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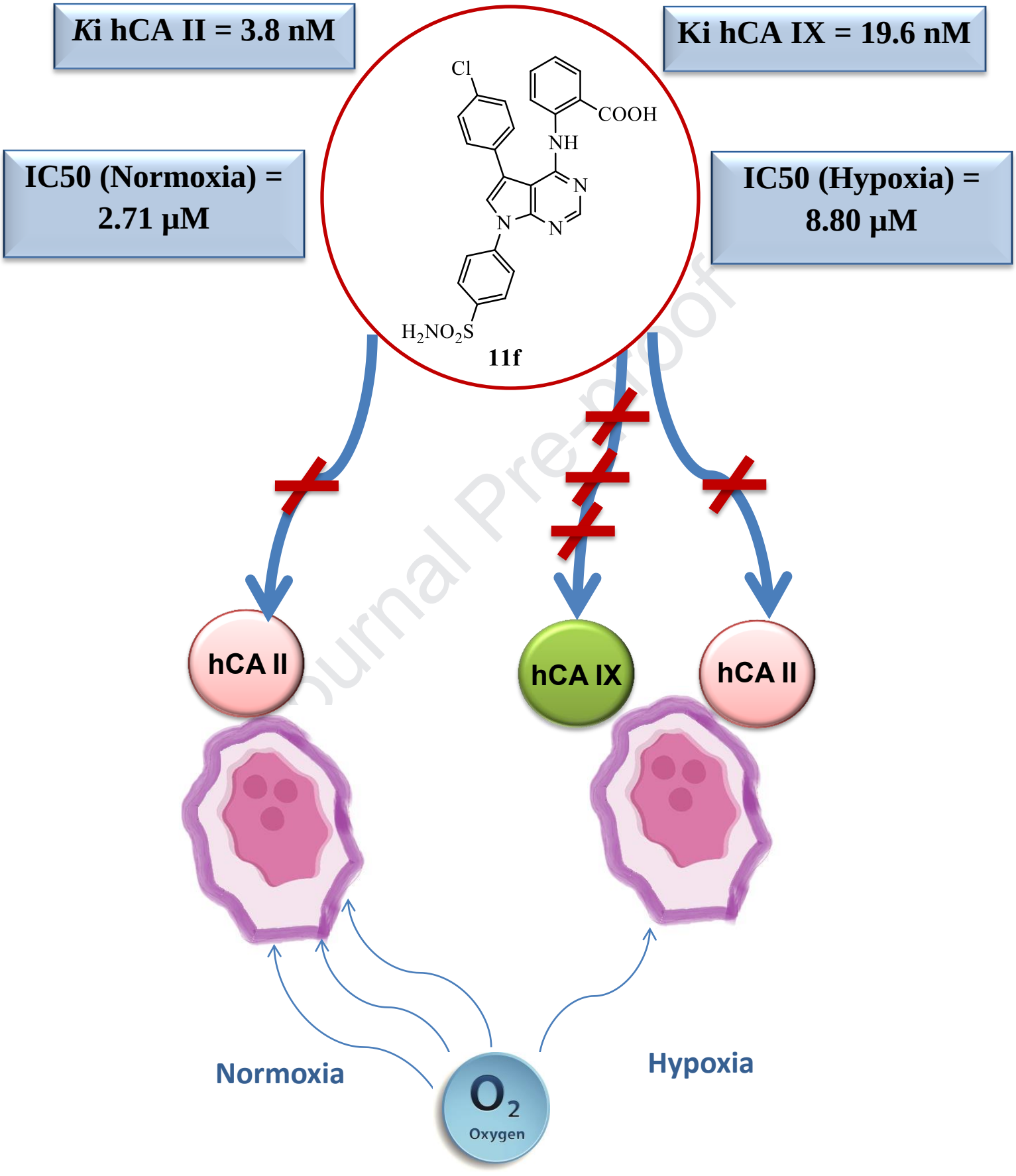


Pyrrolo and Pyrrolopyrimidine Sulfonamides act as Cytotoxic Agents in Hypoxia *via* Inhibition of Transmembrane Carbonic Anhydrases

Omneya M. Khalil [a], Aliaa M. Kamal [a,b,*], Silvia Bua [c], Heba El Sayed Teba [b,*], Yassin M. Nissan [d,e], Claudiu T. Supuran [c,*]

[a] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 11562, Cairo, Egypt.
[b] Organic Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 11787, Giza, Egypt.
[c] Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Polo Scientifico, Via U. Schiff 6, 50019, Sesto Fiorentino (Firenze), Italy.
[d] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, 11562, Cairo, Egypt.
[e] Pharmaceutical Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 11787, Giza, Egypt.

Keywords: cytotoxic; carbonic anhydrase inhibitor; pyrrolopyrimidine; sulfonamide.

*Corresponding author: Aliaa M. Kamal, Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 11562, Cairo, Egypt.
*Corresponding author: Heba El Sayed Teba, Organic Chemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), 11787, Giza, Egypt.
Email: hteba@msa.eun.eg
*Corresponding author: Claudiu T. Supuran, Department of NEUROFARBA, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Polo Scientifico, Via U. Schiff 6, 50019, Sesto Fiorentino (Firenze), Italy.

Abstract

A series of novel sulfonamide derivatives bearing pyrrole and pyrrolopyrimidine scaffolds were synthesized and screened as carbonic anhydrase inhibitors. The inhibition activity of the synthesized compounds was evaluated against the cytosolic human carbonic anhydrase isoforms I and II and the transmembranal isoforms IX and XII. Several candidates showed potent inhibitory activity against IX and XII isoforms. Furthermore, *ex vivo* screening of cytotoxic selectivity and activity of the most potent derivatives were carried out against normal cells (WI38) and cervical cancer cell line (HeLa) under normal and hypoxic conditions using acetazolamide as reference drug. Compound **11b** potency was nearly three folds higher in hypoxic than normoxic condition whereas that of compound **11f** was nearly four folds higher in hypoxic vs. normoxic HeLa cells. All the screened derivatives exhibited

less potency on normal cells (WI38). Molecular docking was carried out to discover the possible binding mode of compounds within the active site of isoform CA IX.

1. Introduction

According to the American cancer society, cancer was estimated to cause 1700 deaths daily [1]. The rapid increase in cancer cases and social burdens represent real crisis for public health and health systems worldwide [2]. Using conventional non selective chemotherapeutic agents affects both cancer and normal rapidly dividing cells causing many problems such as severe side effects, high patient risks, repeated treatment and increased possibility of cancer cells to acquire multidrug resistance [3].

Sulfonamides represent an essential class of drugs having various biological activities such as antibacterial [4], antiviral [5, 6], anti-inflammatory [7, 8], diuretic [9, 10], hypoglycemic [11], antithyroid, protease inhibitors [8, 12], antioxidant, antiacetylcholinesterase [13], lactoperoxidase inhibitors [14], butyrylcholinesterase inhibitors [15], and anti-cancer agents [16-19]. The mechanism of action of sulfonamides as anti-cancer agents is by acting as carbonic anhydrase inhibitors (CAIs) which is the same mechanism of action of acetazolamide (AZA), methazolamide and topiramate (Fig. 1) [20]. Sulfamates, sulfamides (sulfonamides isosteres) and sulfonamides, represent the main classes of CAIs [21]. They all coordinate the zinc ion of CA enzyme with their terminal deprotonated nitrogen atom [22].

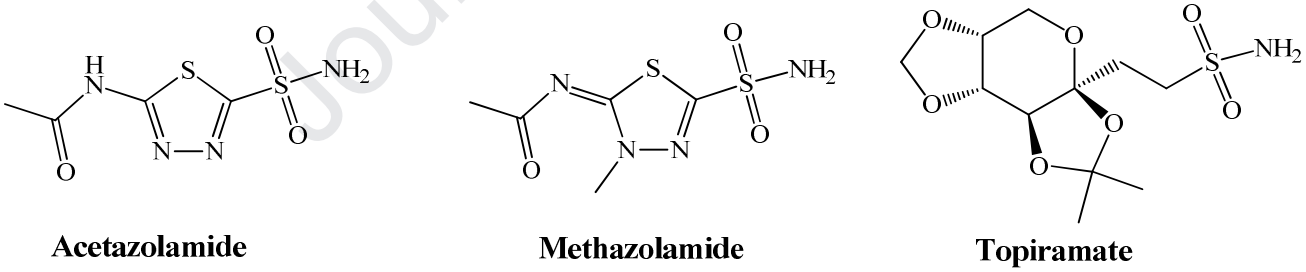


Fig. 1: Sulfonamides with potent CA inhibition activity

Pyrrole and fused pyrrole compounds such as pyrrolopyrimidine derivatives possess a wide spectrum of pharmacological activities mainly acting as anticancer agents [23, 24]. In previous work, a combination of sulfonamide with pyrrole and pyrrolopyrimidine derivative exhibited promising anticancer agents [25-29].

The metalloenzymes carbonic anhydrases (CAs, EC 4.2.1.1) catalyze the reversible hydration of CO_2 to bicarbonate and protons in all living organisms [8, 30-38]. They are involved in various physiological functions such as intra and extra cellular pH regulation, respiration, inorganic carbon transport and bio mineralization [39-42]. Up till now, 15 isoforms of CAs were discovered in humans, and they differ in tissue distribution, subcellular localization and catalytic activity. Five CAs are cytosolic (CA I–III, VII and XIII), two are mitochondrial (CA VA and VB), one is secreted (CA VI) and the others are membrane-bound (CA IV, IX, XII, and XIV) [22]. CA enzymes active site contains a Zn^{+2} ion which is coordinated by three imidazole groups of histidines and one hydroxide ion in a slightly distorted tetrahedral geometry [8]. Carbonic anhydrase IX (CA IX) is a cell surface protein that is overexpressed in cancer cells, being involved in solid tumor acidification but it is not expressed in most normal cells [22, 43, 44]. CA IX was first identified on the surface of the human cervical carcinoma cell line (HeLa) [45]. Expression of CA IX is found in cancers of lung [46], colon [47], breast [48], brain [49] and cervix [50] among others. Expression of CA IX is induced by hypoxia, the enzyme functioning as a pro-survival factor which protects cancer cells against acidosis and hypoxia by its ability to maintain an intracellular pH (pHi) favorable for survival and growth of tumor cells [51]. Recently, the synthesis of new CA IX-selective inhibitors is receiving a considerable attention to furnish a novel targeted strategy in cancer therapy [51-53].

Structure activity relationship of sulfonamides showed that the sulfamoyl group is essential for CA inhibitory activity. Amino group of SO_2NH_2 must remain unsubstituted to retain both *in vitro* and *in vivo* activities. Sulfamoyl group must be attached to aromatic ring and introduction of any additional substituents to the ring decreases the activity [54].

In this study, we synthesized novel sulfonamide derivatives incorporating pyrrole and pyrrolopyrimidine scaffolds. All the synthesized compounds were evaluated for their carbonic anhydrase inhibition activity against four CA isoforms, the cytosolic, widespread hCA I and II, and the transmembranal, tumor-associated CA IX and XII using the well-known CAI acetazolamide (AZA). *In vitro* anticancer screening of the most potent derivatives was carried out against cervical cell line (HeLa) under normal and hypoxic conditions. Docking study was carried out within the active site of hCA IX isoenzyme. Synthesis of the target sulfonamides was represented in Schemes1 and 2.

