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RESEARCH ARTICLE

Anticancer Activity and *In Silico* ADMET Properties of 2,4,5-Trisubstitutedthiazole Derivatives

Bilal A. Al-Jaidi[1,*], Soha Taher Telfah[2], Sanaa K. Bardaweel[3], Pran Kishore Deb[2,*], Pobitra Borah[4], Kathari-gatta N. Venugopala[5,6], Yazan A. Bataineh[2] and Qutaiba Ahmed Al Khames Aga[2]

[1]Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Yarmouk University, P. O. Box - 566, Irbid (21163), Jordan; [2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Philadelphia University, P.O BOX (1), Amman (19392), Jordan; [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Jordan, Amman 11942, Jordan; [4]Pratiksha Institute of Pharmaceutical Sciences, Chandrapur Road, Panikhaiti, Guwahati-781026, Assam, India; [5]Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Ahsa 31982, Kingdom of Saudi Arabia; [6]Department of Biotechnology and Food Technology, Durban University of Technology, Durban 4001, South Africa

Abstract: ***Background*:** Recently, a series of **15** compounds with 2,4,5-trisubstitutedthiazole scaffold having 2-amino/amido/ureido functional groups attached with 5-aryl and 4-carboxylic acid/ester groups (**1-15**) were reported from our research group as novel potential inhibitors of carbonic anhydrase III (CA III) enzyme. Several research studies revealed the potential role of CA inhibitors as anticancer agents, giving us the impetus to further explore these compounds for their potential as anticancer agents.

***Objectives*:** The objective of this study is to investigate the potential of 2,4,5-trisubstitutedthiazole derivatives (**1-15**) for their possible cytotoxic activity (*in vitro*), and to calculate (*in silico*) the absorption, distribution, metabolism, excretion and toxicity (ADMET) properties to evaluate the drug-likeness of these compounds.

***Methods*:** Cytotoxic activity (*in vitro*) was carried out on two breast cancer cell lines (MCF7 and MDA231), and the lymphoblastoid human erythroleukemia cell line (K562) using 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT) assay. Doxorubicin was used as a positive control. ADMET properties were calculated (*in silico*) using the QikProp module of Schrodinger.

***Results*:** Compounds **6** and **9** with a phenylureido group at 2-position, and a methyl-carboxylate moiety at 4-position having *para*-tolyl and benzyl moiety, respectively at the 5-position of the thiazole ring showed significant cytotoxicity against all the three cell lines. In particular, compound **6** with *para*-tolyl group at 5-position exhibited the most potent inhibitory effect on the viability of MCF7, MDA231 and K562 cells, with IC_{50} values of 22, 26 and 11 μM, respectively. Notably, all the highly active compounds possess a phenyluriedo group at 2--position with a methyl ester group at 4-position, indicating the probable role of these substituents in the target interaction and inducing cytotoxicity. Interestingly, compounds **1-4** and **10-13** with a free amino group at 2-position did not show any cytotoxic effect on the K562 cell line, while exhibiting mild to moderate cytotoxicity against the MCF7 and MDA231 cell lines. However, none of the tested compounds showed any activity against normal human dermal fibroblast cells indicating the safety/tolerability of the examined concentrations. Furthermore, these compounds also exhibited satisfactory ADMET properties (*in silico*), without violating Lipinski's rule of five.

***Conclusion*:** The most active compounds **6** and **9** predicted to have good oral absorption and low human serum protein binding, exhibiting no reactive functional group and probable CNS activity compared with 95% of the known oral drugs as predicted (*in silico*) by QikProp. Thus, compounds **6** and **9** can be considered as lead molecules for further modification and discovery of novel anticancer agents with nanomolar potency.

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1. INTRODUCTION

Carbonic anhydrases (CAs, EC4.2.1.1) are ubiquitous metalloproteinases that mainly catalyse the zinc-dependent hydration of carbon dioxide (CO_2) to produce bicarbonate and a proton, and thus maintain the regulation of cellular pH homeostasis and ion transport [1-3]. Based on tissue distribution, subcellular localization and enzyme kinetics, at least 15 human isoforms have been reported to date [4, 5]. Of them, five cytosolic (CAs I, II, III, VII, XII-I), four membrane bound (CAs IV, IX, XII, XIV), one mitochondrial (CA V) and secreted isozyme (CA VI) are catalytically active; and the three CA-related proteins (CAs VIII, X, XI) are inactive [1, 6]. Unlike the widely distributed isoforms I and II, carbonic anhydrase III (30kDa) is abundantly found in metabolically active tissues like slow-twitch skeletal muscles, adipocytes, and liver while a small quantity is distributed in other tissues [7-12]. In fact, being

* Address correspondence to these authors at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Yarmouk University, P. O. Box - 566, Irbid (21163), Jordan; E-mail: bilal.aljaidi@yu.edu.jo
Department of Pharmaceutical Sciences, Faculty of Pharmacy, Philadelphia University, P.O BOX (1), Amman (19392), Jordan; E-mails: prankishore1@gmail.com, pdeb@philadelphia.edu.jo

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the slowest catalyst for hydration of CO_2, it is the least understood and explored isoform as compared to the other cytosolic isozymes [13-15]. Except for the antioxidant activity [15-17], the role of CA III in various physiological and pathological conditions is very poorly understood. Recently, angiotensin converting enzyme inhibitors were also found to exhibit inhibition properties against CA III enzyme [18]. However, CA III has been demonstrated to be associated with tumour progression and carcinogenesis [19-22]. It is reported that overexpression of CA III lowers both the extracellular and intracellular pH, which may further activate the focal adhesion kinase (FAK) pathway and promote cell invasive ability of hepatocellular carcinoma (HCC) cells [19]. Interestingly, a recent study also established a similar correlation of CA III with the development of oral cancer, and reported that CA III overexpression promotes epithelial mesenchymal transition and invasive abilities of the oral cancer cells by modulating the FAK/Src pathway [20]. Thus, in continuation to our effort to assess the pharmacological activity of our recently reported novel 5-substituted-2-uriedo- and 2-amidothiazole-4-carboxylate derivatives as CA III inhibitors [23], the current study was designed to further investigate the cytotoxic activity of these compounds on three cancer cell lines, including two human breast cancer (MCF7 and MDA231) and one lymphoblastoid human erythroleukemia cell line (K562).

2. MATERIALS AND METHODS

2.1. Cytotoxicity Assay (MTT Assay)

2.1.1. Cell Lines

Human breast cancer cell lines MDA231 and MCF7, the erythroleukemia K562 cells, and the normal human dermal fibroblast cells were purchased from the American type culture collection (ATCC, USA), and maintained in the DMEM culture medium supplemented with 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 2 mM L-glutamine.

2.1.2. MTT Assay

The 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT) assay was used to evaluate cell proliferation as previously described by Bardaweel *et al.* (2014) [26]. Cells were seeded in a 96-well plate at a seeding density of 8000 cells per 100 µL culture medium, and then incubated at 37ºC for 24 h. Dimethyl sulfoxide (DMSO) was used to dissolve and prepare the stock solutions of the examined compounds. In all wells, the percentage of DMSO never exceeded 1%. Negative control was untreated cells growing in media with DMSO added in the same % as in the test well.

After that, cells were treated with variable concentrations of the examined compounds (0-1000 µM). After 48 h of treatment, cells were incubated with 5 mg/mL of MTT dyes for 3-4 h. The supernatants were removed, and 100 µL of DMSO was added to each well to dissolve formazan crystal. The optical density (OD) was determined at 570 nm utilizing a microplate reader. The proportion of relative cell survival of treated cells against untreated cells (control) was calculated according to the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Optical density of cells treated}}{\text{Optical density of cells untreated}} \times 100$$

2.1.3. Statistical Analysis

Data analysis was performed using the GraphPad Prism software version 7. The differences between treatment groups were determined by *t*-test or one-way analysis of variance (ANOVA) followed by Tukey post hoc t-test as appropriate. Data were expressed as mean ± SD and $p < 0.05$ was considered a statistically significant difference. A non-linear regression analysis was used to calculate IC_{50} values.

