ORIGINAL ARTICLE

CONTENTS

In Silico Study and synthesis of thiazole-based amino coumarin derivatives with promising carbonic anhydrase inhibitory activity

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ABSTRACT

Aim: To develop and assess four newly synthesized compounds designed to target carbonic anhydrases present in solid tumors.

Materials and Methods: The chemical synthesis was done using 6-Aminocoumarin, chloro acetyl chloride, triethylamine, thiourea, glacial acetic acid, and aromatic aldehyde derivatives. To investigate cytotoxic activity, we performed docking simulations with the 2015.10 Molecular Operating Environment program and used the MTT technique.

Results: Compounds IIIc and IIIb showed strong cytotoxicity against tumor cells (IC_{50} = 17.67 and 21.57 μM) and lower toxicity toward normal cells (IC_{50} = 86.63 and 135.01 μM), indicating better selectivity than cisplatin. Docking results revealed higher binding affinity than acetazolamide, due to effective interactions with key amino acids. The substituted thiazole ring also enhanced molecular flexibility and receptor binding.

Conclusions: This study evaluated newly synthesized aminocoumarin–thiazole hybrid compounds as potential anticancer agents against breast cancer. Compounds IIIa–d showed cytotoxic activity with IC_{50} values of 17.67–42.63 μM, with IIIc and IIIb being the most potent. In silico results supported their strong binding potential. These compounds demonstrated lower toxicity in normal cells compared to cisplatin, indicating selective action against cancer cells. Therefore, IIIc and IIIb may serve as promising candidates for breast cancer treatment, although further in vivo studies are required.

KEYWORDS: carbonic anhydrase inhibitors, 6-Aminocoumarin, Antineoplastic, *in silico* study

Wiad Lek. 2026;79(9):2034-2045. doi: 10.36740/WLek/226777 DOI

INTRODUCTION

Cancer is a complex group of diseases characterized by the unchecked growth and spread of abnormal cells. These cells are capable of producing tumors, invading nearby cells, and spreading to other parts of the body [1, 2]. Numerous new diagnoses are made each year, and it remains a major cause of death worldwide, making it an important global health concern [3, 4]. More than 19.3*10^6 persons worldwide were diagnosed with cancer in 2020, according to the World Health Organization, and 10*10^6 of them died from the disease [5, 6]. At the start of the century, effective cancer management appears to be a major issue. This challenge stems from the struggle to discover novel targeted therapies that can inhibit cancer cell multiplication without harming healthy cells [7]. The focus of current scientific efforts is on elucidating the fundamental mechanisms behind cancer development and identifying potential therapeutic targets [8]. Because it has a significant impact on tumor growth, researchers are focusing progressively more on understanding the tumor microenvironment [9]. The presence of hypoxia in the TME around the tumor is induced by the increased oxygen requirements of rapidly reproducing malignant cells [10]. Moreover, highly proliferating cancer cells use anaerobic glycolysis and oxidative phosphorylation of glucose to produce energy. To maintain an alkaline pH and prevent intracellular H' ion accumulation, this metabolic pathway creates considerable volumes of metabolic acid, which include many protein complexes [11]. The cell surface enzymes known as carbonic anhydrases (CAs) are increased in hypoxic environments. These zinc metalloenzymes, which are members of the α-CA family, contribute to the reversible hydration of carbon dioxide into proton and bicarbonate ion [12]. The α-family comprises 15 identified isozymes of human carbonic anhydrases (hCAs). These isoforms differ in their susceptibility to different kinds of modulators, cellular location, protein structure, and catalytic activity [13]. The metabolic and pH-regulating machinery of tumor cells is considerably improved by hCA XII and IX, which are mostly prevalent in hypoxic cancers and stimulate

tumor cell proliferation [14]. As a result, research has concentrated on creating inhibitors that specifically target this enzyme as a means of overcoming medication resistance to chemotherapy [15-17]. The coumarin molecule is one of the most significant inhibitors of CA. Coumarins (2H-chromen-2-one) are a diverse family of heterocyclic compounds that contain a benzopyrone moiety. This moiety is formed by the fusion of an α-pyrone ring with a benzene ring. Coumarins can be derived from both natural and synthetic sources [18]. Coumarin and its derivatives show a fascinating activity of pharmacological properties such as anticancer [19], antifungal [20, 21], antibacterial [22], and antimalarial [23, 24]. The antineoplastic activity of coumarins is due to the ability of these substances to inhibit DNA repair enzymes, induce cell cycle inhibition, induce cell death, and reduce angiogenesis [25, 26]. The biological activities of five-membered heterocyclic compounds, particularly those that contain multiple heteroatoms, are diverse in the field of medicinal chemistry [27]. Thiazole is a heterocyclic molecule with the chemical formula C_3H_3NS, comprising both nitrogen and sulfur. Thiazole rings are aromatic and planar [28]. Its high reactivity, caused by the acidic proton at C-2, makes it a desirable precursor for creating a huge number of novel chemicals. Thiazole derivatives' chemical, physical, and pharmacological features have long fascinated synthetic and biological scientists. Numerous novel compounds with distinct therapeutic potentials have been generated by modifying the thiazole ring at various places, including antioxidants, antineoplastics, antibiotics, diuretics, and anti-inflammatory medications [29]. Because of their diverse pharmacological potential, coumarin–thiazole analogues exhibit a wide range of biological activities, including anti-inflammatory [30]. Anticancer [31]. Antioxidant [32] and carbonic anhydrase inhibitors [33].

AIM

This study focuses on the development and evaluation of four newly synthesized compounds formulated to inhibit carbonic anhydrase isoforms commonly expressed in solid tumor cells. The primary focus is on the objective to develop potent anti-cancer medications through chemical synthesis using 6-aminocoumarin, chloroacetyl chloride, triethylamine, thiourea, glacial acetic acid, and aromatic aldehyde derivatives.

