≡N) at 2211 and 2179 cm^{-1} for compounds **1** and **2**, respectively. ^1H NMR spectrum of **1** in DMSO-d_6 revealed signals at 1.7 ppm (CH_3) and 6.3 ppm (NH_2). While, the ^1H NMR spectrum of **2** revealed signals at 1.08 ppm (CH_3) and 6.8 ppm (NH_2).

Treatment of compounds **1** and **2** with triethylorthoformate in the presence of acetic anhydride yielded compounds **3** (Scheme 3) and **11** (Scheme 4), respectively. The formation of compounds **3** and **11** was supported from their microanalytical and spectral data. Their IR spectra showed the absence of the bands corresponding to the (NH_2) and the presence of bands at 2218 and 2204 cm^{-1} corresponding to the cyano group. Also, the ^1H NMR spectrum of the compounds in DMSO-d_6 showed the presence of a 3-proton triplet at 1.3 ppm and a 2-proton quartet at 4.3 ppm for the ethoxy group.

When compound **3** was treated with hydrazine hydrate at room temperature, compound **1** was obtained instead of the expected imino–amino derivative **10**. This can explained on the basis of formation of an intermediate, which upon elimination of ethoxymethylene-hydrazone [28] furnished compound **1**. This was confirmed by IR, mixed melting point and similar Rf value on the TLC.

When compound **3** or **11** was stirred with sulfanilamide or sulfamethoxazole at room temperature in ethanol, a nucleophilic substitution reaction took place yielding compounds **4**, **5** (Scheme 3) or **12**, **13** (Scheme 4), respectively. The compounds were confirmed by the presence of the (C≡N) band in the IR spectrum in range of 2202–2223 cm^{-1}. In addition, the ^1H NMR spectrum of the compounds confirmed that the reaction took place by the presence of the aromatic protons.

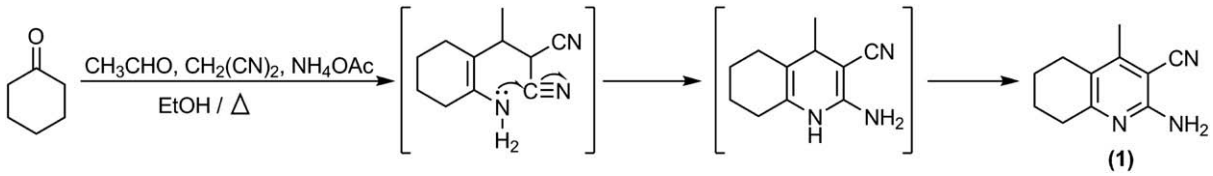
The reaction of compound **1** or **2** with the sulfonamide isothiocyanate derivatives in dimethylformamide, intramolecular cyclization took place giving the pyrimido[4,5-*b*]quinoline derivatives **6**–**9** (Scheme 3) and **16**–**19** (Scheme 4), respectively. Structures of compounds **6**–**9** and **16**–**19** were supported by elemental analysis and spectral data. The IR spectrum of the compounds showed the absence of the (C≡N) bands and the presence of (C=S) bands in the range of 1254–1285 cm^{-1}. In addition, ^1H NMR spectra of these compounds revealed the presence of multiplets at the range of 6.8–7.9 ppm which could be assigned to the aromatic protons.

Refluxing compound **12** or **13** in pyridine caused intramolecular cyclization and furnished the pyrimido[4,5-*b*]quinoline derivative **14** or **15**, respectively (Scheme 4). The structure of compounds **14** and **15** was confirmed by the absence of the (C≡N) bands in the IR spectrum. In addition, ^1H NMR spectrum of these compounds revealed the presence of singlet of imino (NH) and multiplets of the aromatic protons.

2.2. In vitro anticancer screening

The newly synthesized compounds were evaluated for their in vitro cytotoxic activity against the breast cancer cell line, MCF7. Doxorubicin which is one of the most effective anticancer agents was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability.

Table 1 shows the in vitro cytotoxic activity of the synthesized compounds where the compounds exhibited significant activity compared to the reference drug Doxorubicin.



Scheme 1.

From these results, it can be seen that although the variation in biological activity between the compounds was not very high, yet the following points can still be concluded.

Concerning the tetrahydroquinoline derivatives, it was found from the results that the compounds having a substituted sulfonamide – with methyl isoxazolyl ring – **5** and **13** were more potent than the unsubstituted ones **4** and **12**. Compound **13** was the most potent in this group.

While for the fused tetrahydroquinoline derivatives **6**–**9** and **16**–**19**, it was found that the unsubstituted sulfonamide derivatives **6** and **16** were more potent than the substituted ones **7**–**9** and **17**–**19**. The order of activity for the substituted derivatives was as follows: the guanido derivatives **7** and **17** having the highest potency followed by the diazine derivatives **9** and **19** and finally the isoxazole derivatives **8** and **18**. Compound **6** was the most potent in this group.

2.3. Docking studies

Previous literature shows that carbonic anhydrase inhibition is one of the anticancer mechanisms of sulfonamides, and this was clearly showed by Abbate et al. [7], who stated that the potent anticancer sulfonamide drug (E7070) (Fig. 3) – 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.