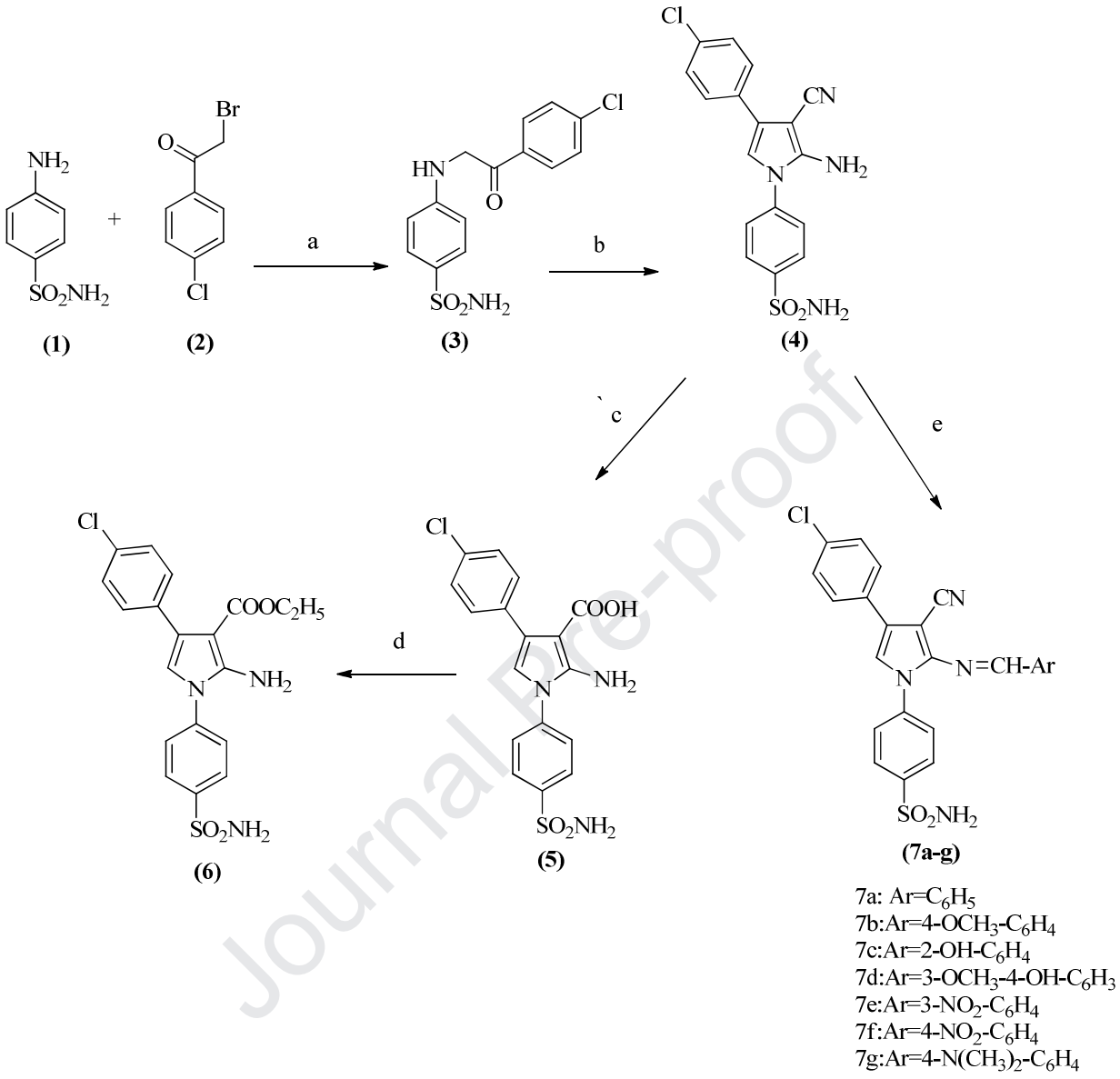
2. Results and discussion

2.1. Chemistry

The synthesis of sulfonamide derivatives **4-7a-g** is displayed in scheme 1. Reaction of sulfanilamide **1** with 4-chlorophenacyl bromide **2** yielded the corresponding 4-(2-(4-chlorophenyl)-2-oxoethylamino)benzenesulfonamide **3** which upon reaction with malononitrile in presence of a base gave the pyrrole derivative **4**. The ^{1}H NMR spectrum revealed the presence of a singlet signal at δ 4.77 ppm due to CH_2 group and a singlet D_2O exchangeable signal at δ 6.60 corresponding to NH group. The IR spectrum of compound **4** exhibited a characteristic absorption band of CN group at 2187 cm^{-1}. In addition, the ^{1}H NMR spectrum exhibited the appearance of a singlet D_2O exchangeable signal at δ 6.14 ppm assigned to NH_2 group and a singlet signal at δ 7.03 ppm due to CH of the pyrrole ring. Acid hydrolysis of compound **4** using 60% H_2SO_4 yielded the acid derivative **5** while condensation of compound **4** with different aromatic aldehydes yielded the corresponding schiff's bases **7a-g**. IR spectrum of compound **5** showed the disappearance of CN group and appearance of a broad absorption band at 3550-2720 cm^{-1} assigned to OH of the carboxylic group and absorption band at 1683 cm^{-1} corresponding to C=O group. ^{1}H NMR spectrum revealed the appearance of a singlet D_2O exchangeable signal at δ 9.85 ppm assigned to OH group of the carboxylic group. The ^{1}H NMR spectra of compounds **7a-g** revealed the presence of singlet signals for (N=CH) in the range of δ 8.88-9.41 ppm. Esterification of compound **5** was carried out using absolute ethanol to yield the ester derivative **6**. IR spectrum of **6** indicated the disappearance of the broad absorption band of OH group and the shifting of the C=O band to 1723 cm^{-1}. Moreover, the presence of CH_3 and CH_2 groups was proved by ^{1}H NMR spectrum by the characteristic triplet and quartet signals at δ 1.11 and 3.77 ppm respectively with the same *J* values and by ^{13}C NMR spectrum at 14.57 and 61.51 ppm.

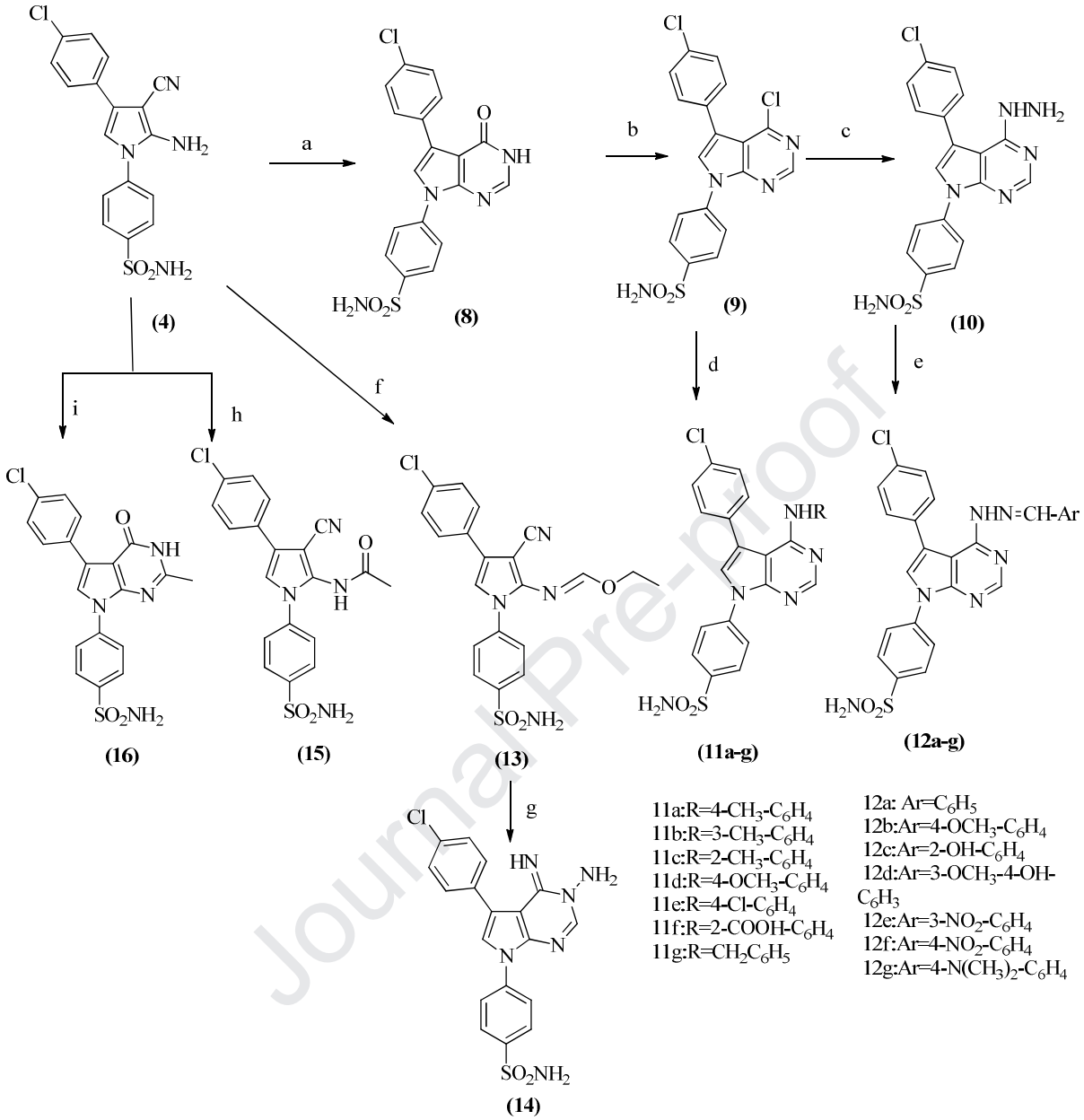
In scheme 2, reaction of amino cyanopyrrole derivative **4** with formic acid afforded the pyrrolopyrimidinone **8** which underwent chlorination with phosphorous oxychloride followed by reaction with different amines to afford compounds **9**, **10** and **11a-g** respectively. Cyclization and formation of **8** was confirmed by IR spectrum which showed the appearance of C=O absorption band at 1657 cm^{-1} in addition to the disappearance of CN band of its precursor. ^{1}H NMR spectrum exhibited the appearance of a singlet signal at δ 8.00 ppm corresponding to CH of pyrimidine and a singlet D_2O exchangeable signal at δ 12.31 ppm due to NH/OH proton. ^{13}C NMR spectrum showed the appearance of a signal at 158.93 ppm corresponding to C=O group. Structure of compound **9** was

proved via ^{1}H NMR spectrum which showed the disappearance of the singlet D_2O exchangeable signal assigned to NH proton at δ 12.31 ppm. The ^{1}H NMR spectrum of compound **10** revealed the presence of a singlet D_2O exchangeable signal due to NH proton at δ 12.30 ppm. The ^{1}H NMR spectra of **11a-g** showed the characteristic signal of NH in the range of δ 12.31-12.33 ppm. For compounds **11a-c**, the appearance of signals at the range of δ 2.12-2.31 ppm confirmed the presence of CH_3 group. Compound **11d** showed signal at δ 3.75 ppm corresponding to OCH_3 group. Appearance of signal at δ 4.72 ppm in compound **11g** is due to CH_2 group. ^{13}C NMR spectra of compound **11f** showed the presence of signal at 158.92 ppm assigned to C=O. Compounds **12a-g** were synthesized via reacting the hydrazino derivative **10** with different aromatic aldehydes. The ^{1}H NMR spectra of **12a-g** revealed the presence of singlet signals in the range of δ 8.33-9.01 ppm for (N=CH) protons in addition to singlet D_2O exchangeable signals in the range of δ 11.16-12.31 ppm assigned to NH protons. Reacting compound **4** with triethylorthoformate gave the compound **13** which upon hydrazinolysis yielded the aminoimino pyrrolopyrimidine **14**. ^{1}H NMR spectrum of compound **13** showed the appearance of triplet and quartet signals at δ 1.04 and 3.45 ppm respectively with the same *J* values corresponding to CH_3 and CH_2 groups in addition to two singlet signals at δ 8.54 and 8.71 ppm with integration of half proton each (N=CH proton) due to the presence of two geometrical isomers in nearly equal ratios and that was confirmed with TLC. Structure of compound **14** was proved on the basis of its ^{1}H NMR spectrum which indicated the appearance of a singlet D_2O exchangeable signal at δ 6.14 ppm assigned to NH_2 group and a singlet signal at δ 7.96 ppm corresponding to CH of pyrimidine. To obtain the 2-methylpyrrolopyrimidinone, compound **4** was refluxed with acetic anhydride. After short time, a new spot on TLC appeared and after working up the reaction mixture, it was found that the newly formed compound was the acetylated aminocyano derivative **15**. Increasing reaction time up to 24 hours gave the pyrrolopyrimidinone **16**. IR spectrum of **15** showed the appearance of absorption band at 1730 cm^{-1} corresponding to C=O group while the ^{1}H NMR spectrum indicated the presence of singlet signal at δ 2.51 corresponding to CH_3 group in addition to a singlet D_2O exchangeable signal at δ 10.24 ppm assigned to NH group. Cyclization and formation of the pyrrolopyrimidinone **16** was confirmed by IR via disappearance of the absorption band due to CN group at 2226 cm^{-1}. ^{1}H NMR spectrum revealed the appearance of a singlet signal at δ 2.45 ppm corresponding to CH_3 group in addition to a singlet D_2O exchangeable signal at δ 12.27 ppm assigned to NH group.