MATERIALS AND METHODS

The anhydrous solvents and reagents were supplied by companies such as Merck, Riedel-de Haën, Sigma-Aldrich, and Hangzhou Hyper Chemicals. The melting temperatures were determined using the Thomas Hover device and capillary tube technique. TLC was used to confirm the reaction procedures; the resulting chemicals were purified, and their retention factors (Rf) were calculated. A combination of 8:2 chloroform and methanol, as well as 3:2 ethyl acetate and hexane, was used as the mobile phase. FT-IR scanning and spectral analysis were performed using a Shimadzu spectrophotometer. KBr discs were created at Ferdowsi University in Iran. Proton NMR spectra were obtained using a Bruker 300 MHz apparatus utilizing DMSO Proton NMR spectra were obtained using a Bruker 300 MHz apparatus utilizing DMSO as a solvent.

GENERAL PROCEDURE

A solution of 6-aminocoumarin in a mixture of benzene and DMF (3:1) was prepared, to which triethylamine (TEA) was introduced. The reaction mixture was chilled in an ice bath, and chloroacetyl chloride, previously diluted with 5ml of benzene, was added dropwise while stirring continuously. The amide derivative was generated upon completion of the reaction. The resulting product was then treated with thiourea in ethanol to create 2-aminothiazole. In the presence of glacial acetic acid, the final primary amine of thiazole reacted with aromatic aldehyde derivatives to form imines. The synthesis of target compounds (IIIa-IIId) and their intermediates is shown in Figure 1.

SYNTHESIS OF COMPOUND I

The 2-chloro-N-(2-oxo-2H-chromen-6-yl) acetamide [compound I] is compiled as follows [34].

(1 g, 0.006 mmol) of 6-aminocoumarin was dissolved in a solution mixture of dimethylformamide and benzene (1:3, 40 mL). The mixture was then treated with TEA (0.85 mL, 0.006 mmol). Chloroacetyl chloride (0.48 mL, 0.006 mol in 5 mL benzene) was added dropwise to the reaction mixture while it was continuously agitated for an hour in an ice bath and then refluxed for 6 hours at 76°C. The product was then precipitated by the addition of excess cold water, filtered, washed with diethyl ether, and recrystallized from ethanol to produce compound (I).

SYNTHESIS OF COMPOUND II

6-((2-((4-chlorobenzylidene)amino)thiazol-4-yl)amino)-2H-chromen-2-one [compound II] has been compiled according to [34].

Thiourea (0.160 g, 0.002 mmol) was introduced to a solution of 0.5 g (0.002 mmol) of Compound I in 40 mL

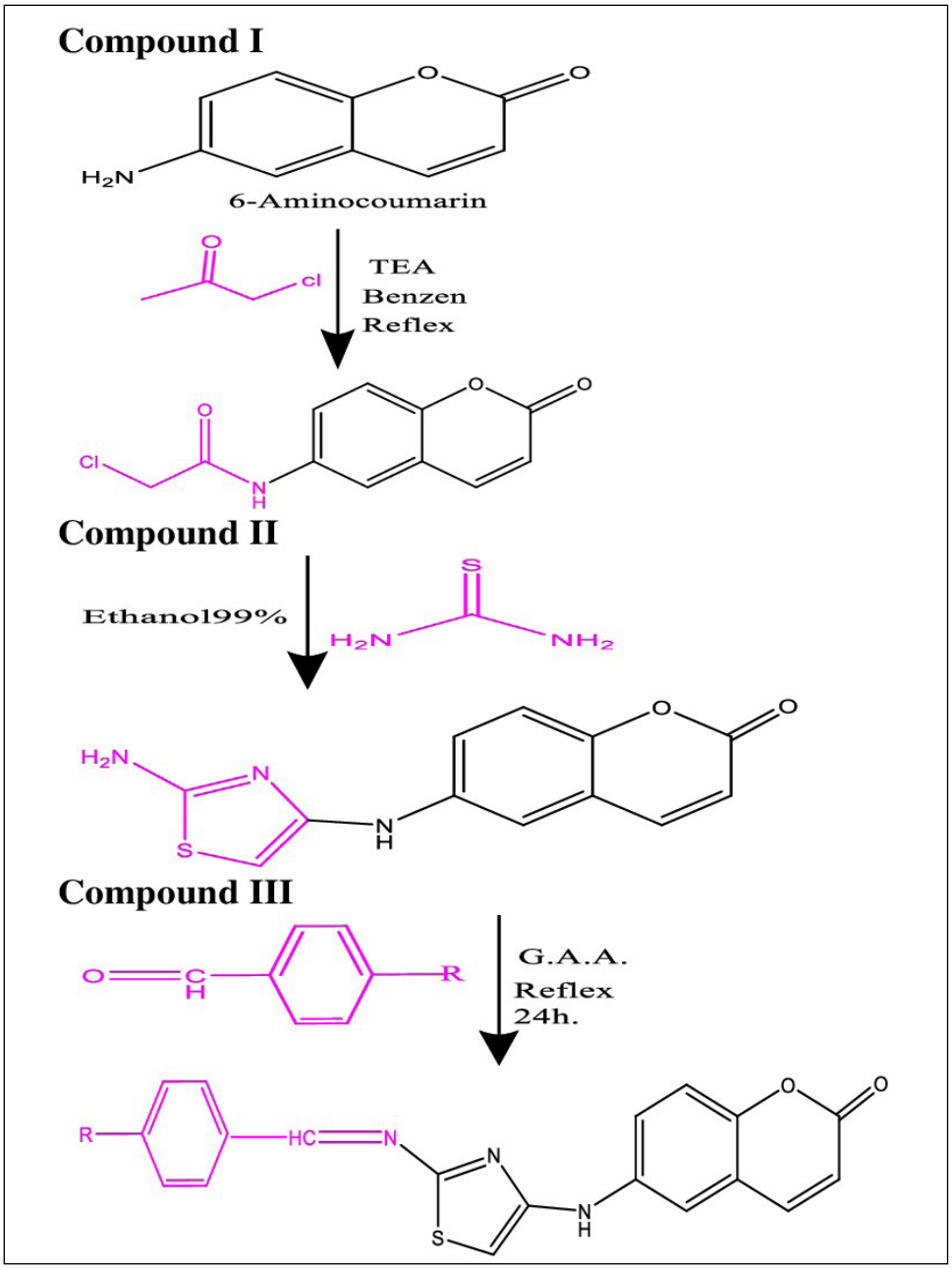


Fig. 1. Synthesis of target compounds (IIIa-IIId) and their intermediates from 6-aminocoumarine. IIIa if R = Br. IIIb if R = Cl, IIIc if R = OH, IIId if R = OCH_3
Source: Own materials

of 99% pure ethanol, and the mixture was constantly agitated until the thiourea dissolved. The reaction mixture was refluxed for 10 hours, the solvent was evaporated, the product was repeatedly washed with diethyl ether, and it was recrystallized from ethanol.