The X-ray crystal structure of the adduct of human carbonic anhydrase II (hCA II) with E7070 revealed similar interactions between the inhibitor and the active site as those reported by Supuran et al. [14,17]. 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 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) [14,17].

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

Docking of the synthesized compounds was performed and it was found that the compounds with an unsubstituted sulfonamide moiety exhibit similar interactions to those previously reported for E7070 and stated above. Fig. 6 shows an example of the interaction map of compound **12** with an unsubstituted sulfonamide moiety docked pose with nearby binding site amino acids of hCA II.

While, it has been seen from the docking of the substituted sulfonamide derivatives that they did not fit properly in the active site of the enzyme and accordingly do not form the similar interactions that have been reported in the literature or obtained from the X-ray crystallography of the compounds used as carbonic anhydrase inhibitors. Fig. 7 shows an example of the docking of compound **5** having a substituted sulfonamide moiety.

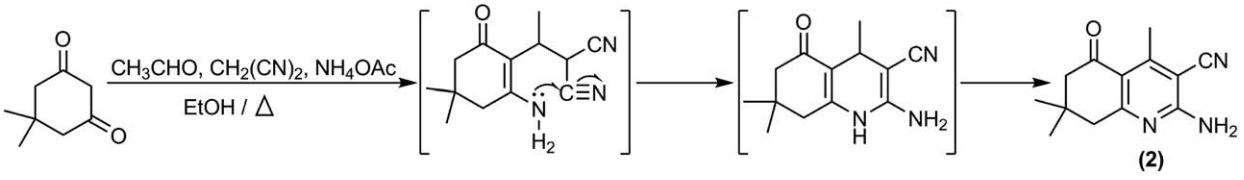
3. Conclusions

We report here the synthesis of new quinoline and pyrimidoquinoline derivatives containing a substituted and unsubstituted sulfonamide moiety. In addition, it was clearly observed that the compounds with either a substituted or unsubstituted sulfonamide moiety exhibit significant anticancer activity. The docking of the compounds showed that the compounds with an unsubstituted sulfonamide moiety may act also as CA inhibitors and this may at least in part contribute to their anticancer activity. While, the compounds with a substituted sulfonamide moiety would not seem to have CA inhibitory activity based on computer modeling and docking studies and this may suggest that the anticancer activity of these compounds is mainly due to another mechanism.

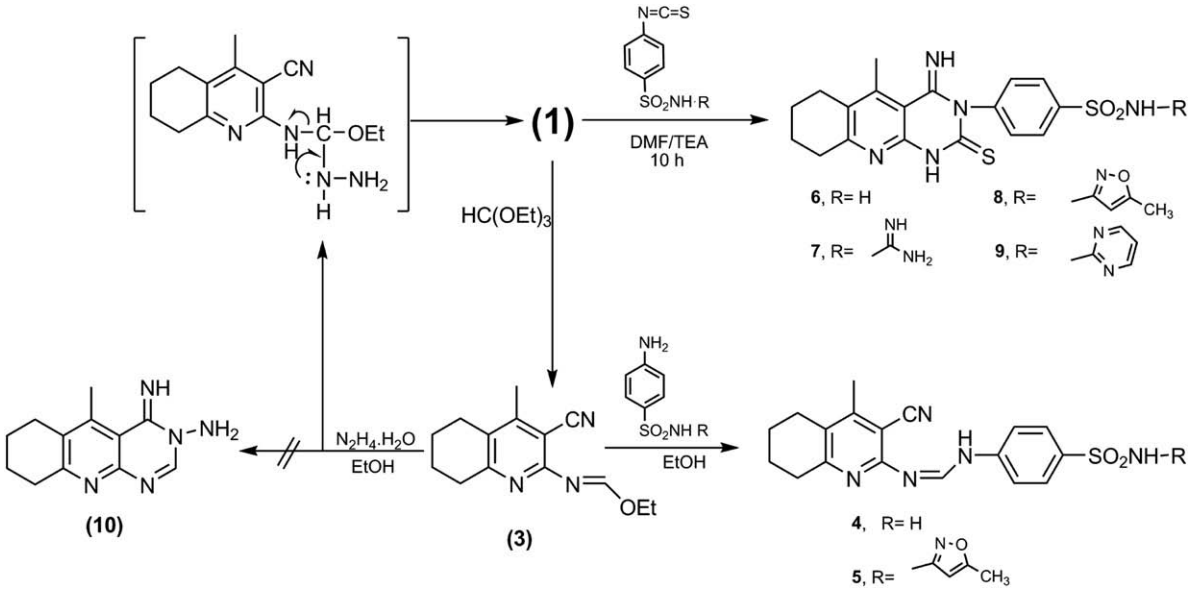
4. Experimental

4.1. Chemistry

Melting points are uncorrected and were determined on Buchi melting point apparatus (B-540). Elemental analyses (C, H, N) were performed on Perkin–Elmer 2400 analyzer (Perkin–Elmer, Norwalk, CT, USA) at Microanalytical Laboratories of the Faculty of Science, Cairo University. All compounds were within ±0.4% of the theoretical values. The IR spectra were measured on Nicolet 380 FT-IR spectrometer. ^{1}H NMR spectra were obtained on a Bruker proton NMR-Avance 300 (300, MHz), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet. Mass spectra (EI) were run on HP Model MS-5988 (Hewlett Packard).