a- Absolute ethanol, TEA, reflux 6 h; b- malononitrile, $NaOC_2H_5$, Dioxane, reflux 8 h; c- 60% H_2SO_4, reflux 24 h; d- absolute ethanol, Conc. H_2SO_4, reflux 8 h; e-substituted aldehyde, absolute ethanol, acetic acid, reflux 2-8 h.

Scheme 1



a- Formic acid, reflux 6 h; b- $POCl_3$, reflux 8 h; c- NH_2NH_2, absolute ethanol reflux 8 h; d- Amine derivatives, dry pyridine, reflux 12 h; e- Aromatic aldehydes, absolute ethanol, acetic acid, reflux; f- $CH(OC_2H_5)_3$, reflux 6 h; g- NH_2NH_2, absolute ethanol reflux 8 h; h- Ac_2O, reflux 5 min; i- Ac_2O, reflux 24 h.

Scheme 2

2.2.Biological evaluation

2.2.1. Carbonic anhydrase inhibition

Screening of CA inhibition activity of the synthesized sulfonamide derivatives was carried out by a stopped flow assay method [55-62] and was used to assess the inhibitory activity against four isoforms of the enzyme, hCA I and II (cytosolic isoforms) and hCA IX and XII (membrane associated isoforms). The reference drug used for this assay was acetazolamide (AZA), the well-known CA inhibitor. Results of the assay are displayed in Table 1.

Table 1: The inhibition activity of compounds **4-16** against hCA I, II, IX, and XII using AZA as reference drug.

Compound	*Ki*[a] (nM)				SI[b]	
	hCA I	hCA II	hCA IX	hCA XII	hCA II / IX	hCA II / XII
4	7550.6	9243.4	2874.1	88.5	3.22	104.45
5	128.4	56.8	75.2	54.1	0.76	1.05
6	8339	5245.3	1868.9	151.9	2.81	34.53
7a	2564.4	1350.2	3048.9	247.9	0.44	5.45
7b	3851.5	2281.8	2707.7	207.8	0.84	10.98
7c	1846	844.3	975.4	40.1	0.87	21.05
7d	2035.8	1461.9	785.1	71.8	1.86	20.36
7e	217.3	129.7	1315.9	73.5	0.10	1.76
7f	160.8	89.5	1136.4	68.8	0.08	1.30
7g	5481.1	6456.5	2717.4	239.6	2.38	26.95
8	504.6	129.1	57.2	71.6	2.26	1.80
9	372.8	47.1	75.6	66.5	0.62	0.71
10	477.8	151.8	85.6	78.9	1.77	1.92
11a	309.2	1.9	10.6	28.7	0.18	0.07
11b	318.5	28	33.7	36	0.83	0.78
11c	361.3	34.3	27.8	44.9	1.23	0.76
11d	86.2	23	66.1	46	0.35	0.50
11e	406.2	34.4	70.4	57.9	0.49	0.59
11f	261.4	3.8	19.6	45.2	0.19	0.08
11g	195.7	88	129	83.1	0.68	1.06
12a	146.6	55.5	157.4	125.7	0.35	0.44
12b	323.9	127.5	159.6	133	0.80	0.96
12c	47.2	9.8	206.3	165.4	0.05	0.06
12d	2035.2	907.4	2966.6	304.3	0.31	2.98
12e	3380.2	1440.2	2687.4	326.7	0.54	4.41
12f	2827.5	1213.1	2553	288.9	0.48	4.20
12g	4744.7	2508.3	3031.1	303.1	0.83	8.28
13	2362.5	1444.2	1016	183.6	1.42	7.87
14	405.6	142.2	149.9	32.2	0.95	4.42
15	7812.7	5197.4	835.6	130.5	6.22	39.83
16	518.5	134.6	72.3	93.8	1.86	1.43
AZA	250	12.5	25	5.7	0.5	2.19

[a] *Ki* is the mean from 3 different assays. (errors are in the range of ± 5-10% of the reported values)
[b] SI (selectivity index) is a ratio between the *Ki* values observed for two hCA isoforms (low value index is indicative of weak selectivity).

Sulfonamides **5, 7e, 7f, 11d, 11g, 12a** and **12c** showed good activity against the cytosolic isoform hCA I, superior to that of AZA ranging from 47.2 to 217.3 nM. Moreover, compounds **11a,**

11f and **12c** exhibited potent inhibitory effect towards the cytosolic isoform hCA II compared to AZA ranging from 1.9 to 9.8 nM.

hCA IX was highly inhibited by compounds **11a** and **11f** with *Ki* values of 10.6 and 19.6 nM, respectively compared to 25 nM for AZA. Compounds **5, 8, 9, 10, 11b, 11c, 11d, 11e** and **16** showed moderate activity in the range of 27.8-85.6 nM while other compounds showed poor activity towards hCA IX. With regards to the tumor associated target isoform hCA XII, all compounds were less potent than AZA. Sulfonamides **5, 7c, 11a, 11b, 11c, 11d, 11f, 11e** and **14** exhibited moderate hCA XII inhibitory activity. The remaining derivatives showed poor activity.

To measure the differential inhibitory activity of the tested candidates towards isoforms hCA II/IX and hCA II/XII, the selectivity index (SI) was calculated (Table 1). The higher the inhibitory activity against the tumor associated hCA IX and hCA XII isoforms over hCA II isoform, the more effective and safe a drug would be. Compounds **4, 5, 6, 7b, 7c, 7d, 7g, 8, 9, 10, 11b, 11c, 11g, 12g, 13, 14, 15** and **16** exhibited high hCA II/IX SI values higher than that of AZA.

2.2.2. *In vitro* anticancer activity

It is worth to be mentioned that, HeLa, cervical cancer cells showed increased CA IX expression upon hypoxic conditions. To study the efficacy of the synthesized compounds as anticancer agents *via* carbonic anhydrase inhibition, the *in vitro* anticancer activity of the most potent hCA IX inhibitors was screened against the cervical cancer cells (HeLa) under normal and hypoxic conditions using AZA as reference drug applying MTT method [63-66]. Moreover, the toxicity of the synthesized compounds was evaluated by measuring their IC_{50} against normal cells WI-38. Results are listed in Table 2 and Figure 2.

Table 2*: In vitro* anticancer screening against cervical cancer cell line (HeLa) under normal and hypoxic conditions & normal cell line (WI-38)

Compound	IC_{50} (µM)*		
	HeLa		WI-38
	Normoxia	Hypoxia	
8	40.22±1.66	32.65±1.61	69.57±4.27
11a	8.86±0.35	6.65±0.25	26.56±1.51

11b	9.05±0.42	3.17±0.11	24.71±0.93
11c	12.35±0.88	13.33±0.81	16.17±0.75
11f	8.80±0.41	2.71±0.06	22.02±1.36
AZA	7.82±0.22	2.83±0.04	14.45±0.77

*Each value is the mean of three values ± standard error

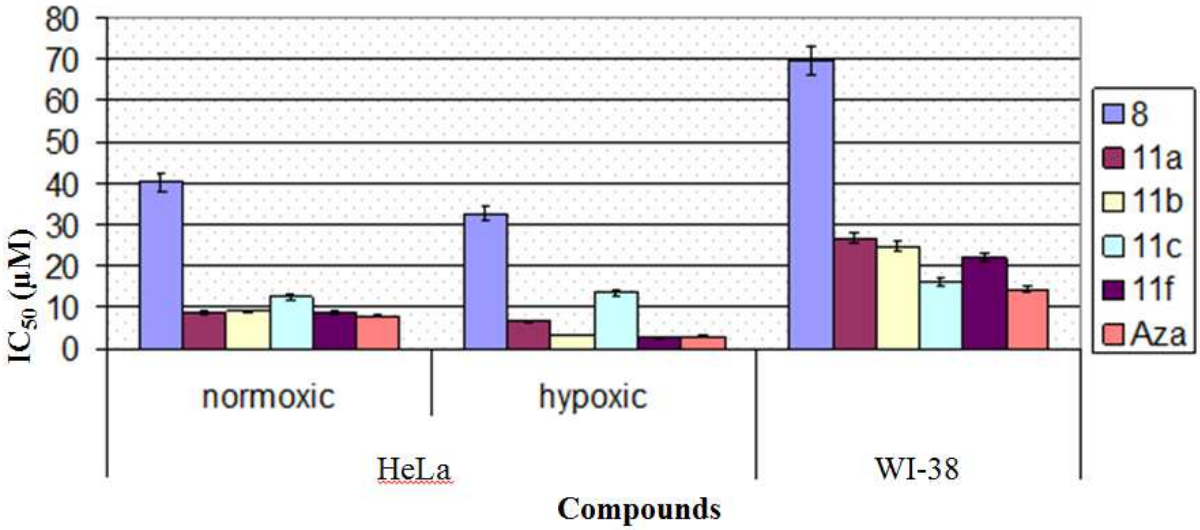


Fig. 2: Graph showing IC_{50} (µM) of the tested compounds against HeLa

Anticancer activity of the candidates was very promising as all the tested candidates showed potency under hypoxic conditions greater than normoxic conditions. Compounds **11f** and **11b** were nearly triple the potency when subjected to hypoxic conditions. The aryl amino pyrrolopyrimidine derivative **11f** was the most potent with IC_{50} 2.71 µM under hypoxic conditions. It was obvious that the presence of carboxylic group in *o*-position in **11f** increased the activity. Replacement of the carboxylic group with methyl group in different positions in compounds **11a-c** decreased the activity where the *m*-methyl derivative **11b** is nearly with the same activity as AZA while the p-methyl derivative **11a** and the o-methyl derivative **11c** showed lower activity with IC_{50} 3.17, 6.65 and 13.33 µM, respectively. The pyrrolopyrimidinone **8** was the least active as anticancer agent among the tested compounds.

The cytotoxic concentration that kills 50% of normal cells WI-38 for the test compounds was determined by MTT cytotoxic assay. All the test compounds exhibited selectivity towards cancer cells than normal cells.

