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Synthesis of New Acetazolamide Analogues Bearing Thiazole Moiety with Promising Carbonic Anhydrase Inhibitory Activity

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ABSTRACT

Objective: Breast cancer is remarkably predominant among female malignancies. Numerous targets exist for breast cancer therapy; carbonic anhydrase enzyme is one such target due to its crucial function in the tumor microenvironment. Aim: Design four new compounds based on the core scaffolds of acetazolamide and thiazole. Methodology: The synthesis process of these compounds involves four steps including: acidic amide hydrolysis, N-chloroacetylation, thiazole ring cyclization, and finally Schiff base reaction. The structures of the final products were confirmed using proton nuclear magnetic resonance spectroscopy. Furthermore, their cytotoxic activities were evaluated. Results: In vitro MTT cytotoxic assays against MCF-7 (breast cancer) and MCF-10A (normal breast) cell lines, demonstrating encouraging anticancer potential, and promising selective compounds. Conclusion: Each of the designed compounds was successfully synthesized and demonstrated both potent anticancer activity and high selectivity.

KEYWORDS: cancer, carbonic anhydrase, acetazolamide, thiazole, MTT assay.

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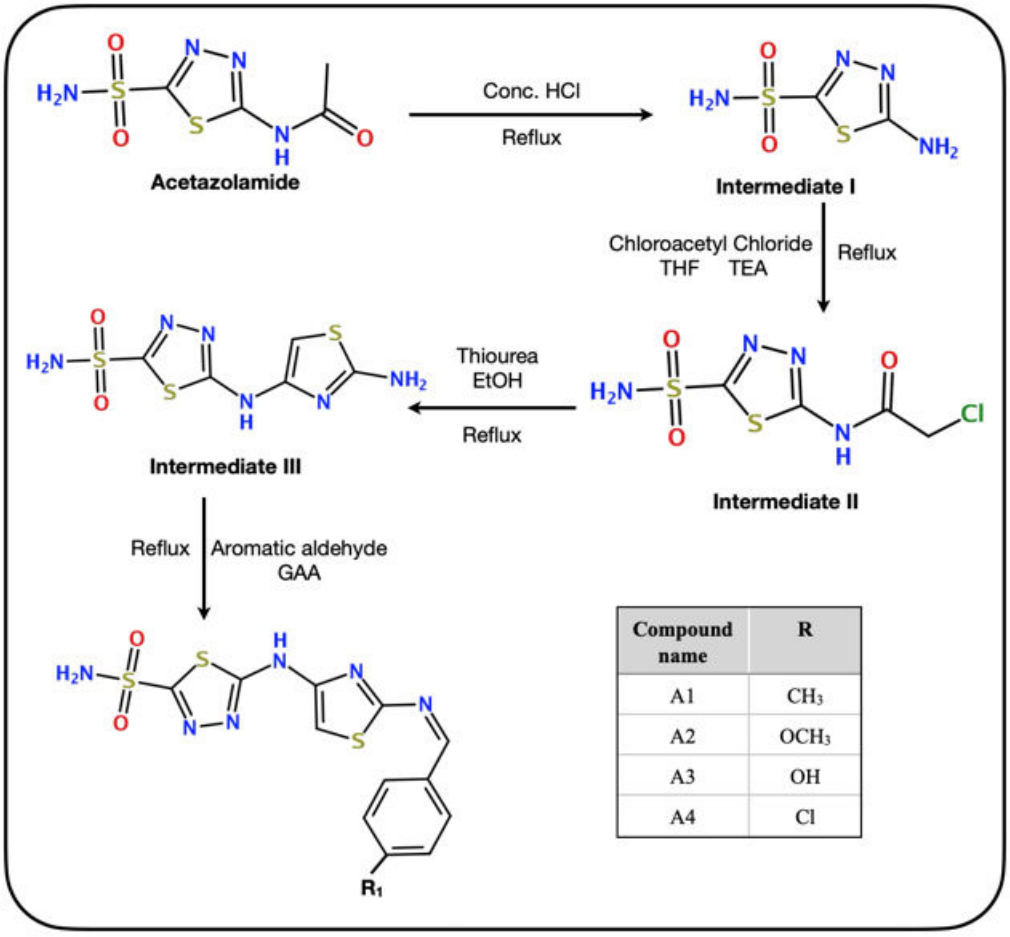
INTRODUCTION