Scheme 2.



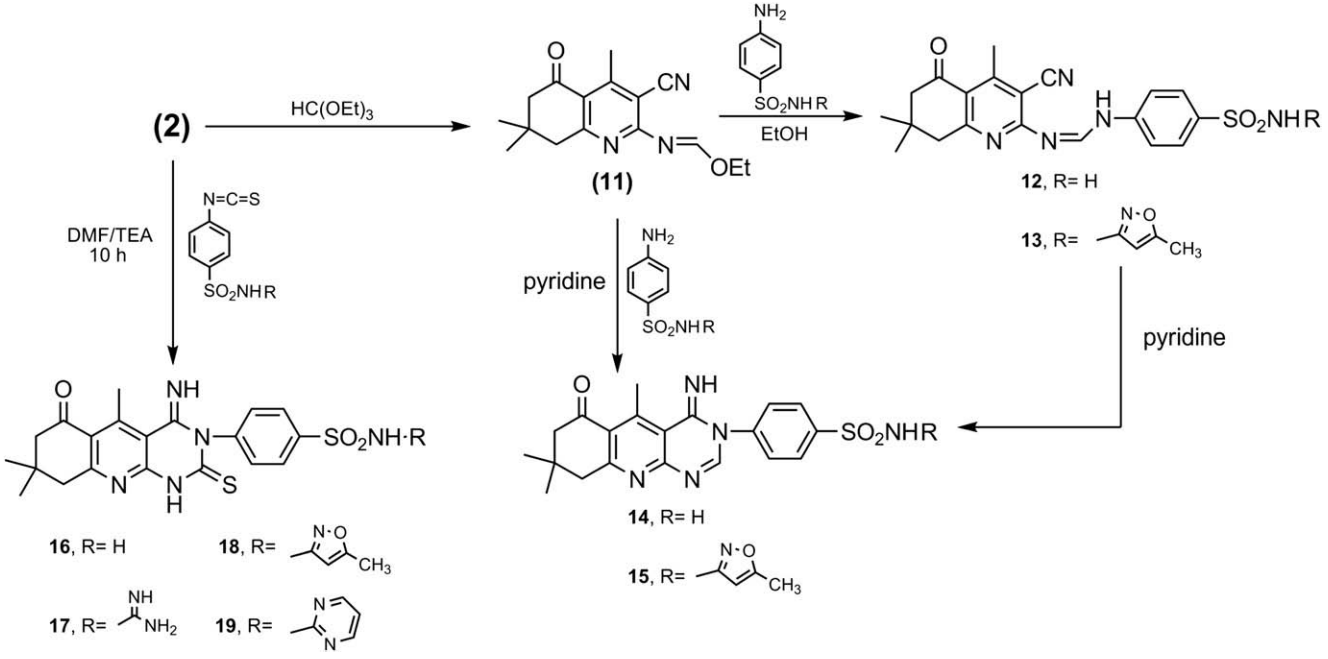
Scheme 3.

*4.1.1. 2-Amino-4-methyl-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**1**) and 2-amino-4,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinoline-3-carbonitrile (**2**)*

A mixture of cyclohexanone (0.98 g, 0.01 mol) or 5,5-dimethylcyclo-hexane-1,3-dione (1.4 g, 0.01 mol) with acetaldehyde (0.44 g, 0.01 mol), malononitrile (0.06 g, 0.01 mol), and ammonium acetate (1.15 g, 0.015 mol) in ethanol (20 ml) was refluxed for 1 h. The reaction mixture was then stirred for 3 h at room temperature and the obtained solid was recrystallized from dioxane to give **1** and **2**, respectively. **1**: Yield, 45%; m.p. 278–280 °C; IR, cm^{-1}: 3403, 3322 (NH_2), 2932, 2872 (CH aliph.), 2211 (C≡N). ^1H NMR (DMSO-d_6) δ: 1.71 [s, 3H, CH_3], 2.23–2.61 [m, 8H, $4CH_2$], 6.37 [s, 2H, NH_2, exchangeable with D_2O]. MS, *m*/*z* (%): 187 [M^+] (100). Anal. Calcd. for $C_{11}H_{13}N_3$: C, 70.56; H, 7.00; N, 22.44. Found: C, 70.30; H, 6.78; N, 22.70. **2**: Yield, 44%; m.p. 188–190 °C; IR, cm^{-1}: 3324, 3216 (NH_2), 2962, 2879 (CH aliph.), 2179 (CN), 1676 (C═O). ^1H NMR (DMSO-d_6) δ: 1.00, 1.02 [2s, 6H, $2CH_3$], 1.08 [s, 3H, CH_3], 2.21–2.49 [m, 4H, $2CH_2$ cyclo], 6.82 [s, 2H, NH_2 exchangeable with D_2O]. MS, *m*/*z* (%): 229 [M^+] (7.5), 217 (100). Anal. Calcd. for $C_{13}H_{15}N_3O$: C, 68.10; H, 6.59; N, 18.33. Found: C, 68.39; H, 6.22; N, 18.55.