SYNTHESIS OF COMPOUNDS IIIA-D

6-((2-((4-substitutedbenzylidene)amino)thiazol-4-yl) amino)-2H-chromen-2-one has been synthesized according to [35].

A reaction mixture of compound (II) (0.002 mmol, 0.5 g) and the desired aldehyde (0.002 mmol) from Table 1 was mixed with a few drops of glacial acetic acid, all dissolved in ethanol (30 mL). Next, the mixture underwent refluxing for about 24 hours. The generated precipitate was then filtered and washed multiple times with diethyl ether before being recrystallized from ethanol, resulting in compounds (IIIa-d). Table 1 shows type of aldehyde compounds and their amounts were used in the synthesis of compounds IIIa-d.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism software, version 10, to assess the influence of various factors on the study parameters. Additionally, Microsoft Office Professional Plus 2021-Excel was utilized to determine the significance of differences in the mean and the standard deviation values observed in this study.

Table 1. Type and amount of aldehyde compounds employed in the synthesis of compounds IIIa-d

Symbol of Product	Aldehyde Derivative	Weight (g)
IIIa	4-Bromobenzaldehyde	0.37
IIIb	4-Chlorobenzaldehyde	0.28
IIIc	4-hydroxybenzaldehyde	0.24
IIId	4-methoxybenzaldehyde	0.27

Source: Own materials

Table 2. Cytotoxic activity (IC50, μM) of 6-aminocoumarin derivatives and reference medicines against MCF7 and MCF10a

Compound	IC50(μM) against MCF7	IC50(μM) against MCF10a
Acetazolamide	67.53	53.91
Cisplatin	15.09	13.55
IIIa	42.63	145.52
IIIb	21.57	135.01
IIIc	17.67	86.63
IIId	41.84	135.45

Source: Own materials

Table 3. Molecular docking of the test ligands (IIIa-IIId) in comparison to acetazolamide displays the S-score, RMSD, and primary amino acids that are involved in the ultimate ligand interactions

Compound name	R Group	S Score	RMSD	No. of Binding Sites	Binding amino Acids
Acetazolamide		-5.18	1	2	Zn301, ThrA199
IIIa	p-Br	-9.98	1.8	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIIb	P-Cl	-9.95	1.8	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIIc	p-OH	-10.12	1.5	5	Zn301, Thr A199, His A96, His A119, Asn A62
IIId	p-OCH3	-10.6	1.7	6	Zn301, Thr A199(2), His A96, His A119, His A94

Source: Own materials

SPECTROSCOPIC DATA

Compound I $C_{11}H_8ClNO_3$: FT.IR (cm^{-1}): 1616 (C=O stretching band of amide), 1703 (ester's C=O stretching band), 3296 (amide's NH stretching band), 3167, 3097 (C-H stretching band of aromatic ring), 1575-1447 (C=C stretching band of lactone and aromatic ring), 684 (C-Cl stretching band).

Compound II $C_{12}H_9N_3O_2S$**:** FT.IR (cm^{-1}): 3437-3305 (asymmetric and symmetric stretching bands of NH_2, respectively), 1633 (C=N (imine of thiazole) stretching band), 1517, 1448 (C=C stretching band of lactone and aromatic ring), 1400 (C-N stretching band of aromatic amines).

Compound IIIa $C_{19}H_{12}BrN_3O_2S$: FT.IR (cm^{-1}): 3192 (N-H stretching band of secondary amine), 1726 (ester's C=O stretching band), 1635, 1568 (C=N stretching bands of thiazole and imine), 1512-1436 (C=C stretching band of lactone and aromatic ring), 644 (C-Br). ^{1}H NMR (ppm): 11.5 (1H, singlet, N-H amine), 10 (1H, singlet, HC=N (imine)), 9.3 (singlet of coumarin) 8.6 - 7.3 (7H, multiplet, of aromatic rings), 6.5 (1H, singlet, vinilic proton).

Compound IIIb $C_{19}H_{12}ClN_3O_2S$**:** FT.IR (cm^{-1})**:** 3157 (N-H secondary amine stretching band), 1722 (ester's C=O stretching band), 1680, 1625 (C=N stretching band of thiazole and band of imine), 1587-1485 (C=C stretching band of lactone and aromatic ring), 675 (C-Cl). ^{1}H NMR (ppm): 10 (1H, singlet, N-H of amine), 9.5 (1H, singlet, HC=N (imine)), 8m6 - 7.3 (7H, multiplet, of aromatic rings).

Compound IIIc $C_{19}H_{13}N_3O_3S$**:** FT.IR (cm^{-1}): 3149 (O–H stretching band), 1670, 1633 (C=N stretching band of thiazole and imine), 1557-1442 (C=C stretching band of lactone and aromatic ring), 1510 (C-N stretching band). ^{1}H NMR (ppm): 10.3 (1H, singlet, N-H of amine), 9.8 (1H, singlet, HC=N (imine)), 8.8–6.8 (7H, multiplet, (of aromatic rings).

Compound IIId $C_{20}H_{15}N_3O_3S$: FT.IR (cm^{-1}): 3188 (N-H secondary amine's stretching band), 1718 (ester's C=O stretching band), 1676, 1633 (C=N thiazole and imine stretching band), 1504, 1439 (C=C stretching band of lactone and aromatic ring). ^{1}H NMR (ppm): 10 (1H, singlet, N-H of amine), 9.4 (1H, singlet, HC=N (imine)), 8.3 – 6.8 (7H, multiplet, of aromatic rings), 3.3–3.8 (3H, singlet, OCH_3 of para methoxyphenyl ring).