2.3.Molecular modeling and docking results

Most of the synthesized compounds showed promising activity against hCA I, II, IX and XII isoforms. The transmembranal, tumor associated isoenzyme hCA IX is our target enzyme as the candidates showed excellent inhibition activity against this isoform. The molecular docking of the newly synthesized compounds within the active site of hCA IX isoenzyme was performed and the amino acids interactions and docking patterns were investigated using the protein data bank file (PDB ID: AI3I). The file contains hCA IX enzyme co-crystallized with AZA. The docking procedures were performed by Molecular Operating Environment (MOE, 2015.10) software. Docking setup was first validated by self-docking of the co-crystallized ligand (acetazolamide) in the vicinity of the binding site of the enzyme, with energy score (S) = -9.96 kcal/mol and root mean standard deviation (RMSD) = 0.59. AZA interacts with the active site of hCA IX by one hydrogen bond between Thr 199 and SO2 group, one hydrogen bond between Thr 200 and N3 of thiadiazole ring, one hydrogen bond between Leu 198 and thiadiazole ring and two interactions between Zn and nitrogen atom of NH_2 group and oxygen atom of SO_2 group (coordination) (Fig. 3, 4).

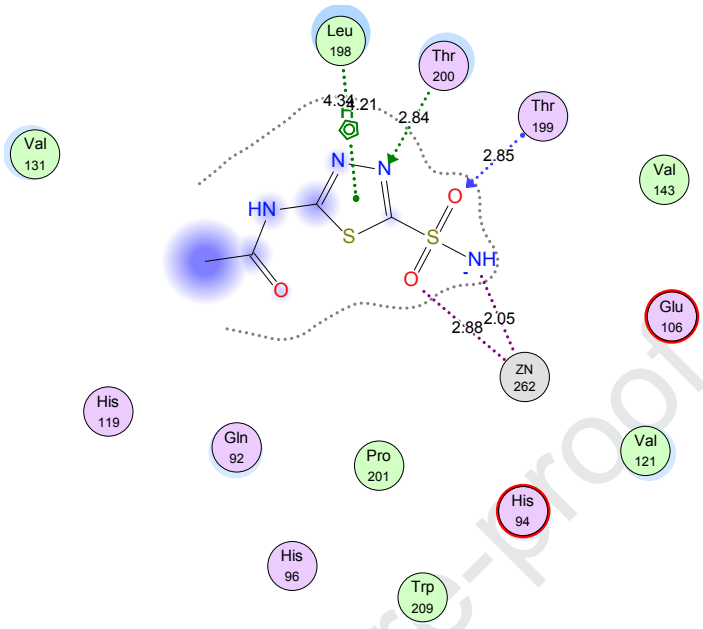
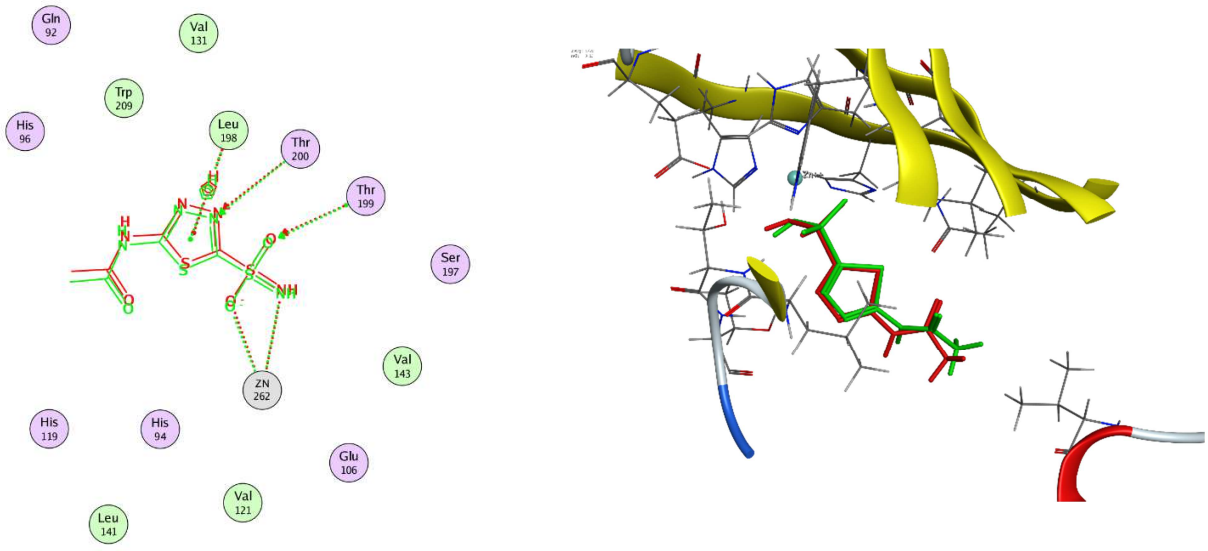


Fig. 3: 2D interaction diagram showing AZA docking pose interactions with CAIX binding site.



(a) (b)

Fig. 4: a) 2D representation, b) 3D representation of the superimposition of the co-crystallized (red) and the docking pose (green) of AZA in CA IX binding site with RMSD of 0.59 A°.

Docking of all the newly synthesized compounds was performed and showed proper fitting in the active site of hCA IX with good energy scores (S), which supports the observed activity of these sulfonamide derivatives as hCA IX inhibitors. The energy score (S) and amino acid interactions of the most potent hCA IX inhibitors are listed in Table 3.

Table 3: Docking results of the synthesized sulfonamide derivatives with hCA IX isoform

Compound	S (kcal/mol)	Amino acids	Interacting groups	Type of interaction	Length (Å)
8	-11.8161		O (S)	Metal complex (Zn)	2.29
			NH (S)	Metal complex (Zn)	2.63
		Gln92	Pyrrole	H-bond (donor)	3.37
		Thr199	O (S)	H-bond (acceptor)	2.81
11a	-12.0662		O (S)	Metal complex (Zn)	3.02
			NH (S)	Metal complex (Zn)	1.74
		Gln92	Pyrrole	H-bond (donor)	3.22
		Thr199	O (S)	H-bond (acceptor)	2.63
11b	-12.4743		O (S)	Metal complex (Zn)	1.77
			NH (S)	Metal complex (Zn)	2.11
		Gln92	Pyrrole	H-bond (donor)	3.24
		Leu135	Arene-H	Pyrimidine	4.52
		Thr199	O (S)	H-bond (acceptor)	2.44
		Trp209	NH (S)	H-bond (acceptor)	3.04
11c	-12.4018		O (S)	Metal complex (Zn)	2.80
			NH (S)	Metal complex (Zn)	2.15
		Gln92	Pyrrole	H-bond (donor)	2.77
		Thr199	O (S)	H-bond (acceptor)	3.13
		Thr199	O (S)	H-bond (acceptor)	3.41
11f	-10.3689		O (S)	Metal complex (Zn)	2.84
			NH (S)	Metal complex (Zn)	2.20
		Gln92	Phenyl	Arene-H	4.40
		Thr199	O (S)	H-bond (acceptor)	3.56
		Thr200	O (S)	H-bond (acceptor)	2.80
AZA	-7.3279		O (S)	Metal complex (Zn)	2.88
			NH (S)	Metal complex (Zn)	2.05
		Leu198	Thiadiazole	Arene-H	4.34
		Thr199	O (S)	H-bond (acceptor)	2.85
		Thr200	N (Thiadiazole)	H-bond (acceptor)	2.84

All compounds showed interaction with the Zn ion through sulfonamide group in coordinate bonds by the deprotonated sulfonamide moiety. Moreover, interactions with Thr 199 through

hydrogen bond were observed. Compound **11c** formed two hydrogen bonds with Thr 199. Compound **11f** formed a hydrogen bond with Thr 200 which mimics the pattern of interaction of AZA with the enzyme. Moreover, all the docked compounds showed the same orientation within the active site of CA IX suggesting the binding pattern of these derivatives within CA IX isoform (Fig. 5).ppppppppppppppppppppppppppppp

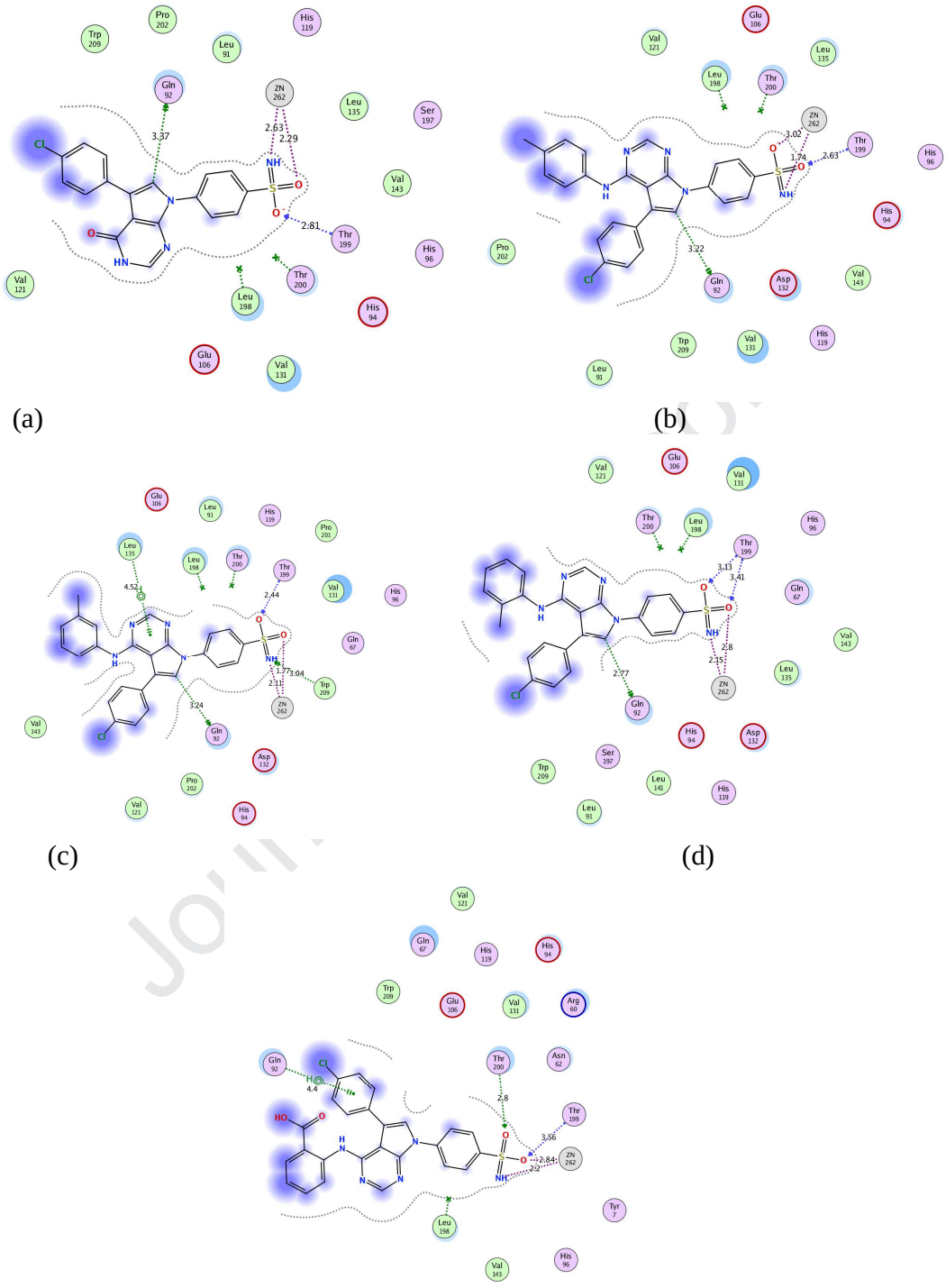


Fig. 5: 2D enzyme-ligand interaction of compounds (a) **8**, (b) **11a**, (c) **11b**, (d) **11c**, (e) **11f** inside the active site of hCA IX

3. Conclusion

In this work, a group of pyrrole and pyrrolopyrimidine sulfonamide derivatives were designed and synthesized as cytotoxic agents. The new compounds were characterized by spectral procedures, and microanalytical analyses. All synthesized compounds were tested as CAIs against hCA I and hCA II (cytosolic isoforms) and hCA IX and hCA XII (transmembrane associated isoforms) using AZA as the reference drug. All compounds showed activity against hCA IX isoform while showed less activity against hCA XII than AZA. Sulfonamide **11a** was 2 and half fold more potent than AZA towards hCA IX with *K*i value 10.6 nM. The most active CAIs were assessed against HeLa cell line under normal and hypoxic conditions. The potency of cytotoxicity for compounds had elevated for all tested derivatives except for **11b**. The effect of tested compounds against WI-38 normal cells revealed much higher IC_{50} values than HeLa cells. The docking of the synthesized compounds was carried out and the docked derivatives showed enzyme-ligand binding interactions with the amino acids within the active site of hCA IX isoform similar to AZA binding interactions. These results support further studies about these new derivatives.