*4.1.2. Ethyl N-3-cyano-4-methyl-5,6,7,8-tetrahydroquinolin-2-ylformimidate (**3**), ethyl N-3-cyano-4,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinolin-2-ylformimidate (**11**)*

A solution of **1** (1.87 g, 0.01 mol) or **2** (0.46 g, 0.002 mol) and triethylorthoformate (10 ml) containing 3 drops of acetic anhydride was refluxed for 8 h. The reaction mixture was cooled and then poured onto ice water. The obtained solid was recrystallized from ethanol to give **3** and **11**, respectively. **3**: Yield, 68%; m.p. 98–100 °C; IR, cm^{-1}: 2940 (CH aliph.), 2218 (C≡N). ^1H NMR (DMSO-d_6) δ: 1.36



Scheme 4.

Table 1
In vitro cytotoxic activity of the synthesized compounds.

Compound	IC_{50}[a] (μg/ml)	IC_{50}[b] (μM)
4	NA	NA
5	1.95	4.33
6	0.95	2.37
7	1.28	2.89
8	2.62	5.44
9	1.48	3.09
12	2.62	6.37
13	1.9	3.86
14	1.5	3.65
15	2.6	5.28
16	4	9.03
17	5.17	13.43
18	NA	NA
19	9.19	17.64
Dox.	0.7	1.2

NA: Compounds having IC_{50} value > 10 μg/ml.
[a] Mean of three results obtained from three experiments.
[b] IC_{50} value: concentration causing 50% inhibition of cell viability.

[t, 3H, CH_3 ethyl], 1.78 [s, 3H, CH_3], 2.23–2.79 [m, 8H, 4CH_2 cyclo], 4.36 [q, 2H, CH_2 ethyl], 8.44 [s, 1H, N=CH]. MS, *m/z* (%): 243 [M^+] (1.78), 187 (100). Anal. Calcd. for $C_{14}H_{17}N_3O$: C, 69.11; H, 7.04; N, 17.27. Found: C, 68.83; H, 7.42; N, 17.50. **11**: Yield, 80%; m.p. 95–97 °C; IR, cm^{-1}: 2962, 2873 (CH aliph.), 2204 (CN), 1678 (C=O). ^1H NMR (DMSO-d_6) δ: 1.02, 1.03 [2s, 6H, 2CH_3], 1.17 [s, 3H, CH_3], 1.30 [t, 3H, CH_3 ethyl], 2.26–2.45 [m, 4H, 2CH_2 cyclo], 4.31 [q, 2H, CH_2 ethyl], 8.46 [s, 1H, N=CH]. MS, *m/z* (%): 285 [M^+] (1.05), 174 (100). Anal. Calcd. for $C_{16}H_{19}N_3O_2$: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.11; H, 6.94; N, 15.02.

*4.1.3. N′-(3-cyano-4-methyl-5,6,7,8-tetrahydroquinolin-2-yl)-N-(4-sulfamoylphenyl)-formimidamide (**4**), N′-(3-cyano-4-methyl-5,6,7,8-tetrahydroquinolin-2-yl)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) formimidamide (**5**), N′-(3-cyano-4,7,7-trimethyl -5-oxo-5,6,7,8-tetrahydroquinolin-2-yl)-N-(4-sulfamoylphenyl)formimidamide (**12**) and N′-(3-cyano-4,7,7-trimethyl-5-oxo-5,6,7,8-tetrahydroquinolin-2-yl)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)formimidamide (**13**)*

A mixture of **3** (0.243 g, 0.001 mol) or **11** (0.285 g, 0.001 mol) and sulfanilamide or sulfamethoxazole (0.001 mol) was stirred overnight at room temperature in ethanol (20 ml). The obtained solid was recrystallized from dioxane to give **4**, **5** or **12**, **13**, respectively. **4**: Yield, 43%; m.p. 224–226 °C; IR, cm^{-1}: 3311, 3207, 3134 (NH, NH_2), 3085 (CH arom.), 2943, 2862 (CH aliph.), 2222 (C≡N), 1369, 1147 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.77 [s, 3H, CH_3], 2.22–2.79 [m, 8H, 4CH_2 cyclo], 6.13 [s, 1H, N=CH], 7.44, 7.80 [2d, 4H, Ar–H, AB system], 9.53 [s, 1H, NH], 11.46 [s, 2H, SO_2NH_2]. MS, *m/z* (%): 369 [M^+] (13.4), 187 (100). Anal. Calcd. for $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18; N, 18.96. Found: C, 58.20; H, 5.05; N, 18.74. **5**: Yield, 46%; m.p. 217–219 °C; IR, cm^{-1}: 3310, 3150 (NH), 3100 (CH arom.), 2935, 2857 (CH aliph.), 2223 (C≡N), 1379, 1184 (SO_2). MS, *m/z* (%): 450 [M^+] (1.7), 187 (100). Anal. Calcd. for $C_{22}H_{22}N_6O_3S$: C, 58.65; H, 4.92; N, 18.65. Found: C, 58.90; H, 5.20; N, 18.91. **12**: Yield, 50%; m.p. 201–203 °C; IR, cm^{-1}: 3334, 3222, 3200 (NH, NH_2), 3083 (CH arom.), 2967, 2874 (CH aliph.), 2202 (CN), 1669 (C=O), 1376, 1157 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.03 [s, 6H, 2CH_3], 1.17 [s, 3H, CH_3], 2.25–2.49 [m, 4H, 2CH_2 cyclo], 7.26–8.23 [m, 4H, Ar–H], 8.85 [s, 1H, N=CH], 10.58 [s, 1H, NH], 11.12 [s, 2H, SO_2NH_2]. MS, *m/z* (%): 411 [M^+] (1.5), 149 (100). Anal. Calcd. for $C_{20}H_{21}N_5O_3S$: C, 58.38; H, 5.14; N, 17.02. Found: C, 58.09; H, 5.41; N, 16.84. **13**: Yield, 45%; m.p. 130–132 °C; IR, cm^{-1}: 3325, 3220 (NH), 3099 (CH arom.), 2959, 2875 (CH aliph.), 2205 (CN), 1658 (C=O), 1370, 1161 (SO_2). MS, *m/z* (%): 493 [M^+] (1.28), 161 (100). Anal. Calcd. for $C_{24}H_{24}N_6O_4S$: C, 58.52; H, 4.91; N, 17.06. Found: C, 58.28; H, 5.20; N, 17.30.

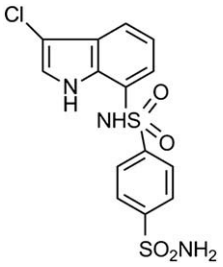


Fig. 3. Compound E7070, a sulfonamide compound in advanced clinical trials as anticancer agent.

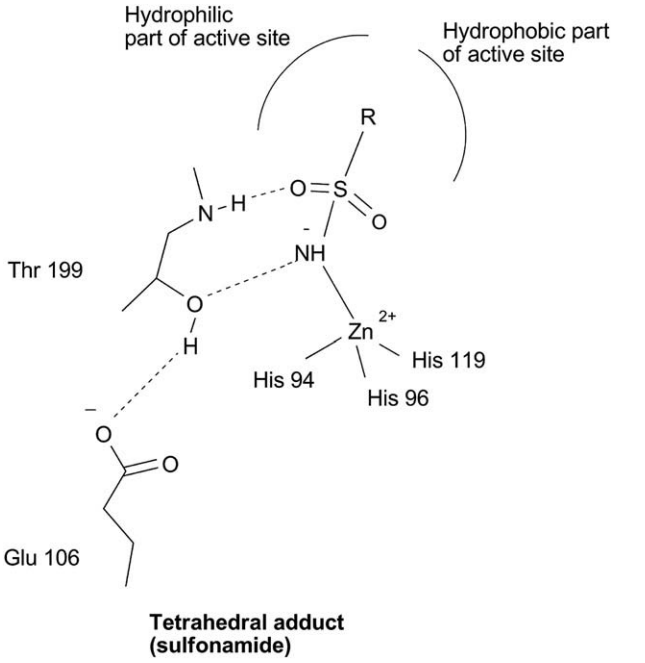


Fig. 4. CA inhibition mechanism by sulfonamides.

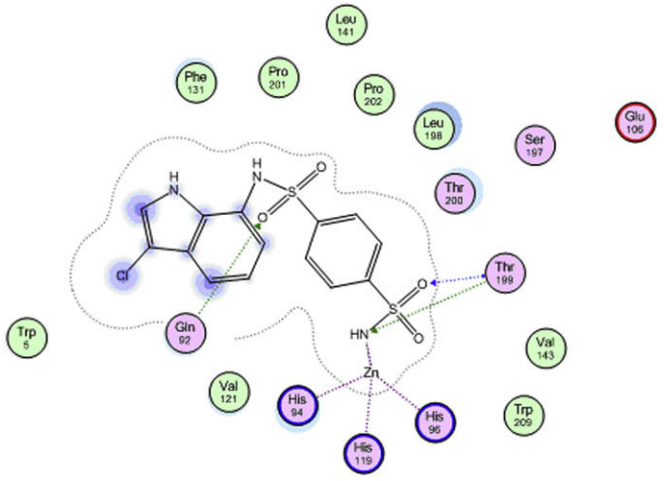


Fig. 5. Interaction map of E7070 with the active site of hCA II showing similar interactions as those previously reported.

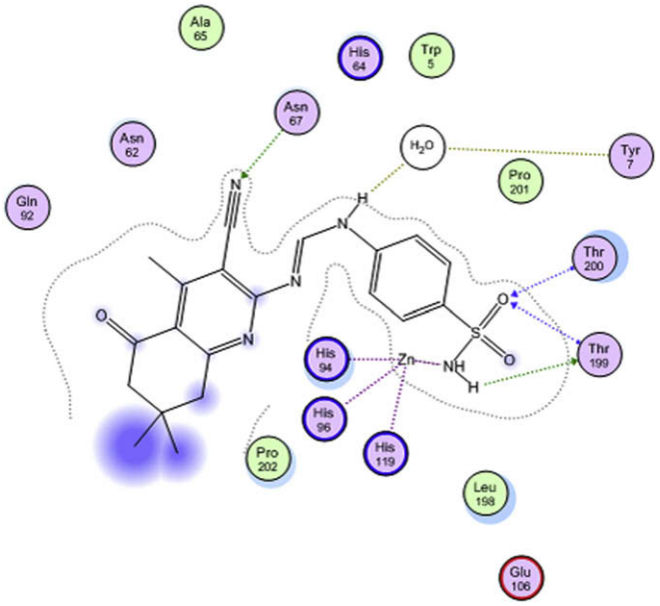


Fig. 6. Interaction map of compound **12** with the active site of hCA II showing similar interactions as those previously reported.

*4.1.4. 4-(4-Imino-5-methyl-2-thioxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]quinolin-3 (4H)-yl)-N-(substituted or unsubstituted)benzenesulfonamides (**6**–**9**) and 4-(4-imino-5,8,8-trimethyl-6-oxo-2-thioxo-1,2,6,7,8,9-hexahydropyrimido[4,5-*b*]-quinolin-3 (4H)-yl)-N-(substituted or unsubstituted)benzenesulfonamides (**16**–**19**)*

A mixture of **1** (0.374 g, 0.002 mol) or **2** (0.345 g, 0.0015 mol) and the corresponding sulfonamide isothiocyanate derivative (0.0015 mol) in dimethylformamide (20 ml) containing 3 drops of triethylamine was refluxed for 10 h. The reaction mixture was cooled and then poured onto ice water. The obtained solid was recrystallized from ethanol to give **6**–**9** and **16**–**19**, respectively. **6**: Yield, 75%; m.p. 227–229 °C; IR, cm^{-1}: 3388, 3324, 3152 (NH, NH_2), 3050 (CH arom.), 2918, 2849 (CH aliph.), 1332, 1150 (SO_2), 1285 (C═S). ^1H NMR (DMSO-d_6) δ: 1.79 [s, 3H, CH_3], 2.22–2.98 [m, 8H, $4CH_2$ cyclo], 7.03–7.82 [m, 6H, Ar–H + SO_2NH_2], 9.75, 12.43 [2s, 2H, 2NH]. MS, *m*/*z* (%): 401 [M^+] (63.5), 55 (100). Anal. Calcd. for $C_{18}H_{19}N_5O_2S_2$: C, 53.85; H, 4.77; N, 17.44. Found: C, 53.50; H, 4.54; N, 17.10. **7**: Yield, 61%; m.p. 213–215 °C; IR, cm^{-1}: 3350, 3138 (NH, NH_2), 3077 (CH arom.), 2927, 2852 (CH aliph.), 1384, 1182 (SO_2), 1277 (C═S). MS, *m*/*z* (%): 443 [M^+] (1.5), 148 (100). Anal. Calcd. for $C_{19}H_{21}N_7O_2S_2$: C, 51.45; H, 4.77; N, 22.11. Found: C, 51.63; H, 4.50; N, 22.40. **8**: Yield, 51%; m.p. 198–200 °C; IR, cm^{-1}: 3147 (NH), 3050 (CH arom.), 2931, 2862 (CH aliph.), 1383, 1142 (SO_2), 1260 (C═S). ^1H NMR (DMSO-d_6) δ: 1.70 [s, 3H, CH_3], 2.10 [s, 3H, CH_3 isoxazole], 2.21–2.89 [m, 8H, $4CH_2$], 6.26 [s, 1H, CH isoxazole], 6.98–7.94 [m, 4H, Ar–H], 12.41, 12.83 [2s, 2H, 2NH], 15.18 [s, 1H, SO_2NH]. MS, *m*/*z* (%): 482 [M^+] (2.0), 187 (100). Anal. Calcd. for $C_{22}H_{22}N_6O_3S_2$: C, 54.75; H, 4.60; N, 17.41. Found: C, 54.50; H, 4.41; N, 17.72. **9**: Yield, 71%; m.p. 240–242 °C; IR, cm^{-1}: 3403, 3320, 3140 (NH), 3090 (CH arom.), 2932, 2852 (CH aliph.), 1389, 1153 (SO_2), 1256 (C═S). MS, *m*/*z* (%): 480 [M^+] (7), 55 (100). Anal. Calcd. for $C_{22}H_{21}N_7O_2S_2$: C, 55.10; H, 4.41; N, 20.44. Found: C, 55.46; H, 4.74; N, 20.12. **16**: Yield, 75%; m.p. 132–134 °C; IR, cm^{-1}: 3340, 3199 (NH, NH_2), 3080 (CH arom.), 2956, 2850 (CH aliph.), 1651 (C═O), 1383, 1154 (SO_2), 1256 (C═S). ^1H NMR (DMSO-d_6) δ: 0.96 [2s, 6H, $2CH_3$], 1.01 [s, 3H, CH_3], 2.21–2.49 [m, 4H, $2CH_2$ cyclo], 7.21–7.95 [m, 6H, Ar–H + SO_2NH_2], 10.32 [s, 1H, NH], 13.82 [s, 1H, NH]. MS, *m*/*z* (%): 443 [M^+] (3.11), 148 (100). Anal. Calcd. for $C_{20}H_{21}N_5O_3S_2$: C, 54.16; H, 4.77; N, 15.79. Found: C, 54.43; H, 5.02; N, 15.97. **17**: Yield, 47%; m.p. 164–166 °C; IR, cm^{-1}: 3426, 3336, 3200 (NH, NH_2), 2955, 2850 (CH aliph.), 1661 (C═O), 1384, 1134 (SO_2), 1254 (C═S). MS, *m*/*z* (%): 485 [M^+] (4), 107 (100). Anal. Calcd. for $C_{21}H_{23}N_7O_3S_2$: C, 51.94; H, 4.77; N, 20.19. Found: C, 52.23; H, 4.36; N, 20.50. **18**: Yield, 59%; m.p. 160–162 °C; IR, cm^{-1}: 3380, 3343 (NH, NH_2), 3055 (CH arom.), 2956, 2840 (CH aliph.), 1658 (C═O), 1389, 1161 (SO_2), 1266 (C═S). ^1H NMR (DMSO-d_6) δ: 0.98, 0.99 [2s, 6H, $2CH_3$], 1.01 [s, 3H, CH_3], 2.11 [s, 3H, CH_3 isoxazole], 2.21–2.82 [m, 8H, $4CH_2$], 6.23 [s, 1H, CH isoxazole], 6.89–7.79 [m, 5H, Ar–H + NH], 9.82 [1s, 1H, 1NH], 11.36 [s, 1H, SO_2NH]. MS, *m*/*z* (%): 524 [M^+] (46), 168 (100). Anal. Calcd. for $C_{24}H_{24}N_6O_4S_2$: C, 54.95; H, 4.61; N, 16.02. Found: C, 54.69; H, 4.89; N, 16.38. **19**: Yield, 71%; m.p. 117–119 °C; IR, cm^{-1}: 3361, 3300 (NH), 2930, 2830 (CH aliph.), 1648 (C═O), 1388, 1155 (SO_2), 1254 (C═S). MS, *m*/*z* (%): 521 [M^+] (2.5), 82 (100). Anal. Calcd. for $C_{24}H_{23}N_7O_3S_2$: C, 55.26; H, 4.44; N, 18.80. Found: C, 55.54; H, 4.09; N, 18.53.