DOCKING INVESTIGATION

The MOE program, version 2015.10, was used to conduct molecular research analysis. To accurately illustrate the

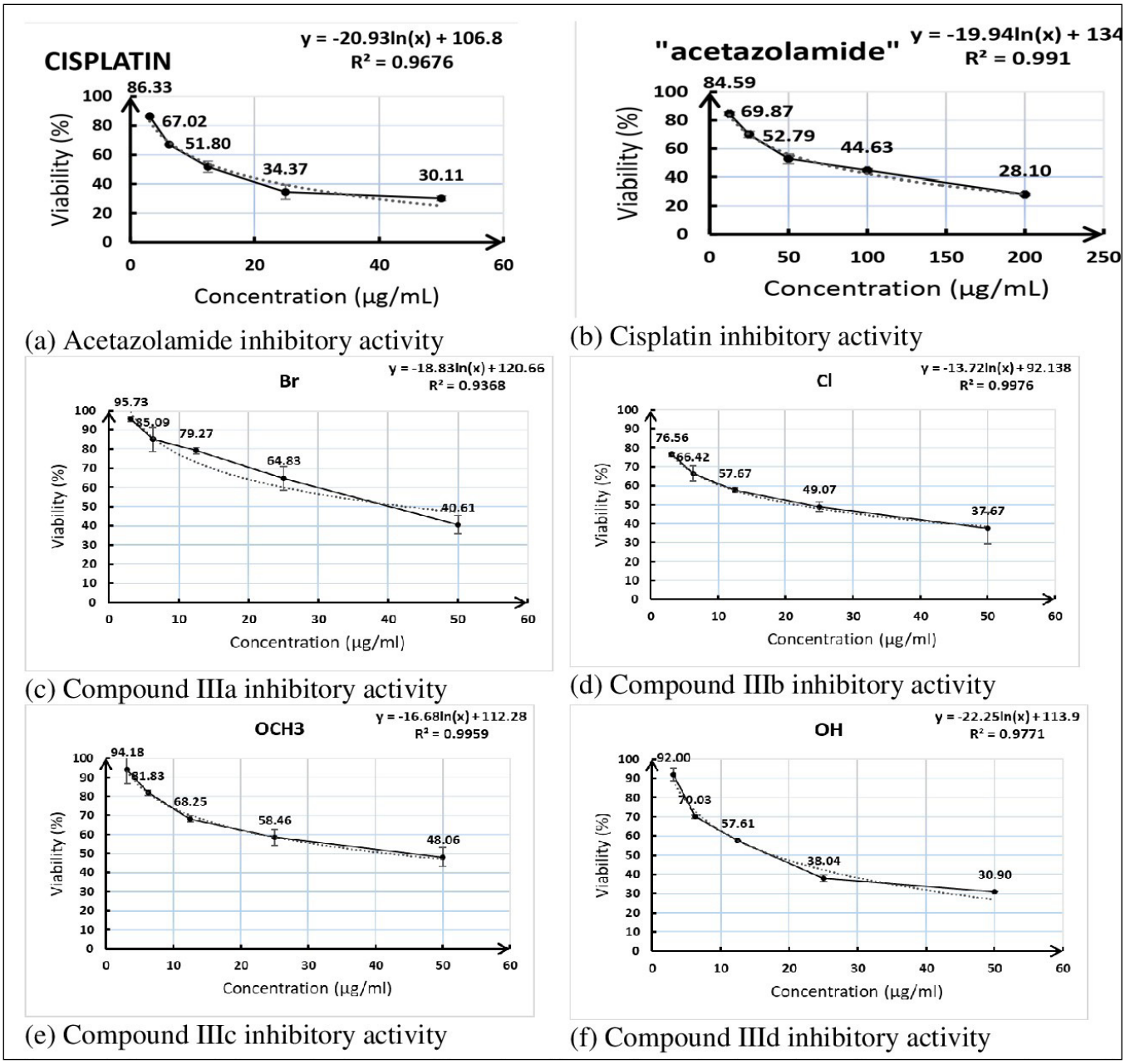


Fig. 2. Cytotoxicity analysis: unveiling the effects of standards and the final compound on MCF7
a - the inhibitory activity of acetazolamide, while; b - the inhibitory activity of cisplatin; c–f - the inhibitory effects of compounds IIIa–IIId
Source: Own materials

ligand formations, the expert program Chem-draw-12.0 was used. Only charges were applied and energy minimization was done after the ligands were protonated in three-dimensional forms inside MOE. Carbonic anhydrase IX's (PDB code: 6fe0) structure was imported into MOE for further analysis after being accessed from the Protein Data Bank. To get the target protein ready, small molecules and non-functional chain fragments were removed. The protein's atomic potentials were changed, and hydrogen bonds were added. The docking process began once the active site was determined and the premade ligands were added to MOE.

CYTOTOXIC STUDY

CELL LINES AND CULTURE

Iran's National Cell Bank (Pasteur Institute) provided the human breast cancer cell line MCF7 and the human normal breast cell line MCF10A. RPMI-1640 medium (Gibco) containing 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) was used to cultivate MCF7 cells. The MCF10A cell line was cultured in DMEM-F12 media with 10% horse serum (Gibco), cholera toxin (100 ng/ml, Sigma), hydrocortisone (0.5 mg/ml, Sigma), EGF (20 ng/ml, Thermofisher), and insulin