Among female cancers, breast cancer is by far the most prevalent. In 157 of the 185 countries that count, breast cancer is the most common type of cancer in women. It killed 670,000 people around the world in 2022 [1]. Hypoxia is a characteristic that most solid tumors have, and it's linked to a fatal outcome in several types of tumors, which includes breast cancer [2]. Intratumoral hypoxia, mainly caused by structural and functional abnormalities in microvasculature, is often associated with a more aggressive phenotype, increased risk of metastasis and resistance to anti-malignancy treatments [3]. From this crucial point there is an urgent need to develop new strategies to overcome hypoxia-induced chemotherapy resistance [4]. Carbonic anhydrases are metalloenzymes which are crucial for maintaining the balance between carbon dioxide (CO2) and bicarbonate (HCO3–) in organisms. There are eight distinct basic groups of these enzymes, which are α, β, γ, δ, ζ, η, θ, and ι [5]. Among the eight distinct types of carbonic anhydrases, the alpha class attracts the greatest focus due to its significance in human pathology [6]. Sixteen distinct alpha-carbonic anhydrase isoforms have been verified, exhibiting significant variation in their cellular location and biophysical characteristics [7]. CA IX expression in several tumor types underlines its importance as a universal marker of tumor hypoxia. Furthermore, its expression is intricately linked to the prognosis of clinical outcomes in many tumor types. These facts confirm the solid promise of CA IX as a target for pharmacological therapy [8]. Human CAXII was first recognized as a cancer biomarker in several malignancies and tumors. The expression of CAXII is modulated by hypoxia and estrogen receptors. CAXII expression has been observed in various tissues, with significantly elevated levels in cancerous and tumor tissues [9]. The traditional carbonic anhydrase inhibitors (CAIs) are the sulfonamides, RSO2NH2, that have been utilized clinically for over 50 years as diuretics and systemically acting antiglaucoma agents. Approximately 30 currently utilized medications (or substances in clinical research) are classified as sulfonamides or sulfamates [10]. Acetazolamide which is an old sulfonamides limits tumor metastasis and associated expression of proteins [11-12]. In order to receive final approval as a combination therapy for solid tumors, SLC-0111, which is a CA IX and XII inhibitor, is now being tested in clinical trials (phase II) [13]. Thiazole is a heterocyclic moiety that contains sulfur and nitrogen. Naturally occurring and synthetically manufactured, it is an essential component of many compounds of medicinal value [14]. The antiparasitic, antifungal, antibacterial, and antiproliferative biological effects of thiazole compounds are well-documented [15].

MATERIALS AND METHODS

Study design

Considering the preceding introduction, new acetazolamide derivatives bearing thiazole ring moiety were constructed and synthesized to function as carbonic anhydrases IX and XII inhibitors. The synthesis process of the designed acetazolamide derivatives illustrated in scheme 1.



Compound name	R
A1	CH_3
A2	OCH_3
A3	OH
A4	Cl

Scheme 1: Chemical synthesis of the designed compounds

Chemical synthesis

Synthesis of 5-Amino-1,3,4-thiadiazole-2-sulfonamide (intermediate I)

Synthesis of intermediate I involve the reaction of 3g of acetazolamide and 20ml concentrated HCl under reflux for three hours then neutralized with NaOH, saturated with NaCl, then washed three times with 50cc THF (tetrahydrofuran). Finally dehydrate the solution by rotatory evaporator [16].

Synthesis of 2-Chloro-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide (intermediate II):

Two solutions were prepared, the first one in a round containing 20ml THF, 0.5g of intermediate I, and 0.4ml triethylamine. The second solution containing 10ml THF and 1.1ml chloroacetylchloride. The second solution added to the first one drop by drop for one hour. Then refluxed for 6hr, excess cold water was added and the precipitate was filtered and washed with diethylether [17].