4. Experimental

4.1. Chemistry

Melting points (^{o}C, uncorrected) were determined on Stuart apparatus and the given values were uncorrected. Progress of the reaction was monitored by thin layer chromatography (TLC) using aluminum sheets precoated with UV fluorescent silica gel (MERCK 60 F 254) and spots were visualized by UV lamp. The solvent system used was dichloromethane: methanol (in different ratios). The IR spectra (cm^{-1}) were determined using KBr discs on a Shimadzu IR 8400s Spectrophotometer, Microanalytical Unit, Faculty of Pharmacy, Cairo University, Cairo, Egypt. ^{1}H-NMR and ^{13}C-NMR spectra were performed on Bruker 400-BB 400 MHz Spectrophotometer, microanalytical unit, Faculty of Pharamacy, Cairo University, Cairo, Egypt, using tetramethylsilane (TMS) as internal standard and chemical shift values were recorded in ppm on δ scales. Peak multiplicities were designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses were performed at the Regional Center for Mycology and Biotechnology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

4.1.1. 4-(2-(4-Chlorophenyl)-2-oxoethylamino)benzenesulfonamide **(3)**

A mixture of sulfanilamide **1** (1.72 g, 0.01 mol) and 4-chloro phenacyl bromide **2** (2.33 g, 0.01 mol) was refluxed in absolute ethanol (20 mL) in presence of catalytic amount of triethylamine for 6 h. The solid obtained was filtered and crystallized from dioxane to give compound **3**.

Yield: 85%; M.p.: 205-207 °C; IR (KBr, cm^{-1}): 3475, 3317, 3244 (NH, NH_2), 3070 (CH arom.), 2931 (CH aliph.), 1685 (C=O), 1311, 1149 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 4.77 (s, 2H, CH_2), 6.60 (s, 1H, NH, D_2O exchangeable), 6.93 (s, 2H, NH_2, D_2O exchangeable), 6.74- 7.53 (2d, 4H, Ar-H, *J*=8.80 Hz, *J*=8.80 Hz), 7.64-8.10 (2d, 4H, Ar-H, *J*=8.60 Hz, *J*=8.60 Hz) ppm. Anal. Calcd. for $C_{14}H_{13}ClN_2O_3S$ (324.78): C, 51.77; H, 4.03; N, 8.63. Found: C, 51.95; H, 4.19; N, 8.90.

4.1.2. 4-(2-Amino-4-(4-chlorophenyl)-3-cyano-1*H*-pyrrol-1-yl)benzenesulfonamide **(4)**

Sodium ethoxide (sodium metal 0.5g in absolute ethanol 10 mL) was added to a mixture of compound **3** (3.24 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in dioxane (20 mL) then was refluxed for 8 h. The reaction mixture was cooled and acidified with dil. HCl till litmus paper is acidic. The obtained solid was filtered and crystallized from dioxane to give compound **4**

Yield: 72%; M.p.:192-194 °C; IR (KBr, cm^{-1}): 3344, 3240 (NH_2), 3093 (CH arom.), 2187 (C≡N), 1319, 1165 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 6.14 (s, 2H, NH_2, D_2O exchangeable), 7.03 (s, 1H, pyrrole), 7.34-7.97 (m, 10H, Ar-H + SO_2NH_2) ppm. ^{13}C NMR (DMSO-d_6, δ): 114.09, 118.10, 121.82, 125.72, 127.51, 127.64, 129.20, 130.34, 131.55, 132.51, 140.05, 143.4, 149.01 ppm. Anal. Calcd. for $C_{17}H_{13}ClN_4O_2S$ (372.83): C, 54.77; H, 3.51; N, 15.03. Found: C, 55.01; H, 3.70; N, 15.21.

4.1.3. 2-Amino-4-(4-chlorophenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrrole-3-carboxylic acid **(5)**

Compound **4** (3.72 g, 0.01 mol) was heated with aqueous sulfuric acid (60 mL, 60% H_2SO_4) at 100 ^oC for 24 h. The reaction mixture was poured into ice /water and the formed solid was crystallized from ethanol to give compound **5**.

Yield: 65%; M.p.: 242-244 °C; IR (KBr, cm^{-1}): 3550-2720 (OH, NH_2), 3130 (CH arom.), 1683 (C=O), 1328, 1171 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 5.56 (s, 2H, NH_2, D_2O exchangeable), 7.58 (s, 1H, pyrrole), 7.04-8.04 (m, 10H, Ar-H + SO_2NH_2), 9.85 (s, 1H, OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 113.10, 115.99, 125.12, 126.09, 127.89, 129.66, 130.09, 131.61,

138.53, 144.41, 159.25, 164.81, 166.95 ppm. Anal. Calcd. for $C_{17}H_{14}ClN_3O_4S$ (391.83): C, 52.11; H, 3.60; N, 10.72. Found C, 52.38; H, 3.76; N, 10.95.

4.1.4. Ethyl-2-amino-4-(4-chlorophenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrrole-3-carboxylate **(6)**

A mixture of compound **5** (3.91 g, 0.01 mol) and absolute ethanol (20 mL) was refluxed in the presence of catalytic amount of conc. H_2SO_4 for 8 h. The reaction mixture was poured onto sodium carbonate solution and the formed solid was crystallized from ethanol to give compound **6**

Yield: 60%; M.p.: 269-271 °C; IR (KBr, cm^{-1}): 3392, 3300 (NH_2), 3096 (CH arom.), 2982 (CH aliph.), 1723 (C=O), 1336, 1163 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 1.11 (t, 3H, *J* = 7.04, 7.08 Hz, CH_3), 3.77 (q, 2H, *J* = 7.04, 7.08 Hz, CH_2), 7.34-7.98 (m, 13H, Ar-H + NH_2 + SO_2NH_2) ppm. ^{13}C NMR (DMSO-d_6, δ): 14.57, 61.51, 118.00, 121.60, 127.90, 129.07, 129.40, 130.76, 131.43, 134.30, 136.30, 138.65, 140.50, 145.10, 165.90 ppm. Anal. Calcd. for $C_{19}H_{18}ClN_3O_4S$ (419.88): C, 54.35; H, 4.32; N, 10.01. Found C, 54.62; H, 4.59; N, 10.27.

4.1.5. General procedures for synthesis of compounds **(7a-g)**

A mixture of compound **4** (3.72 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (20 mL) and glacial acetic acid (1 mL) was refluxed for 2-6 h. The obtained solid was filtered and crystallized from ethanol to give compounds **7a-g** respectively.

4.1.5.1. 4-(2-(Benzylideneamino)-4-(4-chlorophenyl)-3-cyano-1*H*-pyrrol-1-yl) benzenesulfonamide **(7a)**

Yield: 70% (2 h); M.p.: 257-259 °C; IR (KBr, cm^{-1}): 3387, 3271 (NH_2), 3101 (CH arom.), 2206 (C≡N), 1697 (C=N), 1338, 1157 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.51 (s, 1H, pyrrole), 7.54-7.99 (m, 15 H, Ar-H + SO_2NH_2), 9.14 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 79.48, 117.27, 120.22, 124.66, 126.20, 127.09, 128.23, 129.50, 129.64, 129.66, 131.44, 132.65, 133.40, 135.44, 139.90, 143.61, 147.24, 164.04 ppm. Anal. Calcd. for $C_{24}H_{17}ClN_4O_2S$ (460.94): C, 62.54; H, 3.72; N, 12.16. Found: C, 62.31; H, 3.94; N, 12.39.

4.1.5.2. 4-[4-(4-Chlorophenyl)-3-cyano-2-(4-methoxybenzylideneamino)-1*H*-pyrrol-1-yl] benzenesulfonamide **(7b)**

Yield: 80% (2 h); M.p.: 248-250 °C; IR (KBr, cm^{-1}): 3344, 3259 (NH_2), 3101 (CH arom.), 2935 (CH aliph.), 2206 (C≡N), 1593 (C=N), 1311, 1153 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 3.86 (s, 3H, OCH_3), 7.50 (s, 1H, pyrrole), 7.09-7.98 (m, 14H, Ar-H + SO_2NH_2), 9.04 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 56.09, 78.94, 114.40, 115.20, 117.46, 119.69, 124.39, 126.12, 127.05, 128.18, 129.48, 131.75, 132.53, 137.10, 140.01, 143.48, 148.02, 163.43, 163.68 ppm. Anal. Calcd. for: $C_{25}H_{19}ClN_4O_3S$ (490.96): C, 61.16; H, 3.90; N, 11.41. Found: C, 60.89; H, 4.13; N, 11.57.

4.1.5.3. 4-[4-(4-Chlorophenyl)-3-cyano-2-(2-hydroxybenzylideneamino)-1*H*-pyrrol-1-yl]benzenesulfonamide **(7c)**

Yield: 75% (2 h); M.p.: 287-289 °C; IR (KBr, cm^{-1}): 3410, 3255, 3147 (OH, NH_2), 3074 (CH arom.), 2206 (C≡N), 1600 (C=N), 1327, 1165 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.53 (s, 1H, pyrrole), 6.93-7.99 (m, 14H, Ar-H + SO_2NH_2), 9.41 (s, 1H, N=CH), 10.91 (s, 1H, OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 79.44, 117.24, 120.23, 120.90, 124.76, 126.47, 127.15, 128.29, 129.51, 130.00, 131.46, 132.64, 134.00, 135.17, 139.82, 141.00, 143.87, 146.77, 160.06, 161.98 ppm. Anal. Calcd. for: $C_{24}H_{17}ClN_4O_3S$ (476.93): C, 60.44; H, 3.59; N, 11.75. Found C, 60.67; H, 3.80; N, 11.92.

4.1.5.4. 4-[4-(4-Chlorophenyl)-3-cyano-2-(4-hydroxy-3-methoxybenzylideneamino)-1*H*-pyrrol-1-yl] benzenesulfonamide **(7d)**

Yield: 70% (6 h); M.p.: 232-234 °C; IR (KBr, cm^{-1}): 3387 (OH), 3267, 3113 (NH_2), 3074 (CH arom.), 2974 (CH aliph.), 2210 (C≡N), 1585 (C=N), 1338, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 3.80 (s, 3H, OCH_3), 7.50 (s, 1H, pyrrole), 6.92 – 7.98 (m, 13H, Ar-H + SO_2NH_2), 8.96 (s, 1H, N=CH), 10.12 (s, 1H, OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 56.08, 78.69, 111.62, 116.21, 117.51, 119.44, 124.35, 125.44, 126.14, 126.97, 128.17, 129.48, 131.61, 132.51, 140.04, 143.42, 148.07, 148.64, 152.36, 163.35 ppm. Anal. Calcd. for: $C_{25}H_{19}ClN_4O_4S$ (506.96): C, 59.23; H, 3.78; N, 11.05. Found C, 59.50; H, 3.91; N, 11.23.