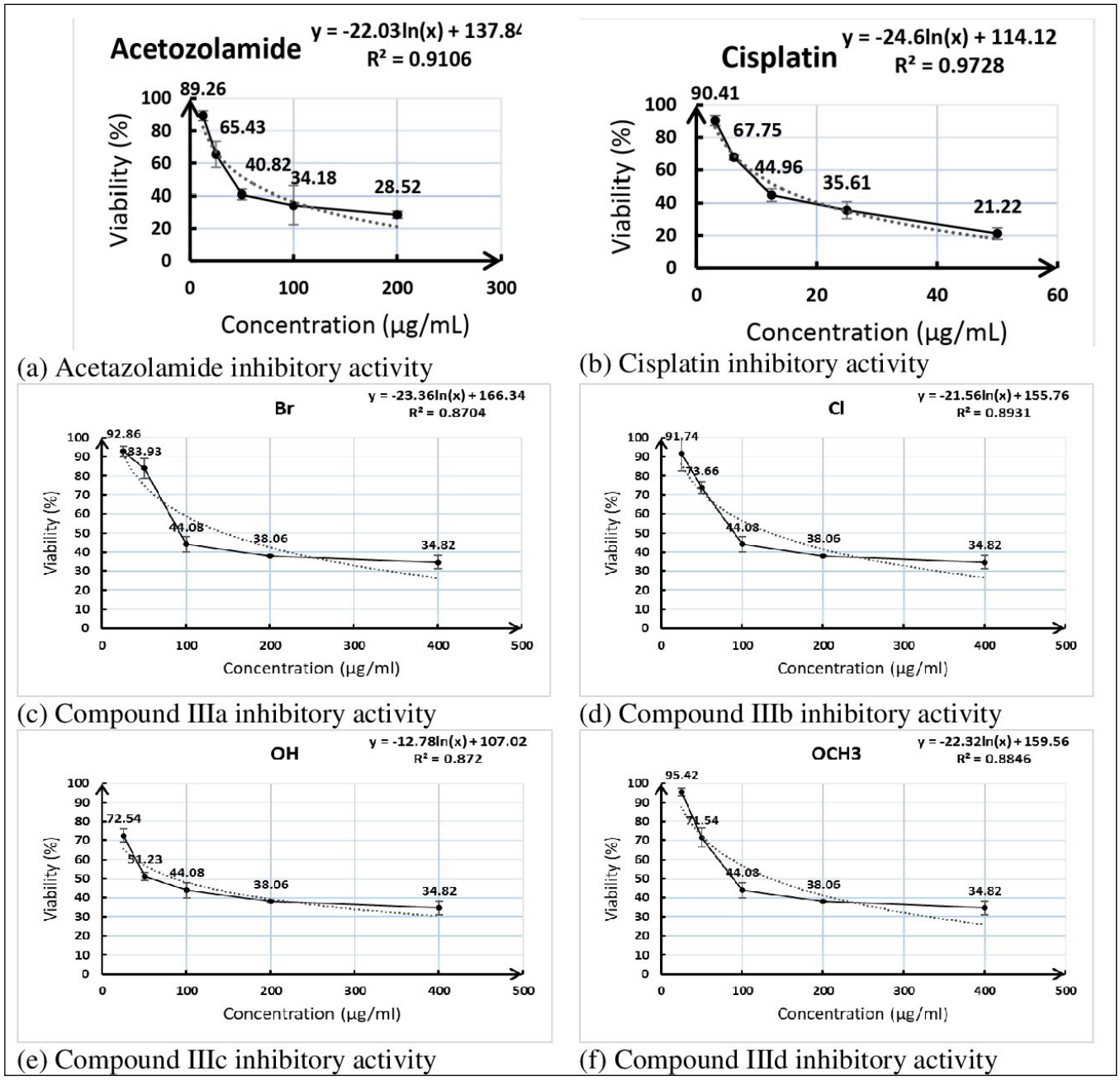


Fig. 3. Cytotoxicity analysis: unveiling the effects of standards and the final compound on MCF10a
a-b - the inhibitory action of acetazolamide and cisplatin, respectively, c–f - the inhibitory effects of compounds IIIa–III
Source: Own materials

(10 µg/ml). Trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution were used to passage the cells, which were grown at 37°C in humidified air with 5% CO_2.

MTT CELL VIABILITY ASSAY

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test (Sigma-Aldrich) was used to measure cell growth and viability. To summarize, dcells were digested with trypsin, collected, adjusted to a density of 1.4 × 104 cells/well, and seeded onto 96-well plates filled with 200 µl fresh media in each well for 24 hours. After forming a monolayer, cancer cells were treated with 50-3.125 µg/ml and normal cells with 400-25 µg/ml of the chemical for 24 hours at 37°C and 5% CO_2. At the final stage of the treatment (24 hours), the monolayer culture was maintained in the original plate, but the supernatant was removed and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added. After that, the plate was incubated at 37°C for a further four hours. MTT solution: 100 µl of dimethyl sulfoxide was added to each well after the cell supernatant was extracted. Until the crystals were totally dissolved, cells were shaken at 37°C. An enzyme-linked immunosorbent

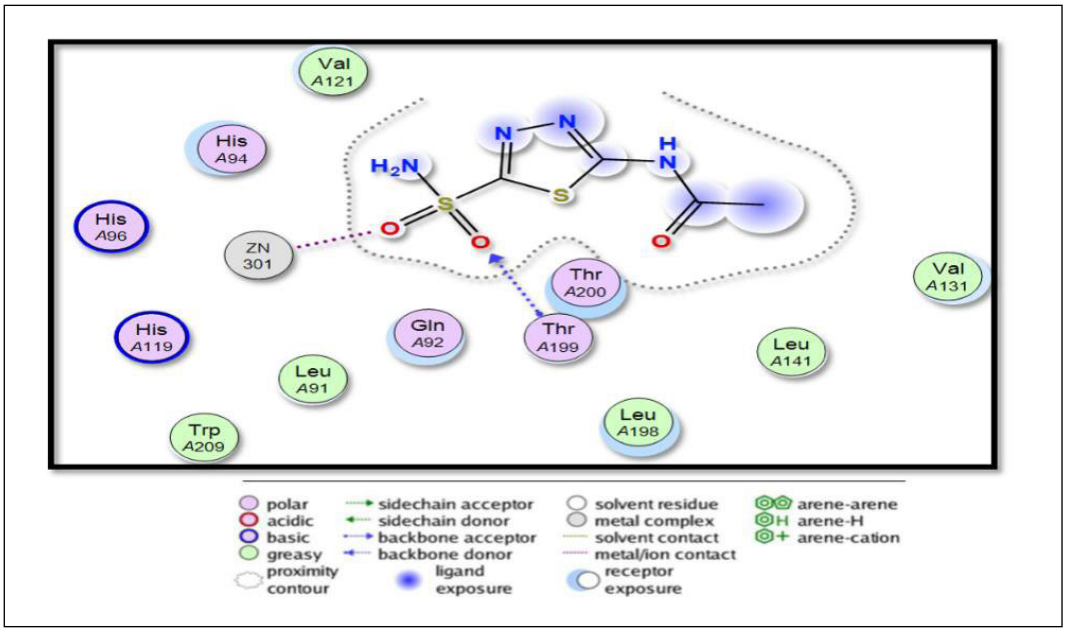


Fig. 4. Docking simulation of reference ligand (acetazolamide) with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