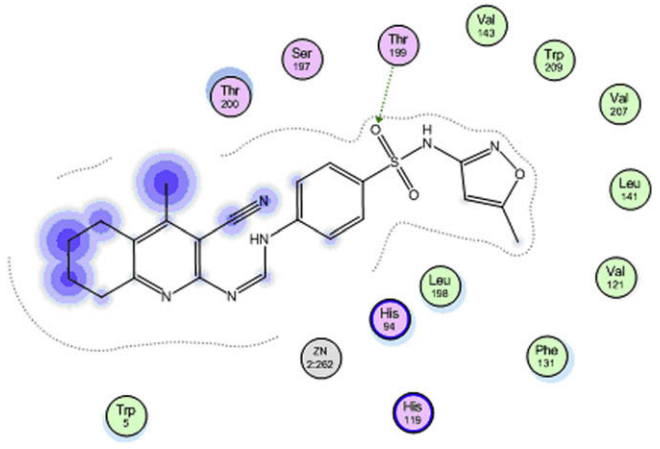


Fig. 7. Interaction map of compound 5 with the active site of hCA II showing different interactions than those previously reported.

*4.1.5. 4-(4-Imino-5,8,8-trimethyl-6-oxo-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolin-3-(4H)-yl)benzenesulfonamide (**14**) and 4-(4-imino-5,8,8-trimethyl-6-oxo-6,7,8,9-tetrahydropyrimido-[4,5-*b*]quinolin-3 (4H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (**15**)*

Method (A). A mixture of compound **11** (0.285 g, 0.001 mol), sulfanilamide or sulfamethoxazole (0.0012 mol) in pyridine (20 ml) was refluxed for 12 h. The reaction mixture was cooled and poured onto ice water. The obtained solid was recrystallized from dioxane to give **14** and **15**, respectively.

Method (B). Compound **12** or **13** (0.001 mol) in pyridine (20 ml) was refluxed for 10 h. The reaction mixture was then poured onto ice water acidified with dil. HCl. The product was filtered and recrystallized from dioxane to give **14** and **15**, respectively (m.p. and m.m.p.).

14: Yield, 55%; m.p. 179–181 °C; IR, cm^{-1}: 3313, 3281, 3125 (NH, NH_2), 3050 (CH arom.), 2958, 2860 (CH aliph.), 1668 (C═O), 1383, 1153 (SO_2). MS, *m*/*z* (%): 412 [M^+] (**4**), 149 (100). Anal. Calcd. for $C_{20}H_{21}N_5O_3S$: C, 58.38; H, 5.14; N, 17.02. Found: C, 58.69; H, 5.40; N, 17.39. **15**: Yield, 61%; m.p. 212–214 °C; IR, cm^{-1}: 3247, 3184 (NH), 3054 (CH arom.), 2962, 2870 (CH aliph.), 1671 (C═O), 1369, 1183 (SO_2). ^1H NMR (DMSO-d_6) δ: 1.02, 1.03 [2s, 6H, $2CH_3$], 1.2 [s, 3H, CH_3], 2.07–2.49 [m, 7H, $2CH_2$ cyclo + CH_3 isoxazole], 6.11 [s, 1H, CH isoxazole], 7.61–7.98 [m, 4H, Ar–H], 8.24 [s, 1H, CH pyrimidine], 10.32 [s, 1H, NH], 11.25 [s, 1H, SO_2NH]. MS, *m*/*z* (%):492 [M^+] (0.5),

161 (100). Anal. Calcd. for $C_{24}H_{24}N_6O_4S$: C, 58.52; H, 4.91; N, 17.06. Found: C, 58.75; H, 5.15; N, 17.33.

4.2. In vitro anticancer screening

The cytotoxic activity was measured in vitro for the newly synthesized compounds using the Sulfo-Rhodamine-B stain (SRB) assay using the method of Skehan et al. [29]. The in vitro anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell microtiter plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time [29]. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given 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-pentaflouro-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.

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

References

[1] J. Drews, Science 287 (2000) 1960–1964.
[2] A.E. Boyd 3rd, Diabetes 37 (1988) 847–850.
[3] C.T. Supuran, A. Scozzafava, Exp. Opin. Ther. Patents 10 (2000) 575–600.
[4] T.H. Maren, Annu. Rev. Pharmacol. Toxicol. 16 (1976) 309–327.
[5] C.T. Supuran, A. Scozzafava, Curr. Med. Chem. – Immunol. Endocr. Metabol. Agents 1 (2001) 61–97.
[6] C.W. Thornber, Chem. Soc. Rev. 8 (1979) 563–580.
[7] F. Abbate, A. Casini, T. Owa, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. 14 (2004) 217–223.
[8] M.M. Ghorab, E. Noaman, M.M. Ismail, H.I. Heiba, Y.A. Ammar, M.Y. Sayed, Arzneimittelforschung 56 (2006) 405–413.
[9] M.M. Ismail, M.M. Ghorab, E. Noaman, Y.A. Ammar, H.I. Heiba, M.Y. Sayed, Arzneimittelforschung 56 (2006) 301–308.
[10] S.A. Rostom, Bioorg. Med. Chem. 14 (2006) 6475–6485.
[11] C.T. Supuran, A. Casini, A. Mastrolorenzo, A. Scozzafava, Mini-Rev. Med. Chem. 4 (2004) 625–632.
[12] A.J. Kivela, J. Kivela, J. Saarnio, S. Parkkila, World J. Gastroenterol. 11 (2005) 155–163.
[13] C.T. Supuran, Nat. Rev. Drug Discov. 7 (2008) 168–181.
[14] C.T. Supuran, A. Scozzafava, Bioorg. Med. Chem. 15 (2007) 4336–4350.
[15] C.T. Supuran, A. Scozzafava, A. Casini, Med. Res. Rev. 23 (2003) 146–189.
[16] A. Casini, A. Scozzafava, A. Mastrolorenzo, L.T. Supuran, Curr. Cancer Drug Targets 2 (2002) 55–75.
[17] S. Pastorekova, S. Parkkila, J. Pastorek, C.T. Supuran, J. Enzyme Inhib. Med. Chem. 19 (2004) 199–229.
[18] F. Saczewski, J. Slawinski, A. Kornicka, Z. Brzozowski, E. Pomarnacka, A. Innocenti, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. 16 (2006) 4846–4851.
[19] F. Saczewski, A. Innocenti, J. Slawinski, A. Kornicka, Z. Brzozowski, E. Pomarnacka, A. Scozzafava, C. Temperini, C.T. Supuran, Bioorg. Med. Chem. 16 (2008) 3933–3940.
[20] W.R. Chegwidden, S.J. Dodgson, I.M. Spencer, EXS 90 (2000) 343–363.
[21] M. Gopal, S. Shenoy, L.S. Doddamani, J. Photochem. Photobiol., B 72 (2003) 69–78.
[22] Y.H. Kim, K.J. Shin, T.G. Lee, E. Kim, M.S. Lee, S.H. Ryu, P.G. Suh, Biochem. Pharmacol. 69 (2005) 1333–1341.
[23] Y.L. Zhao, Y.L. Chen, F.S. Chang, C.C. Tzeng, Eur. J. Med. Chem. 40 (2005) 792–797.
[24] J.P. Liou, Z.Y. Wu, C.C. Kuo, C.Y. Chang, P.Y. Lu, C.M. Chen, H.P. Hsieh, J.Y. Chang, J. Med. Chem. 51 (2008) 4351–4355.
[25] M.M. Ghorab, F.A. Ragab, E. Noaman, H.I. Heiba, E.M. El-Hossary, Arzneimittelforschung 57 (2007) 795–803.
[26] M.M. Ghorab, F.A. Ragab, E. Noaman, H.I. Heiba, E.M. El-Hossary, Arzneimittelforschung 58 (2008) 35–41.
[27] A. Thiry, M. Ledecq, A. Cecchi, J.M. Dogne, J. Wouters, C.T. Supuran, B. Masereel, J. Med. Chem. 49 (2006) 2743–2749.
[28] G.G.G. Tacconi, G. Desimoni, V. Messori, J. Prakt. Chem. 322 (1980) 831–834.
[29] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.