4.1.5.5. 4-[4-(4-Chlorophenyl)-3-cyano-2-(3-nitrobenzylideneamino)-1*H*-pyrrol-1-yl] benzenesulfonamide **(7e)**

Yield: 60% (6 h); M.p.: 238-240 °C; IR (KBr, cm^{-1}): 3383, 3263 (NH_2), 3089 (CH arom.), 2214(C≡N), 1593 (C=N), 1319, 1157 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.53 (s, 1H, pyrrole),

7.58 -8.68 (m, 14H, Ar-H + SO_2NH_2), 9.31 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 80.19, 100.08, 116.97, 121.00, 123.89, 125.00, 126.29, 127.13, 128.29, 129.54, 131.25, 131.32, 132.79, 135.05, 137.02, 139.75, 143.80, 146.03, 148.80, 161.60 ppm. Anal. Calcd. for $C_{24}H_{16}ClN_5O_4S$ (505.93): C, 56.98; H, 3.19; N, 13.84. Found C, 57.14; H, 3.46; N, 14.07.

4.1.5.6. 4-[4-(4-Chlorophenyl)-3-cyano-2-(4-nitrobenzylideneamino)-1*H*-pyrrol-1-yl] benzenesulfonamide **(7f)**

Yield: 60% (6 h); M.p.: 294-296 °C; IR (KBr, cm^{-1}): 3375, 3267 (NH_2), 3074 (CH arom.), 2214 (C≡N), 1593 (C=N), 1327, 1165 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.53 (s, 1H, pyrrole), 7.58 -8.37 (m, 14H, Ar-H + SO_2NH_2), 9.29 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 80.30, 116.99, 121.37, 124.74, 125.22, 126.37, 127.14, 128.31, 129.55, 130.50, 131.19, 132.84, 139.71, 140.91, 143.82, 145.83, 149.90, 161.10 ppm. Anal. Calcd. for: $C_{24}H_{16}ClN_5O_4S$ (505.93): C, 56.98; H, 3.19; N, 13.84. Found C, 57.11; H, 3.42; N, 13.75.

4.1.5.7. 4-[4-(4-Chlorophenyl)-3-cyano-2-(4-(dimethylamino)benzylideneamino)-1*H*-pyrrol-1-yl]benzenesulfonamide **(7g)**

Yield: 65% (6 h); M.p.: 238-240 °C; IR (KBr, cm^{-1}): 3348, 3251 (NH_2), 3082 (CH arom.), 2904 (CH aliph.), 2206 (C≡N), 1585 (C=N), 1311, 1153 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 3.04 (s, 6H, $2CH_3$), 7.50 (s, 1H, pyrrole), 6.78-7.97 (m, 14H, Ar-H + SO_2NH_2), 8.88 (s, 1H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 40.60, 78.08, 111.52, 112.07, 117.87, 118.85, 122.73, 124.12, 126.00, 126.97, 128.10, 129.42, 131.66, 131.77, 132.36, 140.22, 143.22, 149.19, 153.87, 163.16 ppm. Anal. Calcd. for $C_{26}H_{22}ClN_5O_2S$ (504.00): C, 61.96; H, 4.40; N, 13.90. Found C, 61.82; H, 4.56; N, 14.13.

4.1.6. 4-(5-(4-Chlorophenyl)-4-oxo-3*H*-pyrrolo[2,3-*d*]pyrimidin-7(4*H*)-yl) benzenesulfonamide **(8)**

A solution of compound **4** (3.72 g, 0.01 mol) in formic acid (20 mL) was refluxed for 6 h. The reaction mixture was poured onto ice/cold water and the obtained solid was crystallized from ethanol to give compound **8**

Yield: 80%; M.p.: 264-266 °C; IR (KBr, cm^{-1}): 3348, 3300, 3250 (NH, NH_2), 3050 (CH arom.), 1657 (C=O), 1328, 1163 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.41-8.08 (m, 10H, Ar-H + SO_2NH_2), 7.45 (s, 1H, pyrrole), 8.00 (s, 1H, pyrimidine), 12.31 (s, 1H, NH/OH, D_2O exchangeable) ppm. ^{13}C

NMR (DMSO-d_6, δ):106.90, 120.54, 122.12, 124.99, 127.20, 128.46, 130.47, 131.68, 132.44, 139.99, 142.80, 145.43, 148.73, 158.93 ppm. Anal. Calcd. for $C_{18}H_{13}ClN_4O_3S$ (400.84): C, 53.94; H, 3.27; N, 13.98. Found: C, 54.08; H, 3.54; N, 14.19.

4.1.7. 4-(4-Chloro-5-(4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(9)**

A mixture of compound **8** (4.00 g, 0.01 mol) and phosphorous oxychloride (60 mL) was refluxed for 8 h. The reaction mixture was cooled then poured onto ice/water. The formed solid was filtered and crystallized from ethanol to give compound **9.**

Yield: 85%; M.p.: 239-241 °C; IR (KBr, cm^{-1}): 3352, 3232 (NH_2), 3078 (CH arom.), 1330, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.56 (s, 1H, pyrrole), 7.41-8.80 (m, 10H, Ar-H + SO_2NH_2), 8.05 (s, 1H, pyrimidine) ppm. ^{13}C NMR (DMSO-d_6, δ): 116.07, 123.9, 124.97, 126.85, 127.06, 127.4, 128.43, 129.36, 130.22, 132.51, 132.80, 133.15, 143.09, 151.45 ppm. Anal. Calcd. for $C_{18}H_{12}Cl_2N_4O_2S$ (419.28): C, 51.56; H, 2.88; N, 13.36. Found: C, 51.82; H, 3.12; N, 13.59.

4.1.8. 4-(5-(4-Chlorophenyl)-4-hydrazinyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(10)**

A mixture of compound **9** (4.19 g, 0.01 mol) and hydrazine hydrate (99%, 3mL) in absolute ethanol (20 mL) was heated under reflux for 8 h. The reaction mixture was cooled, filtered and the precipitate was dried and crystallized from dimetylformamide to give compound **10**.

Yield: 75%; M.p.: 226-228 °C; IR (KBr, cm^{-1}): 3350, 3217, 3190 (NH, NH_2), 3078 (CH arom.), 1327, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.58 (s, 1H, pyrrole), 8.00 (s, 1H, pyrimidine), 7.45-8.41 (m, 12H, Ar-H + NH_2 + SO_2NH_2), 12.30 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ):106.88, 120.55, 122.09, 125.01, 127.22, 128.45, 129.39, 131.70, 132.40, 139.98, 142.77, 145.41, 148.7, 158.93 ppm. Anal. Calcd. for $C_{18}H_{15}ClN_6O_2S$ (414.87): C, 52.11; H, 3.64; N, 20.26. Found C, 52.38; H, 3.51; N, 20.17.

4.1.9. **General procedures for synthesis of compounds (11a-e)**

A mixture of compound **9** (4.19 g, 0.01 mol) and the appropriate amine (0.01 mol) in dry pyridine (20 mL) was heated under reflux for 12 h and poured onto ice/cold water then acidified with HCl.

The obtained solid was filtered and crystallized from the appropriate solvent to give compounds **11a-e** respectively.

4.1.9.1. 4-(5-(4-Chlorophenyl)-4-(p-tolylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(11a)**

Yield: 80%; M.p.: 207-209 °C; IR (KBr, cm^{-1}): 3348, 3217, 3194 (NH, NH_2), 3078 (CH arom.), 2924 (CH aliph.), 1330, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 2.12 (s, 3H, CH_3), 7.48 (s, 1H, pyrrole), 8.05 (s, 1H, pyrimidine), 6.46-8.58 (m, 14H, Ar-H + SO_2NH_2), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 20.57, 106.89, 114.55, 120.55, 122.55, 124.50, 127.22, 128.47, 129.42, 130.76, 131.70, 132.43, 136.66, 139.99, 141.89, 142.79, 145.43, 148.73, 158.93 ppm. Anal. Calcd. for $C_{25}H_{20}ClN_5O_2S$ (489.98): C, 61.28; H, 4.11; N, 14.29. Found C, 61.04; H, 4.32; N, 14.52.

4.1.9.2. 4-(5-(4-Chlorophenyl)-4-(m-tolylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(11b)**

Yield: 85%; M.p.: 201-203 °C; IR (KBr, cm^{-1}): 3350, 3230, 3190 (NH, NH_2), 3090 (CH arom.), 2950 (CH aliph.), 1330, 1160 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 2.21 (s, 3H, CH_3), 7.58 (s, 1H, pyrrole), 8.13 (s, 1H, pyrimidine), 7.44-8.94 (m, 14H, Ar-H + SO_2NH_2), 12.33 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 21.24, 100.71, 119.04, 124.26, 125.01, 127.68, 128.44, 129.36, 129.73, 130.47, 131.07, 133.25, 138.92, 142.42, 143.51, 144.50, 145.37, 146.64, 148.16, 152.20, 158.92 ppm. Anal. Calcd. for $C_{25}H_{20}ClN_5O_2S$ (489.98): C, 61.28; H, 4.11; N, 14.29. Found C, 60.99; H, 4.28; N, 14.50.

4.1.9.3. 4-(5-(4-Chlorophenyl)-4-(o-tolylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(11c)**

Yield: 80%; M.p.: 262-264°C; IR (KBr, cm^{-1}): 3357, 3210, 3183 (NH, NH_2), 3050 (CH arom.), 2910(CH aliph.), 1330, 1165 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 2.31 (s, 3H, CH_3), 7.60 (s, 1H, pyrrole), 8.04 (s, 1H, pyrimidine), 7.44-8.48 (m, 14H, Ar-H + SO_2NH_2), 12.32 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 21.00, 100.92, 106.85, 118.62, 120.55, 122.09, 124.23, 124.99, 125.78, 126.88, 127.23, 128.46, 129.66, 130.82, 131.72, 133.07, 139.97, 142.75, 145.40,

148.71, 158.00 ppm. Anal. Calcd. for $C_{25}H_{20}ClN_5O_2S$ (489.98): C, 61.28; H, 4.11; N, 14.29. Found C, 61.13; H, 4.34; N, 14.43.

4.1.9.4. 4-(5-(4-Chlorophenyl)-4-((4-methoxyphenyl)amino)-7*H*-pyrrolo[2,3-*d*] pyrimidin-7-yl)benzenesulfonamide **(11d)**

Yield: 75%; M.p.: 216-218 °C; IR (KBr, cm^{-1}): 3475, 3344, 3224 (NH, NH_2), 3074 (CH arom.), 2931 (CH aliph.), 1330, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d6, D_2O, δ): 3.75 (s, 3H, OCH_3), 7.58 (s, 1H, pyrrole), 7.41-8.08 (m, 14H, Ar-H + SO_2NH_2), 8.59 (s, 1H, pyrimidine), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 55.68, 106.88, 115.16, 120.59, 122.01, 123.83, 124.98, 127.24, 129.41, 130.46, 131.72, 132.34, 136.98, 139.99, 142.71, 145.34, 148.70, 149.74, 158.93 ppm. Anal. Calcd. for $C_{25}H_{20}ClN_5O_3S$ (505.98): C, 59.34; H, 3.98; N, 13.84. Found C, 59.60; H, 3.79; N, 14.05.