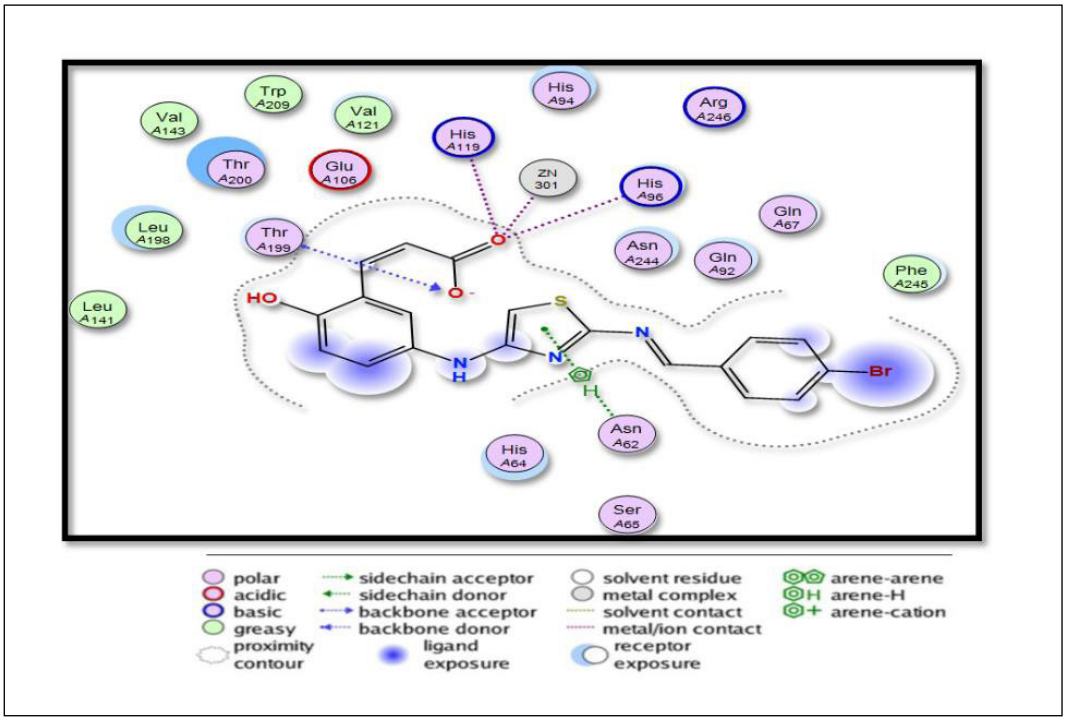


Fig. 5. Docking simulation of compound IIIa with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

assay reader (Model wave xs2, BioTek, USA) was used to measure absorbance at 570 nm to quantify cell viability. The corresponding dose-response curves were used to calculate the concentration of the chemicals that caused 50% of cell death (IC50).

RESULTS AND DISCUSSION

CHEMICAL REACTION

The IR spectra support the electron pair's nucleophilic attack on the primary amine in 6-aminocoumarin towards the carbonyl group of chloroacetyl chloride. The sharp band at 1616 cm^{-1} indicates the amide carbonyl (C=O) bond in the 2-chloroacetamide derivative. Chloroacetyl chloride exhibits selectivity at the α-carbon atom due to higher nucleophilic reactivity toward acid chlorides. This selectivity is due to differences in the electrophilia of the two carbon atoms in chloroacetyl chloride. Steric and electrical elements also have a role in the selecting process as a whole [36]. Compound I is the end product of this reaction. The reaction of the 2-chloroacetamide derivative with thiourea is what creates the thiazole ring. The acetyl chloride's electrophilicity is increased by the hydrogen

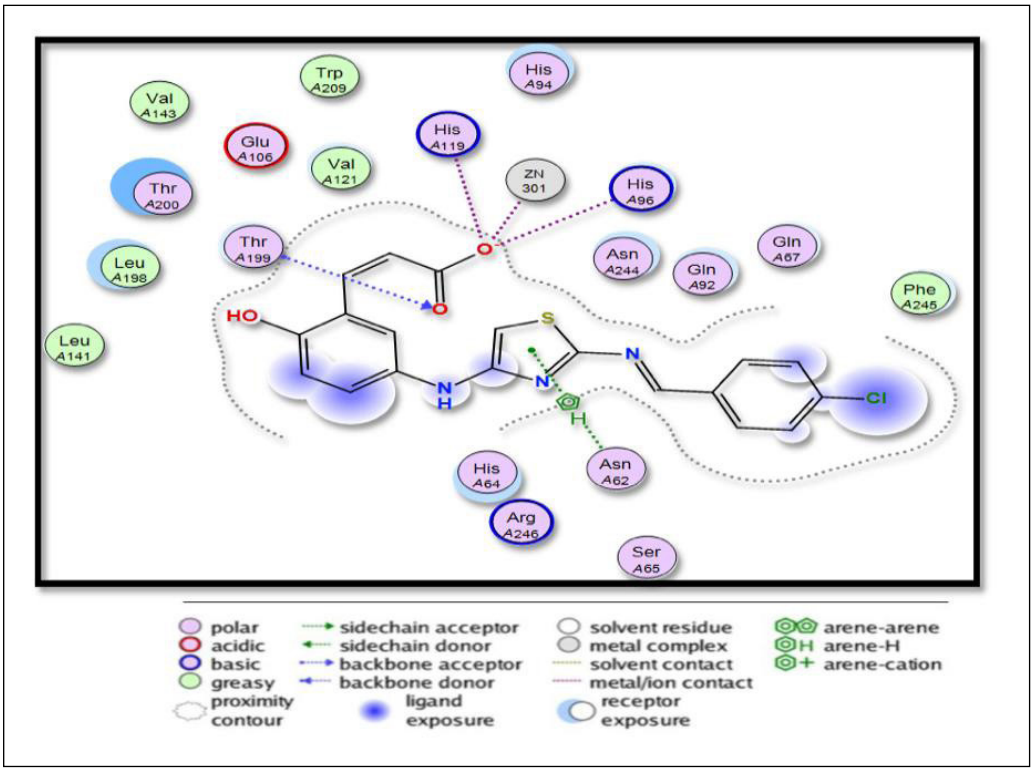


Fig. 6. Docking simulation of compound IIIb with carbonic anhydrase IX (PDB code: 6fe0) *Source: Own materials*

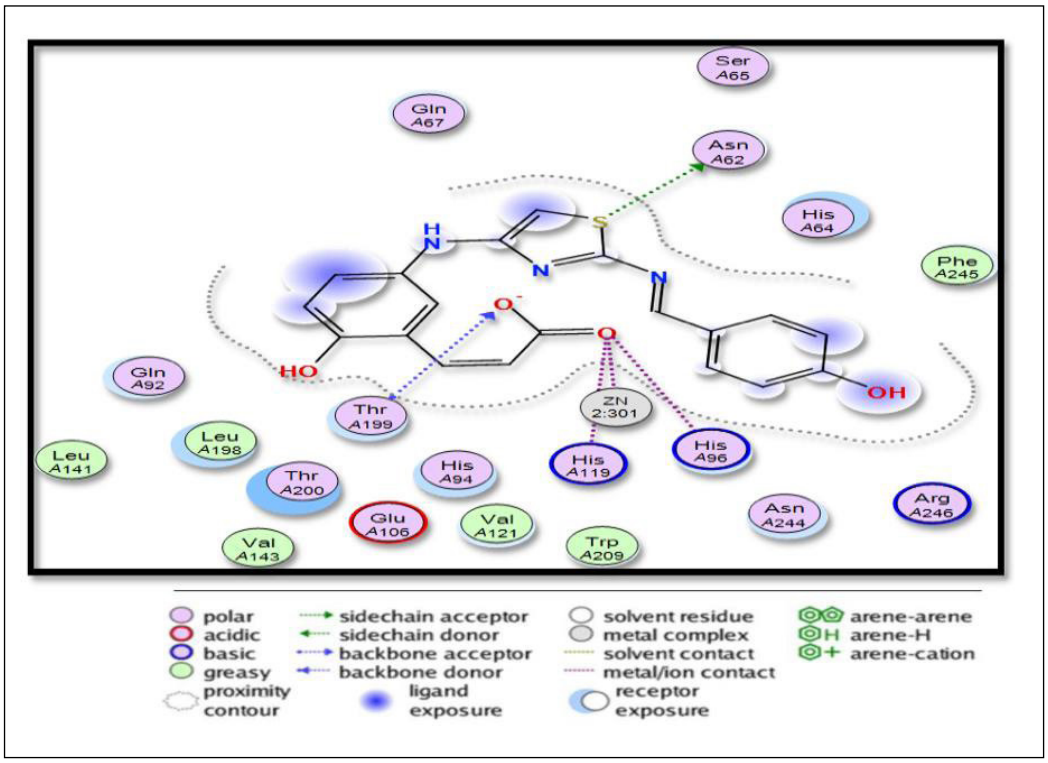


Fig. 7. Docking simulation of compound IIIc with carbonic anhydrase IX (PDB code: 6fe0) *Source: Own materials*

bond that exists between its carbonyl oxygen, making it simpler for the thiourea's amino nitrogen and the sulfur of the chloromethyl carbon to attack and generate a thiazole ring. After that, the removal of an HCl molecule occurs. The creation of the heterocycle is confirmed by the presence of a peak at 1633 cm^{-1}. With the use of glacial acetic acid (GAA) as a catalyst, compounds (IIIa-d) have been produced by Schiff base development from the interacting compound (II) with the corresponding aldehydes. FT-IR spectroscopy confirmed the production of imine bonds, with absorption in the 1568 cm^{-1} region. The mechanism was further supported by the removal of the aldehyde C-H stretch in the 2750–2850 cm^{-1} region and the lack of the aldehyde carbonyl peak in the 1720–1740 cm^{-1} area.

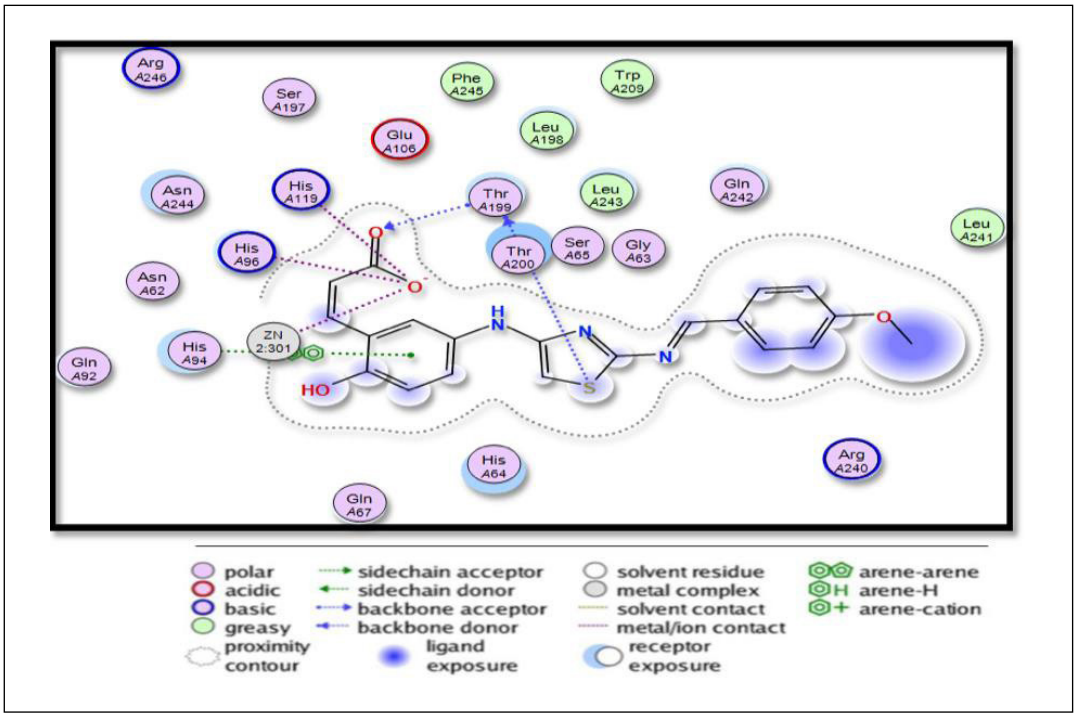


Fig. 8. Docking simulation of compound IIId with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

THE DESCRIPTION AND PHYSICAL CHARACTERISTICS OF THE COMPILED COMPOUNDS AND THEIR INTERMEDIATES

1. 6-aminocoumarin ($C_9H_7NO_2$) as starting material, M. wt = 161.05, green crystals, melting point = 166-168°C, and Rf value = 0.73.
2. Compound I ($C_{11}H_8ClNO_3$), M. wt = 237.64, light yellow crystals, yield = 80%, melting point = 195-198°C, and Rf value = 0.85.
3. Compound II ($C_{12}H_9N_3O_2S$), M. wt = 259.04, yellowish to green powder, yield = 60%, melting point = 221-223 °C, and Rf value = 0.57.
4. Compound IIIa ($C_{19}H_{12}BrN_3O_2S$), M. wt = 426.29, yellow powder, yield = 68%, melting point = 240-242 °C, and Rf value is 0.67.
5. Compound IIIb ($C_{19}H_{12}ClN_3O_2S$), M. wt = 381.83, yellowish to orange powder, yield = 70%, melting point = 230-232 °C, and Rf value is 0.66.
6. Compound IIIc ($C_{19}H_{13}N_3O_3S$), M. wt = 363.39, brown powder, yield = 73%, melting point = 233-235°C, and Rf value = 0.71.
7. Compound IIId ($C_{20}H_{15}N_3O_3S$), M. wt = 377.42, yellow crystals, yield = 75%, melting point = 215-217 °C, and Rf value = 0.60.

CYTOTOXIC EVALUATION

MTT assays were used to evaluate the cytotoxicity of compounds (IIIa, IIIb, IIIc, and IIId) in two cell lines: normal cells (MCF10A) and cancer cells (MCF7). The IC50 values for each medication were determined, and their inhibitory effects were compared to those of acetazolamide. The cytotoxic efficacy of these compounds was compared to that of cisplatin. Compounds IIIa-IIId showed significantly higher cytotoxicity against tumor cells (IC50 = 42.63 µM, 21.57 µM, 17.67 µM, and 41.84 µM, respectively) in comparison with acetazolamide. They also revealed a considerable drop in healthy cell viability (IC50 = 145.52 µM, 135.01 µM, 86.63 µM, and 135.45 µM, respectively) (Table 2).

The results show that each synthesized chemical exhibited excellent inhibitory efficacy and has the potential to be developed into an effective antineoplastic medication. The compounds had IC50 values ranging from 17.67 to 42.63 µM for MCF7 cells and 86.63 to 142.52 µM for MCF10a cells, respectively.

Compounds IIIc and IIIb showed significantly higher cytotoxicity against tumor cells (IC50 = 17.67 µM and 21.57 µM, respectively) and decreased normal cell viability (IC50 = 86.63 µM and 135.01 µM, respectively) compared to cisplatin, indicating higher levels of selectivity and fewer side effects.

These findings show that the synthesized chemicals are effectively selective inhibitors of tumor cells, while causing little harm to healthy cells.

Figure 2 displays the cytotoxicity analysis of several compounds against MCF7 cells, indicated by the mean ± SD of triplicate measurements. Figure 2a shows the inhibitory activity of acetazolamide, while Figure 2b shows the inhibitory activity of cisplatin. Figure 2c–f shows the inhibitory effects of compounds IIIa–IIId.

Figure 3 displays the cytotoxicity analysis of several compounds against MCF10a cells, indicated by the

mean ± SD of triplicate measurements. Figures 3a and 3b indicate the inhibitory action of acetazolamide and cisplatin, respectively. Figure 3(c–f) shows the inhibitory effects of compounds IIIa–IIId.

DOCKING INVESTIGATION

Docking computer simulations demonstrated various binding mechanisms for ligands within the 6fe0 protein's interacting zone. These modes of binding were characterized by specific ligand-protein interactions, including electrostatic interactions and hydrogen bonding. The results showed varied binding strengths for the compounds that have been synthesized, with stronger interactions noted with reference to the target protein compared to acetazolamide. The inhibitory effects of the compounds were clearly identical; all of them bound to the necessary zinc ion for their inhibitory activity, creating hydrogen bonds with the Thr199 residue in the active site of the enzyme. The thiazole moiety was also necessary for attachment to the catalytic region of the enzyme because it interacted with important amino acids that were involved in substrate binding. Table 3 shows for every compound the S. score and RMSD values of the docking study. The S. score values are -9.98, -9.95, -10.12, and -10.6, while the RMSD values are 1.8, 1.8, 1.5, and 1.7, respectively, as listed in Table 3.

Fig. 4 shows the docking simulation of acetazolamide with HCA IX (PDB code 6fe0). Figures 5-8 show the docking simulations of compounds IIIa-IIId with HCA IX (PDB code 6fe0).

CONCLUSIONS

This study sought to explore the efficacy of recently developed aminocoumarin-thiazole hybrid compounds as possible anticancer medicines, with a particular focus on breast cancer. Compounds IIIa-d were meticulously constructed, chemically produced, and simulated to determine their antitumor efficacy. The compounds were tested for cytotoxicity on a cell line and showed IC50 values ranging from 17.67 to 42.63 µM. IIIc and IIIb had the maximum cytotoxicity against breast cancer cell lines (IC50 values of 17.67 and 21.57 µM, respectively). Furthermore, in silico investigations revealed that these compounds had superior S. scores of -10.12 and -9.95, indicating that they could be chemotherapeutic agents. Nonetheless, they showed lesser toxicity in normal cells than cisplatin, meaning that they may do less harm to healthy tissues. The findings show the ability of the developed aminocoumarin-thiazole compounds to selectively target tumor cells while preserving normal cells. The results reveal that compounds IIIc and IIIb may be potential therapy agents for breast cancer; more study is needed to analyze their in vivo anticancer activity more carefully.

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ACKNOWLEDGMENT
The Authors would like to acknowledge the editor's and reviewers' efforts in evaluating this work.

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 17.12.2025
ACCEPTED: 03.08.2026

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