Synthesis of 2-Chloro-N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide (intermediate III)

In this step two solutions were prepared too, the first one in a round containing 20ml absolute ethanol and 0.5g of intermediate II. The second solution containing 0.15 thiourea and 10ml absolute ethanol. The second solution was added slowly to the first one and reflux for 10hr, then the solution was evaporated to give intermediate III. The product was recrystallized from hot ethanol and washed with diethylether [18].

Synthesis of final compounds

One of the four benzaldehyde derivatives (1 mmol) enumerated in (Table 1) was dissolved in 10 ml of absolute ethanol. Subsequently, five drops of glacial acetic acid were introduced, and the mixture was stirred for 15 minutes. Following this, Intermediate 3 (0.3 g, 1 mmol) was dissolved in 15 ml of absolute ethanol and added to the initial solution. The mixture was then refluxed for 8-11 hours, after which the precipitate was filtered and washed with diethyl ether [19].

Table 1: Benzaldehyde derivatives used in step four with their weight and molecular weight

Compound name	R group	Aldehyde compound used		
		Name	M.Wt	Weight(g)
A1	CH3	4-methylbenzaldehyde	120	0.120
A2	OCH3	4-methoxybenzaldehyde	136	0.136
A3	OH	4-hydroxybenzaldehyde	122	0.122

A4	Cl	4-chlorobenzaldehyde	140	0.140

Methods of identification

Thin layer chromatography

Thin-layer chromatography was conducted on 0.2 mm thick aluminum plates previously coated with silica gel G60 F254 (E Merck, Germany) to assess the purity of the products and track the progress of the reaction. Compounds were disclosed following exposure to UV light. Mobile phase consist of one part of methanol and nine parts of chloroform was used [20-21].

Nuclear magnetic resonance

The 1H-NMR spectra were obtained from the Chemistry Department of the Faculty of Sciences/Basrah University /Iraq. The instrument model is the Bruker 400 MHz-Avance Neo, and the solvent utilized is DMSO.

In-vitro cytotoxicity

The two cell line, Michigan cancer foundation/cell line 7 (MCF7), and Michigan cancer foundation/10th patient specimen/sub-line A (MCF10a) were used in this study. Both are human breast cell lines, the first one, MCF7, is cancer cell line, while the other is non-tumorigenic. The MTT assay was used, which involves the conversion of the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple formazan by the action of mitochondrial reductase [22-23].

RESULTS AND DISCUSSION

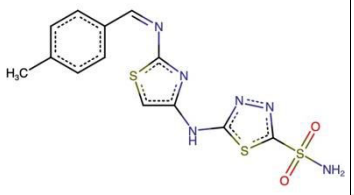
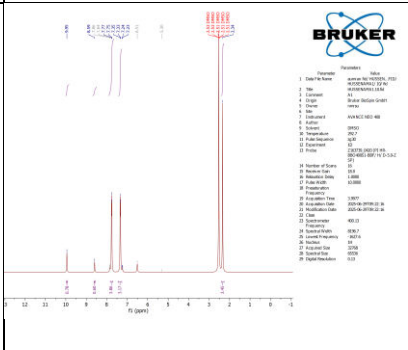
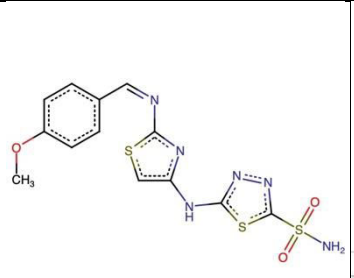
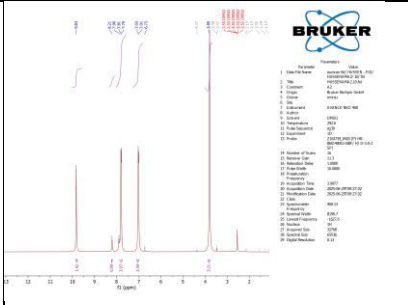
The first step of the synthesis process involves amide hydrolysis to give primary amine (intermediate I) and carboxylic acid (acetic acid), in this step sulfonamide group did not break due to its high stability [24]. In the second step primary amine converted again to amide, through the attack of the primary amine of intermediate I to the chloroacetylchloride carbonyl carbon, because that carbon is more electrophilic than the other carbon of chloroacetylchloride [25]. The next step involves thiazole ring cyclization, which is the cornerstone of this study [26]. The last step is Shiff base reaction that yield four different compounds, with four different functional group, CH_3, OCH_3, OH, and Cl. The physical properties of these compounds are illustrated in (Table 2).