2.2. ADME Properties Calculation (*In silico*)

The ADME properties of compounds **1-15** were calculated *in silico* (in fast mode) with default options using the QikProp v5.2 module of Schrödinger (2017-2) by following our previously published protocol [27-32]. QikProp not only predicts the physically significant descriptors and pharmaceutically relevant properties of any organic molecule but also provides ranges for comparing a particular molecule's properties with those of 95% of known drugs that are available in the market.

3. RESULTS AND DISCUSSION

3.1. Cytotoxicity Assay (MTT assay)

Recently, we have reported a series of 15 5-substituted-2-uriedo- and 2-amidothiazole-4-carboxylate derivatives as promising CA III inhibitors [23]. All the 15 compounds were screened for their cytotoxic potential against two human breast cancer cell lines (MCF7 and MDA231), and the lymphoblastoid human erythroleukemia cell line (K562) by using doxorubicin as a positive control. In the MTT assay (Table **1**), compounds **6, 8** and **9** with a phenylureido group at 2-position and methyl-carboxylate moiety at 4-position having aromatic moiety at the 5-position of the thiazole ring showed good cytotoxicity against all the three cell lines. In particular, compound **6** with *para*-tolyl group at 5-position exhibited potent inhibitory effect on the viability of MCF7, MDA231 and K562 cells, with IC_{50} values of 22, 26 and 11 µM, respectively. Replacement of the *para*-tolyl group with a benzyl group at 5-position of the scaffold resulted in a little drop in activity against all the three cell lines for compound **9** (IC_{50} MCF7 = 35 µM, MDA231 = 44 µM and K562 = 22 µM). A representative dose-response curve for anticancer activity of compound **9** tested against MCF7 cell line is presented in Fig. **1**. Introduction of the bulky *para*-methyl-sulfonyl group at 5-position of compound **8** further reduced the activity (IC_{50} MCF7 = 100 µM, MDA231 = 157 µM and K562 = 105 µM). Interestingly, the replacement of methyl-ester group with a carboxylic group at 4-position drastically reduced the activity for compound **14** (IC_{50} MCF7 = 692 µM, MDA231 = 244 µM and K562 = 237 µM) as compared to compound **6**, indicating the significant role of ester group in inducing cytotoxic effect.

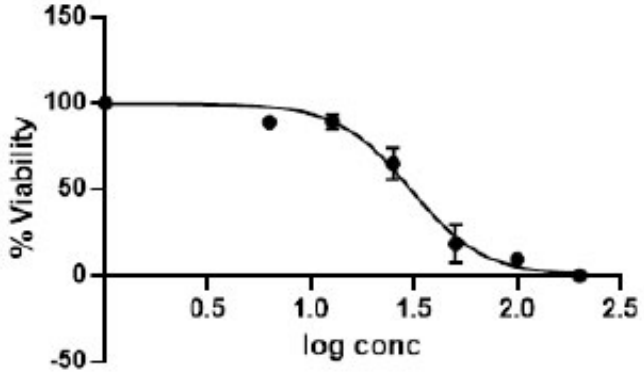

Fig. (1). A representative dose-response curve for anticancer activity of compound 9 tested against MCF7 cell line.

Table 1. The cytotoxic activity of the compounds (1-15) on breast cancer (MCF7 and MDA231) and lymphoblastoid human erythroleukemia (K562) cell lines. SD did not exceed 5%.

Compound* No.	R_1	R_2	IC_{50} (μM)		
			MCF7	MDA231	K562
1	$-C_6H_5$	H	582	248	> 1000
2	$-p-CH_3-C_6H_4$	H	739	539	> 1000
3	$-p-SO_2CH_3-C_6H_4$	H	434	121	> 1000
4	$-CH_2-C_6H_5$	H	572	256	> 1000
5	$-C_6H_5$	$-p-OCH_3-C_6H_4CO$	309	207	123
6	$-p-CH_3-C_6H_4$	$-C_6H_5NHCO$	22	26	11
7	$-p-SO_2CH_3-C_6H_4$	$-p-NO_2-C_6H_4CO$	213	204	> 1000
8	$-p-SO_2CH_3-C_6H_4$	$-C_6H_5NHCO$	100	157	105
9	$-CH_2-C_6H_5$	$-C_6H_5NHCO$	35	44	22
10	$-C_6H_5$	H	411	212	> 1000
11	$-p-CH_3-C_6H_4$	H	416	922	> 1000
12	$-p-SO_2CH_3-C_6H_4$	H	938	982	> 1000
13	$-p-Cl-m-NO_2-C_6H_3$	H	382	717	> 1000
14	$-p-CH_3-C_6H_4$	$-C_6H_5NHCO$	528	739	> 1000
15	$-p-SO_2CH_3-C_6H_4$	$-p-NO_2-C_6H_4CO$	692	244	237
Doxorubicin	-	-	3	8	12

*The chemistry, synthesis and characterization data of all the compounds, including their CA-III activity, have been recently reported from our lab (Bilal *et al.*, *J. Enzyme Inhib. Med. Chem.*, **2020**, *35* (1), 1483–1490).

Table 2. Important ADMET properties of compounds 1-15 predicted (*in silico*) by QikProp.

Comp. No.	#rtvFG	CNS	SASA	donorHB	accptHB	QPlogPo/w	QPPCaco	QPlogBB	#metab	QPlogKhsa	% Human Oral Absorption	Rule of Five
1	0	0	468.948	2	4	1.67	611.851	-0.613	1	-0.178	86.598	0
2	0	0	500.514	2	4	2.012	613.017	-0.646	2	-0.038	88.618	0
3	0	-2	555.264	2	8	0.624	108.026	-1.529	1	-0.442	66.997	0
4	0	0	488.392	2	4	2.199	689.535	-0.692	2	-0.142	90.625	0
5	0	0	674.291	1	6.75	3.489	1160.107	-0.636	2	0.24	100	0
6	0	-1	697.574	1	4.5	4.527	744.42	-0.899	3	0.762	100	0
7	0	-2	764.206	0	9	1.959	19.013	-3.024	2	-0.214	61.306	0
8	0	-2	750.649	1	8.5	2.925	133.903	-1.903	2	0.171	82.136	0
9	0	-1	682.337	1	4.5	4.486	771.712	-0.972	3	0.656	100	0
10	0	-1	417.481	3	4	1.278	55.039	-0.929	1	-0.575	65.585	0
11	0	-1	449.713	3	4	1.552	55.009	-0.981	2	-0.452	67.18	0
12	0	-2	504.506	3	8	0.276	9.722	-1.835	1	-0.726	46.238	0
13	0	-2	471.761	3	5	1.159	8.642	-1.627	2	-0.475	50.496	0
14	0	-2	645.912	3	5.5	2.808	46.079	-1.27	3	-0.205	73.163	0
15	0	-2	712.259	2	11	1.101	2.152	-3.173	2	-0.535	39.345	0

#rtvFG = Number of reactive functional groups. The presence of these groups can lead to false positives in HTS assays and to decomposition, reactivity or toxicity problems *in vivo* (recommended values 0–2); **CNS** = Predicted central nervous system activity [recommended values -2 (inactive) to 2 (active)]; **SASA** = Total solvent accessible surface area (SASA) in square angstroms using a probe with 1.4 Å radius (recommended values 300.0–1000.0); **donorHB** = Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (recommended values 0-6); **acceptHB** = Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (recommended values 2-20); **QPlogPo/w** = Predicted octanol/water partition coefficient (recommended values -2 to 6.5); **QPPCaco** = Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport. (< 25 poor and > 500 great); **QPlogBB** = Predicted brain/blood partition coefficient (recommended values −3 to 1.2); **#metab** = Number of likely metabolic reactions; range 95% of drugs (1-8); **QPlogKhsa** = Prediction of binding to human serum albumin (recommended values −1.5 to 15); **% Human Oral Absorption** = Predicted human oral absorption on 0–100% scale (recommended values <25% is poor and >80% is high). **Rule of Five** = Indicates the number of violations of Lipinski's rule of five. The rules are: MW < 500, QPlogPo/w < 5, donorHB < 5, accptHB < 10. Compounds that satisfy these rules are considered drug-like (maximum is 4).

However, among the compounds with a methyl ester group at the 4-position, compound **5** and **7** with *para*-methoxybenzamido and *para*-nitrobenzamido group, respectively, at the 2-position of the scaffold, showed moderate cytotoxic effect on all the three cell lines, further indicating the probable advantageous role of the uredo group as compared to the benzamido group in inducing cytotoxicity. Moreover, the decreased activity of compound **15** (particularly against MCF7 cell line) with a *para*-nitrobenzamido group at 2-position and a *para*-methylsulfonyl phenyl group at 5- position also indicates the important role of having a methyl ester group at 4--position and a phenyl-uredo group at 2-position of the scaffold for inducing cytotoxicity. In case of K562 cell line, only compounds **6** and **9** were found to be the most active whereas compounds **5**, **8** and **15** showed moderate activity. Although, compounds **1-4** and **10-13** with a free amino group at 2-position exhibited mild to moderate toxicity against MCF7 and MDA231 cell lines but did not show any cytotoxic effect on the lymphoblastoid human erythroleukemia (K562) cell line. Evidently, these compounds on human dermal fibroblast cells did not result in any significant growth reduction indicating the safety/tolerability of the examined concentrations.

3.2. ADME Properties Calculation (*In silico*)

Prediction of pharmacokinetic properties like absorption, distribution, metabolism, excretion and toxicity (ADMET) at the early stage of drug discovery plays a crucial role in determination of drug-likeness of any compound to save time, economy and resources while increasing the success rate at the advance stage of drug development [24, 25]. The predicted (*in silico*) ADMET properties that are considered to influence the solubility, oral absorption, cell permeation, bioavailability, and plasma protein binding of compounds **1-15** were found to be in the acceptable ranges compared with 95% of the known oral drugs as predicted by QikProp (Table **2**). Interestingly, these compounds also satisfied Lipinski's rule of five. In particular, the most active compounds **6** and **9** have shown promising drug-like properties with 100% probable oral absorption.

CONCLUSION

In the present study, a series of novel 5-substituted-2-uriedo- and 2-amidothiazole-4-carboxylate derivatives (**1-15**) were investigated for their possible cytotoxic activity on two breast cancer cell lines (MCF7 and MDA231), and the erythroleukemia (K562) cell line using MTT assay. All the compounds have shown activity at a low to moderate micromolar range. However, none of these compounds showed any activity against normal human dermal fibroblast cells indicating the safety/tolerability of the examined concentrations. Compounds **6** and **9** exhibited significant cytotoxic activity against all three cell lines. Notably, all these highly active compounds possess a phenyluriedo group at 2- position with a methyl ester group at 4-position, indicating the probable role of these substituents in target interaction and inducing cytotoxicity. Furthermore, these compounds also exhibited satisfactory ADMET properties (*in silico*) without violating Lipinski's rule of five. In particular, compounds **6** and **9** predicted to have good oral absorption and low human serum protein binding, exhibiting no reactive functional group and probable CNS activity compared with 95% of known oral drugs as predicted (*in silico*) by QikProp. Thus, compounds **6** and **9** can be considered as lead molecules, and further synthesizing of different analogues with a phenyluriedo group at 2-position may lead to the discovery of novel anticancer agents with nanomolar potency.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

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