4.1.9.5. 4-(5-(4-Chlorophenyl)-4-((4-chlorophenyl)amino)-7*H*-pyrrolo[2,3-*d*] pyrimidin-7-yl)benzenesulfonamide **(11e)**

Yield: 75%; M.p.: 234-236 °C; IR (KBr, cm^{-1}): 3474, 3300, 3232 (NH, NH_2), 3072 (CH arom.), 1328, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.58 (s, 1H, pyrrole), 7.44-8.80 (m, 14H, Ar-H + SO_2NH_2), 8.38 (s, 1H, pyrimidine), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 101.40, 116.03, 117.55, 123.99, 125.00, 127.30, 128.99, 129.45, 130.46, 130.75, 132.59, 138.17, 140.25, 142.06, 148.88, 150.66, 152.14, 157.39 ppm. Anal. Calcd. for $C_{24}H_{17}Cl_2N_5O_2S$ (510.40): C, 56.48; H, 3.36; N, 13.72. Found C, 56.73; H, 3.48; N, 13.98.

4.1.9.6. 2-((5-(4-Chlorophenyl)-7-(4-sulfamoylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4-yl)amino)benzoic acid **(11f)**

Yield: 70%; M.p.: 229-231 °C; IR (KBr, cm^{-1}): 3474, 3300, 3232 (OH, NH, NH_2), 3074 (CH arom.), 1662 (C=O), 1327, 1161 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 7.59 (s, 1H, pyrrole), 7.96-8.15 (m, 14H, Ar-H + SO_2NH_2), 8.34 (s, 1H, pyrimidine), 9.20 (s, 1H, OH, D_2O exchangeable), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 101.25, 106.88, 117.88, 120.56, 122.07, 124.99, 127.32, 128.44, 129.50, 130.46, 131.70, 132.38, 132.41, 132.73, 139.98, 142.42, 142.76, 145.39, 148.71, 156.21, 158.92 ppm. Anal. Calcd. for $C_{25}H_{18}ClN_5O_4S$ (519.96): C, 57.75; H, 3.49; N, 13.47. Found C, 58.01; H, 3.71; N, 13.68.

4.1.9.7. 4-(4-(Benzylamino)-5-(4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl) benzenesulfonamide **(11g)**

Yield: 70%; M.p.: 140-142 °C; IR (KBr, cm^{-1}): 3400, 3379, 3300 (NH, NH_2), 3062 (CH arom.), 2970 (CH aliph.), 1327, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 4.72 (s, 2H, CH_2), 7.60 (s, 1H, pyrrole), 7.24-8.34 (m, 15H, Ar-H + SO_2NH_2), 8.48 (s, 1H, pyrimidine), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 44.47, 100.69, 101.72, 117.29, 118.58, 124.91, 127.28, 127.51, 128.76, 130.84, 131.68, 132.88, 133.02, 135.05, 139.33, 140.29, 142.13, 143.20, 158.92 ppm. Anal. Calcd. for $C_{25}H_{20}ClN_5O_2S$ (489.98): C, 61.28; H, 4.11; N, 14.29. Found C, 61.45; H, 4.40; N, 14.50

4.1.10. General procedures for synthesis of compounds **(12a-g)**

A mixture of compound **10** (4.14 g, 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in absolute ethanol (20 mL) and small amount of glacial acetic acid (1 mL) was refluxed for 6 h. the obtained solid was filtered off and recrystallized from the appropriate solvent to give compounds **12a-g** respectively.

4.1.10.1. (*E/Z)* 4-(4-(2-Benzylidenehydrazinyl)-5-(4-chlorophenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-7-yl)benzenesulfonamide **(12a)**

Yield: 65%; M.p.: 276-278 °C; IR (KBr, cm^{-1}): 3350, 3317, 3300 (NH, NH_2), 3070 (CH arom.), 1334, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 6.85-8.20 (m, 15H, Ar-H + SO_2NH_2), 7.48 (s, 1H, pyrrole), 8.18 (s, 1H, pyrimidine), 8.33 (s, ½ H, ½ N=CH), 8.43 (s, ½ H, ½ N=CH), 11.16 (s, ½ H, ½ NH, D_2O exchangeable), 11.97(s, ½ H, ½ NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 101.68, 103.22, 118.67, 120.47, 122.29, 124.27, 125.32, 126.69, 128.21, 130.82, 131.62, 133.03, 134.56, 136.06, 142.21, 143.48, 145.29, 149.83, 151.86, 152.73, 156.67 ppm. Anal. Calcd. for $C_{25}H_{19}ClN_6O_2S$ (502.98): C, 59.70; H, 3.81; N, 16.71. Found C, 59.87; H, 4.03; N, 16.89.

4.1.10.2. (*E/Z)* 4-(5-(4-Chlorophenyl)-4-(2-(4-methoxybenzylidene)hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12b)**

Yield: 75%; M.p.: 152-154 °C; IR (KBr, cm^{-1}): 3479, 3350, 3309 (NH, NH_2), 3074 (CH arom.), 2966 (CH aliph.), 1323, 1165 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 3.83 (s, 3H, OCH_3), 7.05-8.08 (m,

14H, Ar-H + SO_2NH_2), 7.45 (s, 1H, pyrrole), 8.00 (s, 1H, pyrimidine), 8.63 (s, ¾ H, ¾ N=CH), 9.87 (s, ¼ H, ¼ N=CH), 12.31 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 55.81, 106.83, 114.84, 114.95, 120.58, 122.03, 125.03, 126.88, 127.24, 128.45, 130.01, 130.48, 131.74, 132.33, 139.96, 142.69, 145.34, 148.69, 158.94, 161.01, 162.14, 164.70 ppm. Anal. Calcd. for $C_{26}H_{21}ClN_6O_3S$ (533.00): C, 58.59; H, 3.97; N, 15.77. Found C, 58.38; H, 4.11; N, 15.89.

4.1.10.3. 4-(5-(4-Chlorophenyl)-4-(2-(2-hydroxybenzylidene)hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12c)**

Yield: 75%; M.p.: 213-215 °C; IR (KBr, cm^{-1}): 3583, 3332, 3305 (OH, NH, NH_2), 3008 (CH arom.), 1315, 1195 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 7.42 (s, 1H, pyrrole), 7.94 (s, 1H, pyrimidine), 6.80-7.78 (m, 14H, Ar-H + SO_2NH_2), 9.01 (s, 1H, N=CH), 11.13 (s, 1H, NH, D_2O exchangeable), 11.40 (s, 1H, OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 116.15, 116.98, 118.52, 119.51, 120.04, 120.16, 126.95, 128.69, 129.24, 131.43, 133.79, 142.89, 156.91, 159.03, 163.37 ppm. Anal. Calcd. for $C_{25}H_{19}ClN_6O_3S$ (518.97): C, 57.86; H, 3.69; N, 16.19. Found C, 58.09; H, 3.84; N, 16.45.

4.1.10.4. 4-(5-(4-Chlorophenyl)-4-(2-(4-hydroxy-3-methoxybenzylidene) hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12d)**

Yield: 70%; M.p.: 278-280 °C; IR (KBr, cm^{-1}): 3475, 3332, 3236 (OH, NH, NH_2), 3078 (CH arom.), 2939 (CH aliph.), 1334, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 3.80 (s, 3H, OCH_3), 7.50 (s, 1H, pyrrole), 6.87 – 8.12 (m, 13H, Ar-H + SO_2NH_2), 8.10 (s, 1H, pyrimidine), 8.58 (s, 1H, N=CH), 9.77 (s, 1H, OH, D_2O exchangeable), 12.30 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 55.98, 110.50, 116.15, 116.98, 118.52, 119.51, 120.04, 120.16, 123.96, 125.96, 126.95, 128.69, 129.24, 131.43, 133.79, 142.89, 148.44, 150.33, 156.91, 159.03, 161.10, 163.37 ppm. Anal. Calcd. for $C_{26}H_{21}ClN_6O_4S$ (549.00): C, 56.88; H, 3.86; N, 15.31. Found C, 57.04; H, 4.09; N, 15.48.

4.1.10.5. 4-(5-(4-Chlorophenyl)-4-(2-(3-nitrobenzylidene)hydrazinyl)-7*H*-pyrrolo [2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12e)**

Yield: 80%; M.p.: 170-172 °C; IR (KBr, cm^{-1}): 3321, 3250, 3182 (NH, NH_2), 3086 (CH arom.), 1346, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 7.45-8.38 (m, 14H, Ar-H + SO_2NH_2), 7.78 (s, 1H, pyrrole), 8.71 (s, 1H, pyrimidine), 8.91 (s, 1H, N=CH), 12.30 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 119.23, 121.79, 123.05, 126.22, 127.24, 127.93, 130.44, 131.06,

131.51, 134.90, 135.29, 135.67, 139.00, 148.61, 148.64, 160.90, 172.47. Anal. Calcd. for $C_{25}H_{18}ClN_7O_4S$ (547.97): C, 54.80; H, 3.31; N, 17.89. Found C, 54.96; H, 3.57; N, 17.69.

4.1.10.6. 4-(5-(4-Chlorophenyl)-4-(2-(4-nitrobenzylidene)hydrazinyl)-7*H*-pyrrolo [2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12f)**

Yield: 65%; M.p.: 285-287 °C; IR (KBr, cm^{-1}): 3350, 3332, 3294 (NH, NH_2), 3086 (CH arom.), 1346, 1161 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 7.09-8.88 (m, 14H, Ar-H + SO_2NH_2), 7.57 (s, 1H, pyrrole), 8.18 (s, 1H, pyrimidine), 8.88 (s, 1H, N=CH), 12.27 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 101.60, 103.45, 118.87, 122.90, 124.56, 125.30, 128.98, 130.54, 131.65, 133.80, 135.76, 136.06, 141.21, 143.32, 145.76, 149.83, 150.86, 152.73, 154.67 ppm. Anal. Calcd. for $C_{25}H_{18}ClN_7O_4S$ (547.97): C, 54.80; H, 3.31; N, 17.89. Found C, 55.03; H; 3.60; N, 17.81.

4.1.10.7. 4-(5-(4-Chlorophenyl)-4-(2-(4-(dimethylamino)benzylidene)hydrazinyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)benzenesulfonamide **(12g)**

Yield: 70%; M.p.: 230-232 °C; IR (KBr, cm^{-1}): 3400, 3355, 3200 (NH, NH_2), 3084 (CH arom.), 2911 (CH aliph.), 1301, 1160 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 3.00 (s, 6H, $2CH_3$), 7.50 (s, 1H, pyrrole), 7.30-8.55 (m, 14H, Ar-H + SO_2NH_2), 8.25 (s, 1H, pyrimidine), 8.88 (s, 1H, N=CH), 12.30 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 40.81, 105.83, 113.80, 114.95, 120.30, 121.03, 124.60, 125.58, 127.14, 128.45, 131.48, 132.53, 137.90, 141.92, 144.37, 147.69, 157.45, 161.13, 162.14, 163.70 ppm. Anal. Calcd. for $C_{27}H_{24}ClN_7O_2S$ (546.04): C, 59.39; H, 4.43; N, 17.96. Found C, 59.51; H, 4.57; N, 18.12.