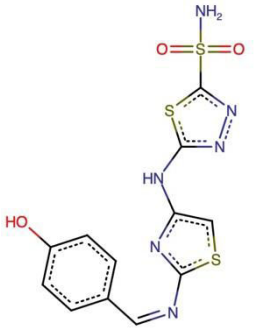
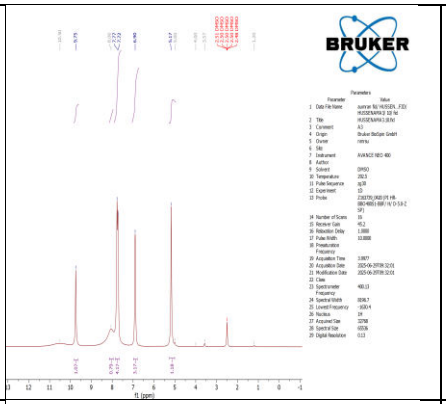
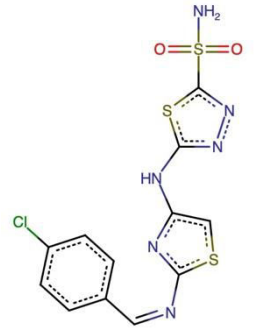
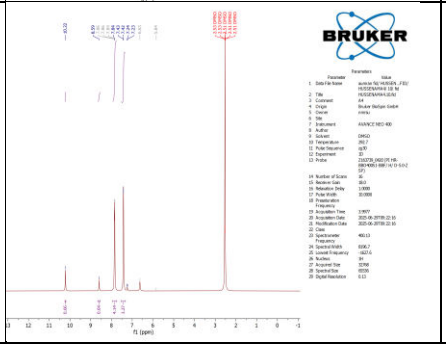
Table 2: Physical properties of the synthesized compounds and their percent of yield

Compound name	R group	Physical properties results		
		Appearance	Melting point	%yield
A1	CH3	Pale orange powder	201-203	61%
A2	OCH3	Pale orange powder	198-199	64%
A3	OH	Burgundy powder	234-235	75%
A4	Cl	Light burgundy powder	199-200	69%

These compounds subjected to 1H-NMR, the result are listed in the table below:

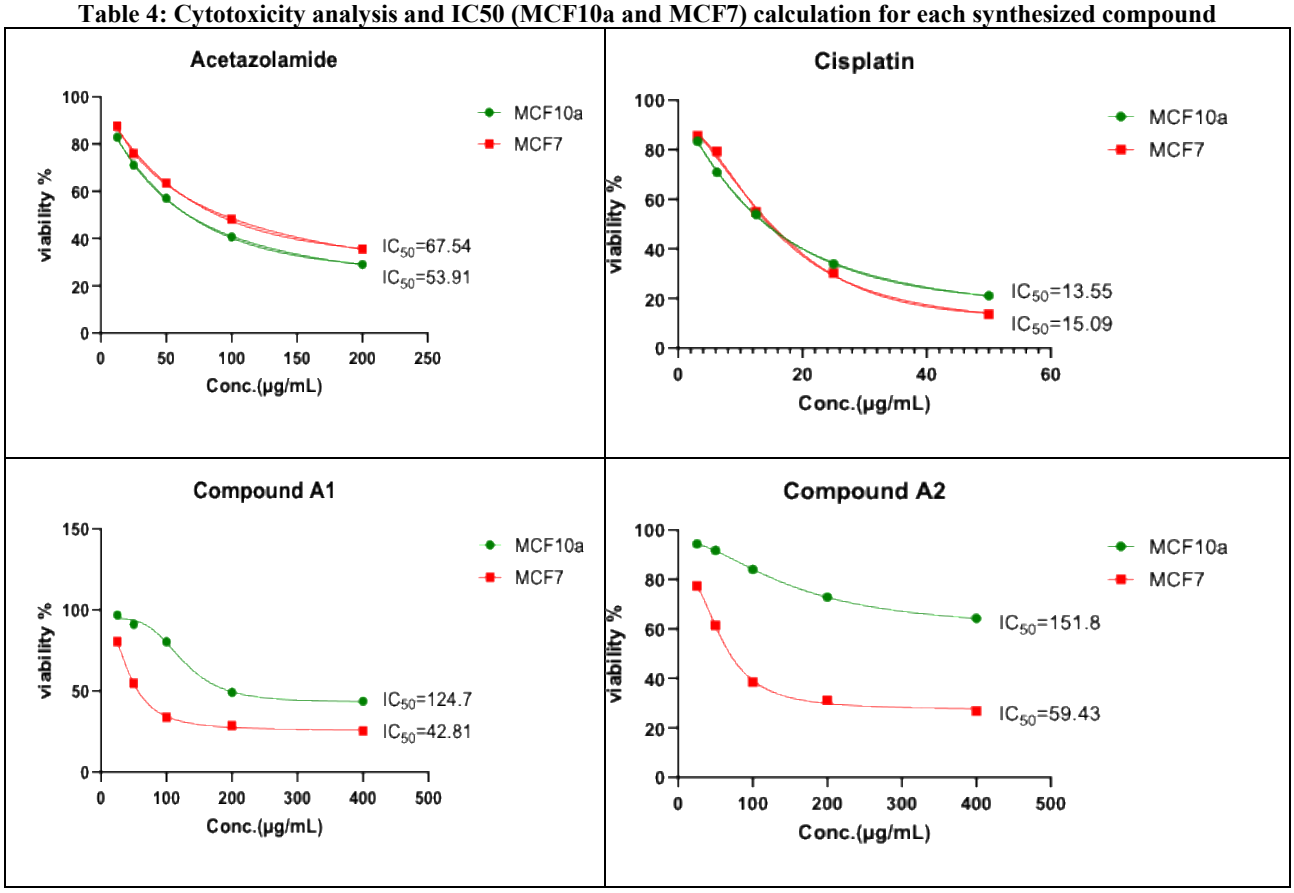
Table 3: ^{1}H-NMR spectrum of each synthesized compound and their explanation [27]

Compound name	Structure	1H-NMR result	Explanations
A1			-Band 2.34= CH3 protons -Band 7.23 to 7.35= two aromatic protons of benzene, and one proton of thiazole -Band 7.75 to 7.77= two aromatic protons of benzene, and two protons of $-NH_2$ -Band 8.59= the Schiff proton -Band 9.95= -NH proton
A2			-Band 3.8= OCH3 protons -Band 6.73 to 7.03= two aromatic protons of benzene, and one proton of thiazole -Band 7.79 to 7.9= two aromatic protons of benzene, and two protons of $-NH_2$ -Band 8.21= the Schiff proton -Band 9.83= -NH proton

A3			-Band 5.17= OH proton -Band 6.9= two aromatic protons of benzene, and one proton of thiazole -Band 7.72 to 7.77= two aromatic protons of benzene, and two protons of -NH_2 -Band 8.06= the Schiff proton -Band 9.75= -NH proton
A4			-Band 7.42 to 7.43= two aromatic protons of benzene, and one proton of thiazole -Band 7.84= two aromatic protons of benzene, and two protons of -NH_2 -Band 8.59= the Schiff proton -Band 10.22= -NH proton

Traditional cancer treatment such as cisplatin, which used as positive reference, lack selectivity and lead to resistance. The MTT assay results shown in Table 4 explain the concentration required for a 50% cell viability reduction for each compound. These results indicate optimum inhibitory effect of the designed compounds comparing with acetazolamide, and acceptable toxicity in comparison with cisplatin [28-29].

Table 4: Cytotoxicity analysis and IC50 (MCF10a and MCF7) calculation for each synthesized compound



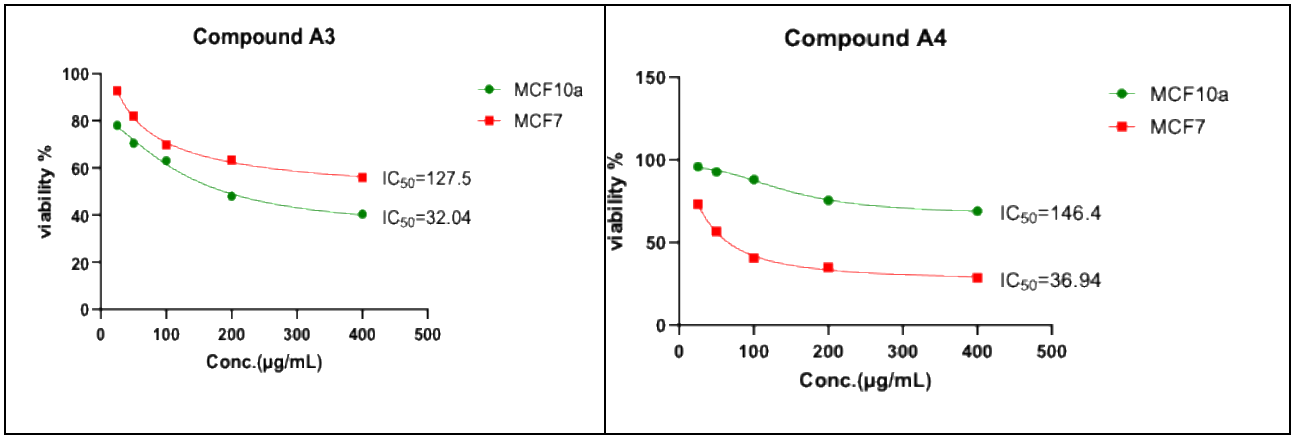


Table 5: P-value calculations for each synthesized compound [30]

Compound name	MCF10a		MCF7	
	Mean IC50	P-value	Mean IC50	P-value
Cisplatin	13.55 ±0.52	Standard(A)	15.09 ±0.77	Standard(A)
Acetazolamide	53.91 ±2.66	Standard(B)	67.54 ±2.71	Standard(B)
A1	124.7 ±7.02	0.0001**	42.81 ±2.36	0.0001**
		0.0001**		0.0046**
A2	151.8 ±9.36	0.0001**	59.43 ±2.62	0.0001**
		0.0001**		0.0498*
A3	127.5 ±7.84	0.0001**	32.04 ±1.57	0.0017**
		0.0001**		0.0001**
A4	146.4 ±8.41	0.0001**	36.94 ±1.83	0.0001**
		0.0001**		0.0001**
* (P<0.05), ** (P<0.01)				

The index of selectivity (SI) is a measurement tool to evaluate the degree of selectivity. The formula: (SI= IC50 normal cells/ IC50 cancer cells) can be employed to predict the compounds' likely selectivity for cancer cells relative to normal cells. The (Table 6) shows the index of selectivity of each synthesized compound alongside the reference compounds [31].

Table 6. SI calculations for each synthesized compound

Compound name	IC50 cancer cell	IC 50 normal cell	SI
Cisplatin	13.55	15.09	0.897
Acetazolamide	53.91	67.54	0.798
A1	124.7	42.81	2.913
A2	151.8	59.43	2.554
A3	127.5	32.04	3.979
A4	146.4	36.94	3.963

SI values approaching 1 indicate limited or no selectivity, however values equal to or beyond 3 imply superior selectivity. The results above suggest that compounds A3 and A4 have superior selectivity, compounds A1 and A2 have good selectivity, while the reference compounds cisplatin and acetazolamide have inferior selectivity [32].

CONCLUSION

This research focuses on the development of new acetazolamide analogues bearing a thiazole moiety, the H-NMR results prove that the synthesis process was successfully completed, since all the peaks are identical to each compound protons. Then the MMT results demonstrates that the synthesized compounds (A1, A2, A3, and A4) have promising anticancer activity with improve selectivity. Indicating that thiazole ring plays a significant role in binding affinity with the enzyme, providing flexibility and enhancing the likelihood of interaction with the enzyme's active site. Additionally, the influence of position and nature of benzoyl substitution on binding orientation.

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