4.1.11. (*E/Z*)-Ethyl N-(4-(4-chlorophenyl)-3-cyano-1-(4-sulfamoylphenyl)-1*H*-pyrrol-2-yl)formimidate **(13)**

A mixture of compound **4** (3.72 g, 0.01 mol) and triethylorthoformate (20 mL) was refluxed for 6 h. the reaction mixture was cooled and then poured onto ice /water. The formed solid was filtered and crystallized from ethanol to give compound **13**

Yield: 80%; M.p.: 181-183 °C; IR (KBr, cm^{-1}): 3340, 3232 (NH_2), 3075 (CH arom.), 2950 (CH aliph.), 2201 (C≡N), 1330, 1160 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6, D_2O, δ): 1.04 (t, 3H, J = 7.00, 7.04 Hz, CH_3), 3.45 (q, 2H, J = 7.00, 7.04 Hz, CH_2), 7.68 (s, 1H, pyrrole), 7.47-8.04 (m, 10H, Ar-H +

SO_2NH_2), 8.54 (s, ½ H, N=CH), 8.71 (s, ½ H, N=CH) ppm. ^{13}C NMR (DMSO-d_6, δ): 14.54, 56.50, 113.99, 121.81, 125.72, 125.80, 126.27, 127.63, 128.07, 128.65, 129.54, 131.63, 132.41, 144.27, 149.10, 162.56, 162.98 ppm. Anal. Calcd. For $C_{20}H_{17}ClN_4O_3S$ (428.89): C, 56.01; H, 4.00; N, 13.06. Found C, 56.23; H, 4.19; N, 13.28.

4.1.12. 4-(3-Amino-5-(4-chlorophenyl)-4-imino-3H-pyrrolo[2,3-d]pyrimidin-7(4H)-yl)benzenesulfonamide **(14) T1**

A mixture of compound **13** (4.28 g, 0.01 mol) and hydrazine hydrate (99%, 3mL) in absolute ethanol (20 mL) was heated under reflux for 8 h. the reaction mixture was cooled and solid formed was filtered and crystallized from the appropriate solvent to give compound **14**

Yield: 75%; M.p.: 173-175 °C; IR (KBr, cm^{-1}): 3344, 3249, 3215 (NH, NH_2), 3093 (CH arom.), 1319, 1176 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 6.14 (s, 2H, NH_2, D_2O exchangeable), 7.03 (s, 1H, pyrrole), 7.96 (s, 1H, pyrimidine), 7.42-8.50 (m, 11H, Ar-H + NH + SO_2NH_2). ^{13}C NMR (DMSO-d_6, δ): 114.84, 118.09, 121.82, 126.68, 127.97, 129.37, 131.74, 132.51, 133.07, 136.85, 140.05, 143.39, 149.01, 160.15. Anal. Calcd. For $C_{18}H_{15}ClN_6O_2S$ (414.87): C, 52.11; H, 3.64; N, 20.26. Found C, 52.39; H, 3.81; N, 20.12.

N-(4-(4-Chlorophenyl)-3-cyano-1-(4-sulfamoylphenyl)-1H-pyrrol-2-yl) acetamide **(15)**

A solution of compound **4** (3.72 g, 0.01 mol) in acetic anhydride (20 mL) was refuxed for 5 min. the reaction mixture was concentrated and the solid separated was crystallized from ethanol to give compound **(15)**

Yield: 80%; M.p.: 170-172 °C; IR (KBr, cm^{-1}): 3447, 3350, 3260 (NH, NH_2), 3101 (CH arom.), 2970 (CH aliph.), 2226 (CN), 1730 (C=O), 1331, 1163 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 2.51 (s, 3H, CH_3), 7.57 (s, 1H, pyrrole), 7.53-8.02 (m, 10H, Ar-H + SO_2NH_2), 10.24 (s, 1H, NH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 22.84, 114.52, 115.63, 121.75, 123.50, 125.81, 127.56, 129.62, 130.82, 131.44, 134.80, 138.61, 139.74, 145.02, 172.37 ppm. Anal. Calcd. For. $C_{19}H_{15}ClN_4O_3S$ (414.87): C, 55.01; H, 3.64; N, 13.50. Found C, 55.30; H, 3.76; N, 13.72.

4.1.13. 4-(5-(4-Chlorophenyl)-2-methyl-4-oxo-3H-pyrrolo[2,3-d]pyrimidin-7(4H)-yl)benzenesulfonamide **(16)**

A solution of compound **4** (3.72 g, 0.01 mol) in acetic anhydride (20 mL) was refuxed for 24 h. the reaction mixture was concentrated and the solid separated was crystallized from dioxane to give compound **16**

Yield: 75%; M.p.: 166-168 °C; IR (KBr, cm^{-1}): 3450, 3300, 3214 (NH, NH_2, OH), 3090 (CH arom.), 2910 (CH aliph.), 1728 (C=O), 1369, 1163 (SO_2) cm^{-1}. ^{1}H NMR (DMSO-d_6, D_2O, δ): 2.45 (s, 3H, CH_3), 7.60 (s, 1H, pyrrole), 7.49-8.12 (m, 10H, Ar-H + SO_2NH_2), 10.27, 12.27 (s, 1H, NH/OH, D_2O exchangeable) ppm. ^{13}C NMR (DMSO-d_6, δ): 21.54, 114.45, 119.50, 122.35, 124.50, 125.44, 128.38, 129.51, 131.36, 132.94, 134.06, 139.16, 140.63, 146.97, 172.35 ppm. Anal. Calcd. For. $C_{19}H_{15}ClN_4O_3S$ (414.87): C, 55.01; H, 3.64; N, 13.50. Found C, 55.27; H, 3.81; N, 13.76.

4.2. Biological evaluation

4.2.1. Carbonic anhydrase inhibition assay

The enzyme inhibition assays of human Carbonic anhydrase (hCA) isoforms I, II, IX and XII were done at Neurofarba Department, University of Florence, Italy. To determine the activity of CA mediated CO_2 hydration, an Applied Photophysics stopped flow instrument has been utilized. The absorbance (λ_{max}; 557 nm) of the color intensity obtained from a solution containing indicator 0.2 mM (phenol red), buffer 20 mM (Hepes) to maintain pH 7.5, and Na_2SO_4 20 mM was measured to determine the initial rates of the CA-catalyzed CO_2 hydration reaction. To analyze the kinetic parameters and inhibition constants, the CO_2 concentrations were adjusted between 1.7 and 17 mM. To calculate the initial velocity, six traces of the first 5-10 % of the reaction progress were measured for each inhibitor. Determination of the uncatalyzed rates was carried out by employing the same procedures and deducted from the total rates. Stock solution of tested compound (0.01 mM) was dissolved in distilled-deionized water and then diluted with the assay buffer to 0.01 nM. The formation of E-I complex was reached through incubating the tested compound and the enzyme at room temperature or 4 ^{o}C before the assay. Enzyme and inhibitor were incubated for 15 min then data were collected. PRISM 3 was utilized to measure the mean from three determinations of inhibition constants. All CA isoforms were recombinant ones obtained in-house [55-62].

4.2.2. *In vitro* anticancer activity

Anticancer activity was carried out at the Confirmatory Unit, VACSERA, Cairo, Egypt. The *in vitro* anticancer activity of the newly synthesized compounds was evaluated against HeLa employing Acetazolamide as the positive standard drug according to MTT method [63-66]. The MTT system is a method for measuring the activity of the living cells through mitochondria dehydrogenases [67]. MTT assay based on using (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide) or MTT which is water soluble yellow dye. Upon adding MTT on viable cells, mitochondrial dehydrogenases cleave the tetrazolium solution, thus they are dissolved in acidified isopropanol to yield purple solution. The purple solution was measured spectrophotometrically so as to estimate the toxicity degree caused by the test material [68].

4.2.2.1. Cell culture protocol

Cervical Cells (HeLa) were obtained from American Type Culture Collection, cells were cultured using Dulbecco's modified Eagle's medium (DMEM) (Invitrogen/Life Technologies) supplemented with 10% fetal bovine serum (FBS) (Hyclone), 10 ug/ml of insulin (Sigma-Aldrich), and 1% penicillin-streptomycin. All of the other chemicals and reagents were purchased from Sigma-Aldrich, or Invitrogen. The culture medium was transferred to a centrifuge tube. In order to remove any traces of serum, the cell layer was washed with 0.25% (w/v) Trypsin-0.53 mM EDTA solution. Trypsin EDTA solution 2.0 to 3.0 mL was added and cells were examined under an inverted microscope until cell layer was dispersed (5 - 15 min). Complete growth medium 6.0 to 8.0 mL was added and cells were aspirated by gentle pipetting. The cell suspension in addition to the medium and cells from previous step was centrifuged (5 to 10 min) at 125 xg. The supernatant was thrown out then fresh growth medium was added to the cell pellet and the cell suspension were transfered to new culture vessels. To induce hypoxia, 25mM stock solution was prepared in sterile water (prepared immediately before use). $CoCl_2$ was used at the final concentration of 100 µM in the regular cell culture media. Cultures were Incubated for 24 hrs at 37°C. Cells were treated with serial concentrations of the test compounds and AZA then incubated for 48 h at 37ºC then proceeded for the MTT assay.

4.2.2.2. MTT cytotoxic assay protocol:

Cells were plated in a volume of 100µl complete growth medium (cells density 1.2 – 1.8 × 10,000 cells/well) and 100 µl of the tested compound per well in a 96-well plate for 24 hours before

the MTT assay. Cultures from incubator were removed into laminar flow hood or other sterile work area. Each vial of MTT [M-5655] to be used was reconstituted with 3 ml of medium or balanced salt solution without phenol red and serum. Reconstituted MTT was added in an amount equal to 10% of the culture medium volume. Cultures were incubated for 2-4 hours depending on cell type and maximum cell density. MTT Solubilization Solution [M-8910] was added to cultures to dissolve the resulting formazan crystals and dissolution was enhanced by mixing in gyratory shaker. Moreover, trituration was helpful for complete dissolution. ROBONIK P2000 was used to measure the color intensity at wavelength of 450 nm. To draw the survival curve for HeLa cell line after specified time, surviving fraction was plotted versus the drug concentration. The half maximal inhibitoy concentration (IC_{50}) was calculated to the test compounds and the reference drug AZA. The surviving fractions were expressed as means ± S.E.M.

4.3. Molecular modeling and docking

All the molecular modeling studies were performed using Molecular Operating Environment (MOE, 2015.10) software. The partial charges were calculated automatically. All minimizations were performed with MOE until an RMSD gradient of 0.05 kcal/molÅ with MMFF94x force field. The X-ray crystallographic structure of CAIX co-crystalized with acetazolamide (PDB ID: 3IAI) was downloaded from the protein data bank available at the RCSB Protein Data Bank, http://www.rscb.org. For each co-crystallized enzyme; water molecules and ligands which are not involved in the binding were removed. Protonate 3D protocol in MOE with its default options was used to prepare the protein. The co-crystalized ligand (acetazolamide) was used to define the binding site for docking. The method used for docking was Triangle Matcher placement with scoring function London dG. The interactions of ligand with the amino acids of the active site of CA IX enzyme were studied. Only one pose was selected for each compound and the selection was based on the number of interactions with the enzyme, docking score, superposition with AZA (the original ligand) and the lengths of H-bonds formed (Table 3).

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Highlights:

- Design and synthesis of pyrrolo and pyrrolopyrimidine sulfonamides.
- *In vitro* screening of carbonic anhydrase inhibition activity against hCA I, II, IX and XII isoforms.
- Cytotoxic activity against HeLa cells under normal and hypoxic conditions
- Molecular docking within the active site of hCA IX isoform.

List of abbreviations

AZA	Acetazolamide
CAI	Carbonic anhydrase inhibitors
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
Gln	Glutamine
hCA	Human carbonic anhydrase
IC_{50}	Inhibitory concentration 50%
Leu	Leucine
MMFF94x	Merk Molecular Force Field
MOE	Molecular Operating Environment
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide
PDB	Protein data bank
pHi	Intracellular pH
RCSB	Research Collaboration for Structural Bioinformatics
RMSD	Relative Mean Square Deviation
SI	Selectivity index
Thr	Threonine
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
Trp	Tryptophan

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: