ORIGINAL ARTICLE

CONTENTS

Synthesis and evaluation of Sulfonamide-Thiazolidinone conjugates as promising anticancer agents via carbonic anhydrase inhibition

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ABSTRACT

Aim: This study focuses on developing and evaluating five newly created compounds targeting carbonic anhydrase isoforms found in solid tumors. Our primary goal is to create exceptionally potent anti-cancer drugs.

Materials and Methods: Sulfanilamide, chloroacetyl chloride, Gamma-aminobutyric acid, thionyl chloride, methanol, hydrazine hydrate, aromatic aldehyde derivatives, glacial acetic acid, and thioglycolic acid were used in the chemical synthesis. We performed docking studies using the Molecular Operating Environment software program version 2015.10, and used MTT assay to predict cytotoxic activity.

Results: The compounds we developed demonstrated impressive antineoplastic action in both in silico experiments as well as cell line experiments. Their toxicity to normal cells varied significantly, but their efficacy against cancer cells differed significantly from cisplatin. When compared to acetazolamide, each of the produced compounds exhibited significant differences in their effects on MCF7 cells. Based on these findings, synthetic compounds may serve as antineoplastic medications. Including the thiazolidinone ring in these compounds enhanced their affinity for the receptor by binding to multiple crucial amino acids that play a significant role in our target's enzymatic activity and substrate binding.

Conclusions: Our synthetic compounds revealed cytotoxicity and inhibitory potencies against carbonic anhydrase. Moreover, they exhibited cytotoxicity.

KEY WORDS: Carbonic Anhydrase Inhibitors, In Silico, Thiazolidin-4-one, Sulfonamide

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INTRODUCTION

Cancer is a complex group of disorders distinguished by the unregulated proliferation and spreading of irregular cells, and these abnormal cells can develop into tumors, infiltrate neighboring tissues, and spread to other areas of the body through a process known as metastasis [1]. Cancer is a significant worldwide public health issue, with millions of new cases diagnosed each year. It is a prominent factor contributing to mortality on a global scale [2]. According to the World Health Organization (WHO), in 2020, over 19.3 million individuals globally received a cancer diagnosis, and 10 million of them died from the disease [3]. With an expected 2.3 million new cases (11.7% of the total), female breast cancer has surpassed lung cancer as the most often diagnosed cancer. Afterward, colorectal 10.0%, prostate 7.3%, and stomach 5.6% cancers were listed in that order of frequency [4]. Although there have been significant improvements in cancer treatment, cancer still poses a considerable challenge [5]. Current research efforts are mainly focused on understanding the fundamental principles of cancer development and discovering new targets for therapy [6]. Nowadays, numerous researchers are attempting to gain a more comprehensive understanding of the tumor microenvironment, which substantially impacts the development of tumors [7]. The tumor microenvironment consists of various elements that create a complex network, including nutrition, oxygen levels, metabolites, pH, and growth factors, so they contribute to therapeutic resistance by providing a protective niche for cancer cells against chemotherapy, radiation therapy, and targeted therapies [8]. Hypoxia is a significant carcinogenic factor present in all solid tumors, contributing to enhanced malignancy and associated with resistance to ionizing radiation and chemotherapy [9]. One of the proteins induced by hypoxia via the hypoxia-inducible factor 1α (HIF-1α) is carbonic anhydrase IX (CA IX), a membrane-associated enzyme. CA IX expression is markedly elevated in human cancer cells while remaining minimal in normal tissues [10]. Human carbonic anhydrases (hCAs) are essential me-

talloenzymes capable of converting CO_2 and H_2O into HCO_3^- and H^+. There are about 16 isoforms, and we will focus on CA IX as it is the most active CA isoform for the CO^2 hydration reaction, playing a significant role in regulating the tumor acid-base balance [11-12]. Targeting CAIX could be a beneficial strategy for delivering cytotoxic medications directly to the desired place, minimizing adverse effects on normal tissues [13]. All currently utilized CA inhibitors are either sulfonamide or a structurally similar sulfamido or sulfamato group. This group acts as a zinc-binding group (ZBG) and mimics the transition state analogue, resembling the tetrahedrally bound zinc to bicarbonate [14-15]. Since 2005, sulfonamides have attracted considerable attention because of their capacity to inhibit carbonic anhydrase, potentially making them suitable for use as anticancer therapies. Additionally, sulfonamide has an essential functionality with various pharmacological applications, such as antihypertensive and antibacterial, antiprotozoal, antifungal, and anti-inflammatory medications [16-17]. The sulfonamides interact with the enzyme in the following manner: Initially, the nitrogen atom in the R-SO2-NH- molecule must establish an ionic bond with the Zn^{2+} ion present in the main site of the carbonic anhydrase. Establishing two hydrogen bonds with the THR-199 amino acid is also necessary [18]. In medicinal chemistry, five-membered heterocyclic molecules, especially those with several heteroatoms, have shown various biological activities [19]. Thiazolidinone is a compound characterized by a sulfur atom at the 1-position, a nitrogen atom at the 3-position, and a carbonyl group at the 2-, 4-, or 5-positions [20]. Compounds that include a Thiazolidinone group have shown diverse pharmacological effects, such as anti-HIV, anti-malarial, anti-inflammatory, and hypoglycemic activity [20]. Incorporating Thiazolidinone into a molecule can enhance its interaction with biological targets. Thiazolidinone fragments are frequently chosen to modify lead compounds in developing anti-tumor medications [22]. These derivatives have the potential to combat cancer through various mechanisms, including initiating apoptosis, preventing the cell cycle, generating reactive oxygen species (ROS), and inhibiting a diverse range of enzymes that are directly associated with the survival of cancer cells, including our target enzyme, CA [23].

AIM

This study focuses on developing and evaluating five newly created compounds targeting carbonic anhydrase isoforms found in solid tumors. Our primary goal is to create exceptionally potent anti-cancer drugs.

MATERIALS AND METHODS

EXPERIMENTAL SECTION

All reagents and anhydrous solvents were supplied by Sigma-Aldrich, Germany, Riedel de Haën, Germany, Hangzhou Hyper Chemicals, and Merck, Germany. We used the Thomas Hover apparatus and the capillary tube method to determine the melting points. Ascending thin-layer chromatography confirmed the reaction steps, purified the synthesized compounds, and measured their retention factor (Rf) values. This was accomplished by the use of a mobile phase that was composed of methanol and acetone at a ratio of one to one [24]. The scanning FT-IR and spectra estimation were carried out utilizing KBr discs by means of a Japanese Shimadzu spectrophotometer at the University of Kufa. Recordings of H^1NMR were made using the Bruker 300 MHz instrument at the University of Mashhad, with DMSO as the solvent.

TYPICAL PROCEDURE FOR THE REACTIONS

We first dissolved the sulfanilamide in a benzene and dimethylformamide solvent; we then added Triethylamine to the solution. The mixture was agitated in a chilled container. Chloroacetyl chloride (CAC) dissolved in benzene is then added in small amounts to the sulfonamide mixture to form acetyl sulfonamide. GABA carboxyl (COOH) esterified using thionyl chloride in cold methanol. The GABA's methyl ester participated in the reaction with acetyl sulfonamide, which resulted in the formation of a secondary amine bond. The compound that was formed as a result of this reaction possessed 99% of hydrazine hydrazide, and this resulted in the creation of hydrazide. The hydrazide underwent a reaction with aromatic aldehyde derivatives in the presence of glacial acetic acid as a catalyst, leading to the synthesis of imines. The imines underwent a reaction with thioglycolic acid to yield sulfonamide derivatives with a heterocyclic ring known as thiazolidin-4-one. The production procedure for synthesizing molecules (IVa–IVe) derived from sulfanilamide is described in Fig. 1.

2-CHLORO-N-(4-SULFAMOYLPHENYL) ACETAMIDE (A) IS SYNTHESIZED ACCORDING TO MINA ET. AL., [25]

Two grams of sulfanilamide, which equals 11.6 mmol, were dissolved in 40 ml of a solution that included 1:3 ratio of dimethylformamide to benzene. Subsequently, we added Triethylamine (1.6 mL, 11.6 mmol). The reaction solution was stirred continually in an ice bath throughout the experiment. Gradually, CAC (0.92 mL, 11.6 mmol in 10 mL benzene) was added while

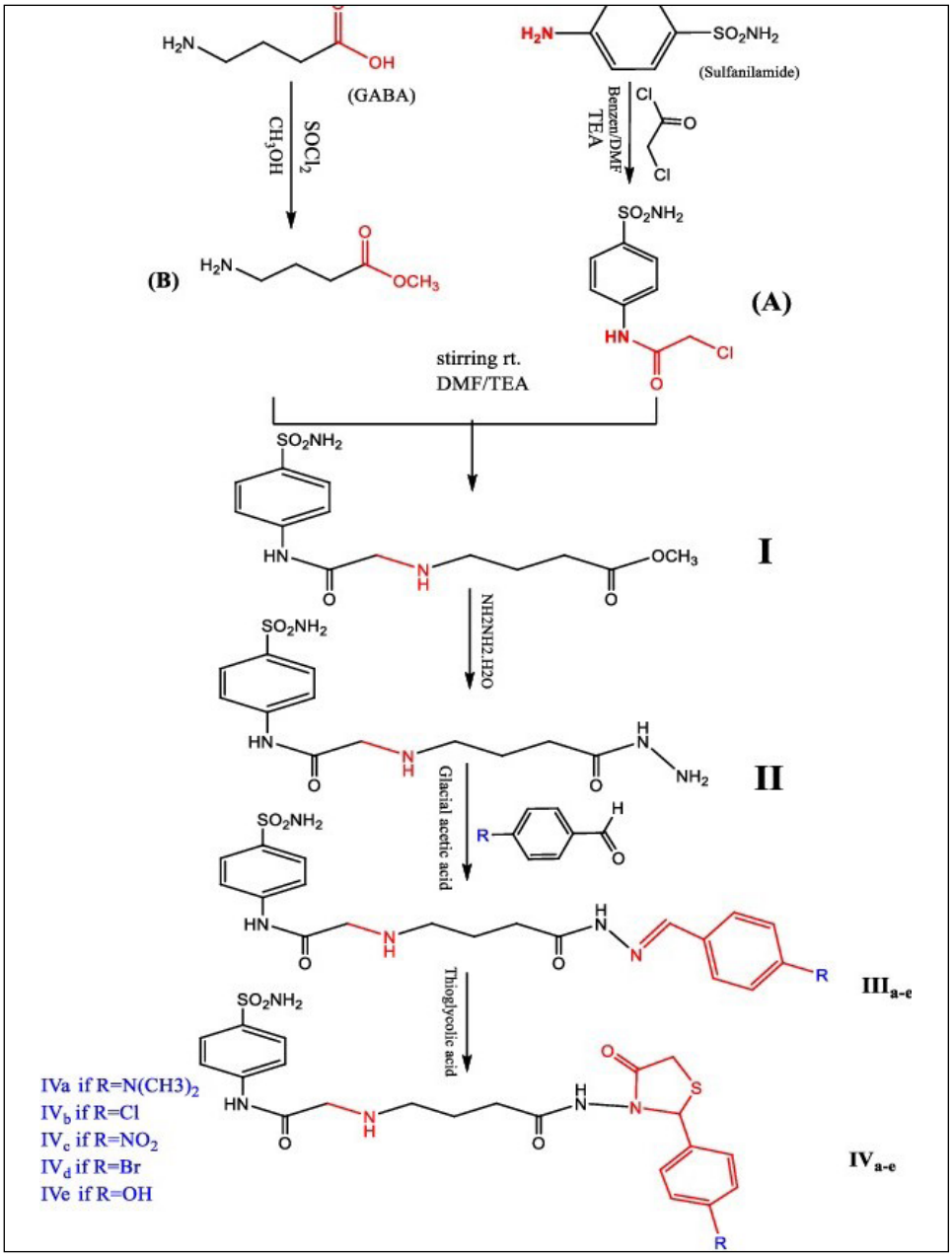

Fig. 1. Synthesis of target compounds.

continuously stirring for one hour. The mixture was then heated to 76°C and subjected to reflux for three hours. We then introduced additional crushed ice water, which led to the formation of a solid substance. Filtering separated this solid, and ethanol-based recrystallization purified it, producing compound A. Table 1 provides %yield, physical data, and Rf values.

METHYL-4-AMINOBUTYRATE HYDROCHLORIDE (B) IS SYNTHESIZED ACCORDING TO LI, J. AND Y. SHA., [26]

Gamma-aminobutyric acid (1g, 9.7 mmol) was dissolved in methanol and then cooled to a temperature of 0°C. We gradually added 0.7 mL (9.7 mmol) of thionyl chloride. The solution was left at room temperature for 45 minutes and then refluxed. After the reaction was finished, the solvent was completely evaporated. Then, the remaining substance was subjected to multiple washes with diethyl ether and purified by recrystallization using a combination of methanol and diethyl ether. This process resulted in compound B. Table 1 provides % yield, physical data, and Rf values.

METHYL 4-((2-OXO-2-((4-SULFAMOYLPHENYL)AMINO)ETHYL) AMINO) BUTANOATE (I) IS SYNTHESIZED ACCORDING TO PARK, H.-S., ET AL., [27]

We used a round-bottom flask to dissolve 0.6 of compound B, which equals 4 mmol in 20 ml of DMF. Before adding compound A (1g, 4 mmol), 0.75 mL of Triethylamine was slowly added to the reaction solution. We stirred the mixture throughout the night. Subsequently, the solvent evaporated, and the resul-

Table 1. Type of aldehyde compounds and their amounts used in the above reaction

Aldehyde compounds	Number of grams
4-Bromobenzaldehyde	0.276
4-Chlorobenzaldehyde	0.213
4-Dimethylaminobenzaldehyde	0.226
4-Hydroxybenzaldehyde	0.185
4-Nitrobenzaldehyde	0.229

tant residue was gathered and subjected to numerous washes with diethyl ether, creating a viscous oil compound I. Table 1 provides % yield, physical data, and Rf values.

2-((4-HYDRAZINYL-4-OXOBUTYL)AMINO)-N-(4-SULFAMOYLPHENYL) ACETAMIDE (II) IS SYNTHESIZED ACCORDING TO JUBIE ET. AL., [28]

The compound (I) (1.5g, 4.5 mmol) was dissolved in absolute ethanol in a flask. We introduced a 0.63 mL solution of 99% hydrazine hydrate, equivalent to 13 mmol, into the flask. The resulting mixture was refluxed for 10 hours at a temperature of 75°C. After finishing the reaction, we evaporated the solvent until it reached half of its initial volume. A solid substance was acquired upon cooling, which underwent filtration, drying, and further purification via recrystallization utilizing 100% ethanol, leading to compound II. Table 1 provides %yield, physical data, and Rf values.

2-((4-(2-BENZYLIDENEHYDRAZINEYL)-4-OXOBUTYL) AMINO)-N-(4-SULFAMOYLPHENYL) ACETAMIDE (IIIA-E) IS SYNTHESIZED ACCORDING TO XAVIER A ET AL., [29]

We carefully added five droplets of glacial acetic acid to a reaction mixture with compound II (1.52 mmol, 0.5 g) and the right aldehydes (1.52 mmol), both dissolved in 25 mL of methanol:

1. Sulfanilamide ($C_6H_8N_2O_2S$) M. WT = 172.20, off white crystals, Melting point in °C = 165. Rf value= 0.90.
2. GABA ($C_4H_9NO_2$) = M. WT = 103.12, white crystals, melting point in °C= 203 and Rf value = 0.35.
3. Compound A ($C_8H_9ClN_2O_3S$) M. WT = is 248.69, yield=76.9%, grey powder, melting point in °C = 203-205 and Rf value is 0.74.
4. Compound B ($C_5H_{12}ClNO_2$) M. WT = is 153.06, yield =96%, white crystal, melting point in °C = 120-122 and Rf value is 0.62.
5. Compound I ($C_{13}H_{19}N_3O_5S_2$) M. WT = 329.37, Brown, yield = 80% and Rf value = 0.81.
6. Compound II ($C_{12}H_{19}N_5O_4S$) M. WT = 329.38, yellowish white powder, yield = 82%, melting point in °C = 50-52 and Rf value = 0.72.
7. Compound IIIa ($C_{21}H_{28}N_6O_4S$) M. WT = 460.55, red crystals, yield = 72%, melting point in °C = 256-258 and Rf value = 0.90.
8. Compound IIIb ($C_{19}H_{22}ClN_5O_4S$) M. WT = 451.93, yellow crystals, yield = 74%, melting point in °C = 209-211 and Rf value = 0.77.
9. Compound IIIc ($C_{19}H_{22}N_6O_6S$) M. WT = 462.84, yellow crystals, yield = 75%, melting point in °C = 307-309 and Rf value = 0.75.
10. Compound IIId ($C_{19}H_{22}BrN_5O_4S$) M. WT = 496.38, yellow crystals, yield = 75%, melting point in °C = 226-228 and Rf value is 0.69.
11. Compound IIIe ($C_{19}H_{23}N_5O_5S$) M. WT = 433.48, white crystals, yield = 60%, melting point in °C = 208-210 and Rf value = 0.78.
12. Compound IVa ($C_{23}H_{30}N_6O_5S_2$) M. WT = 534.65, orange oil, yield = 68% and Rf value = 0.68.
13. Compound IVb ($C_{21}H_{24}ClN_5O_5S_2$) M. WT=526.02, White oil, yield= 70% and RF= 0.66.
14. Compound IVc ($C_{21}H_{24}N_6O_7S_2$) M. WT=536.58, yellow oil, yield=66% and RF=0.71.
15. Compound IVd ($C_{21}H_{24}BrN_5O_5S_2$) M. WT=570.48, White oil, yield=72% and RF=0.69.
16. Compound IVe ($C_{21}H_{25}N_5O_6S_2$) M. WT=507.58, yellow oil, yield=71% and RF=0.73.

We then refluxed the reaction mixture for 2–3 hours. Next, we separated the formed precipitate using filtration and purified it by recrystallization using ethanol. This process resulted in the formation of compounds IIIa–e, table 1 presents the percentage yield, physical data, and Rf values for the compounds.

4-((2-OXO-2-((4-SULFAMOYLPHENYL) AMINO)ETHYL)AMINO)-N-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)BUTANAMIDE (IVA-E) IS SYNTHESIZED ACCORDING TO NEUENFELDT PD ET AL., [30]

The procedure involved heating a mixture consisting of 3 mL of thioglycolic acid and 1 mmol of one of the compounds IIIa-e to a temperature of 60°C. The mixture was continuously stirred until the reaction was fully completed, normally taking around 3 hours. Next, we introduced 5 mL of ethyl acetate into the reaction mixture. Next, we rinsed the organic layer three times with 20 mL of a saturated sodium bicarbonate solution and once with 10 mL of water. After removing any remaining water using anhydrous magnesium sulfate, the mixture was concentrated to produce an oily substance. We rinsed the oil with diethyl ether to get the compounds IVa-e. Table 1 provides the % yield, physical data, and Rf.

Table 2. Cytotoxic activity (IC50, μM) of sulfonamide derivatives and the reference drugs against MCF7 and MCF10a

Compound	MCF7 Results		MCF10a Results	
	IC_{50}(μM) ± SD	p-value	IC_{50}(μM) ± SD	p-value
Acetazolamide	67.53 ± 1.80	Standard	53.91± 5.636	Standard
Cisplatin	15.09 ±2.25	Standard	13.55 ±3.45	Standard
IVa	33.57 ± 1.438	0.0024 a 0.0081 b	567.24± 5.072	0.0001 a 0.0001 b
IVb	34.60 ±1.42	0.0028 a 0.0076 b	511.04±2.422	0.0001 a 0.0001 b
IVc	41.29 ± 1.29	0.0067 a 0.0039 b	452.75± 1.194	0.0001 a 0.0001 b
IVd	28.55±1.342	0.0001 a 0.0096 b	240.51±3.068	0.0001 a 0.0001 b
IVe	30.20 ±2.014	0.0019 a 0.0087 b	490.38 ±3.97	0.0001 a 0.0001 b

Letters a and b: There is a considerable variation compared to cisplatin and acetazolamide, respectively. Not significant (Ns).

Table 3. Results of the investigated compounds' S scores and rmsd values

Compound Name	R group	Docking-Scores	RMSD	Total affinity sites	Molecules that are involved in binding
Acetazolamide	----------	-6.5	1.66	5	Zn301, Thr199, Thr200, His96, His119
IVa	N(CH3)2	-8.8	1.44	7	Zn301, Thr199, Leu198, Gln92, Asn62, His96, His119
IVb	Cl	-9.1	1.9	6	Zn301, Thr199, Leu198, Trp5, His96, His119
IVc	NO2	-8.55	1.7	6	Zn301, Thr199, Leu198, Trp5, His96, His119
IVd	Br	-9.16	1.9	10	Zn301, Thr199, Leu198, Gln67, Gln92, Trp5, His64, His94, His96, His119
IVe	OH	-8.68	1.7	8	Zn301, Thr199, Leu198, Gln92, His64, His94, His96, His119.

SPECTROSCOPIC ANALYSIS [30]

Compound A ($C_8H_9ClN_2O_3S$) FT-IR (cm^{-1}) 3327-3213 (N-H_2 of sulfonamide), 3134 (N-H of amide), 1689 (C=O amide), 1602-1548 (C=C of aromatic ring) and 684 (C-Cl stretching). Compound B ($C_5H_{12}ClNO_2$) FT-IR (cm^{-1}) 3018 (N-H of primary amine salt), 1735 (C=O of ester), 1132 (C-N stretching). Compound I ($C_{16}H_{14}N_4O_3S_2$) FT-IR (cm^{-1}) 3433-3390 (N-H_2 of sulfonamide), 3178 (N-H of amide), 1695 (C=O of ester), 1666 (C=O of amide), 1595-1496 (aromatic C=C). Compound II ($C_{12}H_{19}N_5O_4S$) FT-IR (cm^{-1}) 3294-3190 (N-H_2 of sulfonamide overlap with NH2 of amine), 1668 (C=O of amide). Compound IIIa ($C_{21}H_{28}N_6O_4S$) FT-IR (cm^{-1}) 3442-3375 (N-H_2 of sulfonamide), 3196 (N-H of amide), 2947-2816 (C-H of N-$(CH_3)_2$), 1658 (C=O of amide overlap with C=N), 1365 (N-$(CH_3)_2$ stretching). Compound IIIb ($C_{19}H_{22}ClN_5O_4S$) FT-IR (cm^{-1}) 3520 and 3446 (N-H_2 of sulfonamide), 3229 (N-H of amide), 1660 (stretching of C=N), 1624 (C=O of amide), 817 (stretching of C-Cl). Compound IIIc ($C_{19}H_{22}N_6O_6S$) FT-IR (cm^{-1}) 3444-3292 (N-H_2 of sulfonamide), 3186 (N-H of amide), 1668 (stretching of C=N), 1620 (C=O of amide), 1546-1334 (asymmetric and symmetric of NO_2). Compound IIId ($C_{19}H_{22}BrN_5O_4S$) FT-IR (cm^{-1}) 3477-3415 (N-H_2 of sulfonamide), 3145 (N-H of amide), 1624 (C=O of amide overlap with C=N), 636 (stretching of C-Br). Compound IIIe ($C_{19}H_{23}N_5O_5S$) FT-IR (cm^{-1}) Broad 3396 Overlap of O-H and of NH2 of sulfonamide, 1658 (C=O of amide overlapping with C=N), 1018 (alcoholic C-O). Compound IVa ($C_{23}H_{30}N_6O_5S_2$) FT-IR (cm^{-1}) 3435 (N-H_2 of sulfonamide), 3170 (N-H of amide), 2933 (C-H of alkane), 1718 (C=O of thiazolidinone overlap with C=O of amide), 1294 (C-S stretching). ^{1}H NMR (ppm): 1.09(2H, M, CH_2 of GABA); 2.89 (6H, S, CH_3 of N$(CH_3)_2$); 3.32-3.39 (4H, M, CH_2 of GABA); 3.61 (1H, S, NH of amine); 3.67(2H, S, CH_2-C=O); 3.99-4.06 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.17 (1H, S, CH of thiazolidinone); 6.68 (2H, S, NH_2 of sulfonamide); 7.19-7.82 (8H, M, Aromatic CH); 10.18 (1H, S, NH of amide); 10.52 (1H, S, NH of amide). Compound IVb ($C_{21}H_{24}ClN_5O_5S_2$) FT-IR (cm^{-1}) 3441-3348 (N-H_2 of sulfonamide), 3182 (N-H of amide), 1718 (C=O of thiazolidinone), 1645 (C=O of amide), 1294 (C-S stretching), 669 (stretching of C-Cl). ^{1}H NMR (ppm): 1.22(2H, M, CH_2 of GABA); 3.20 (2H, S, CH_2-C=O); 3.34(1H, S, NH of amine); 3.62-3.65 (4H, M,

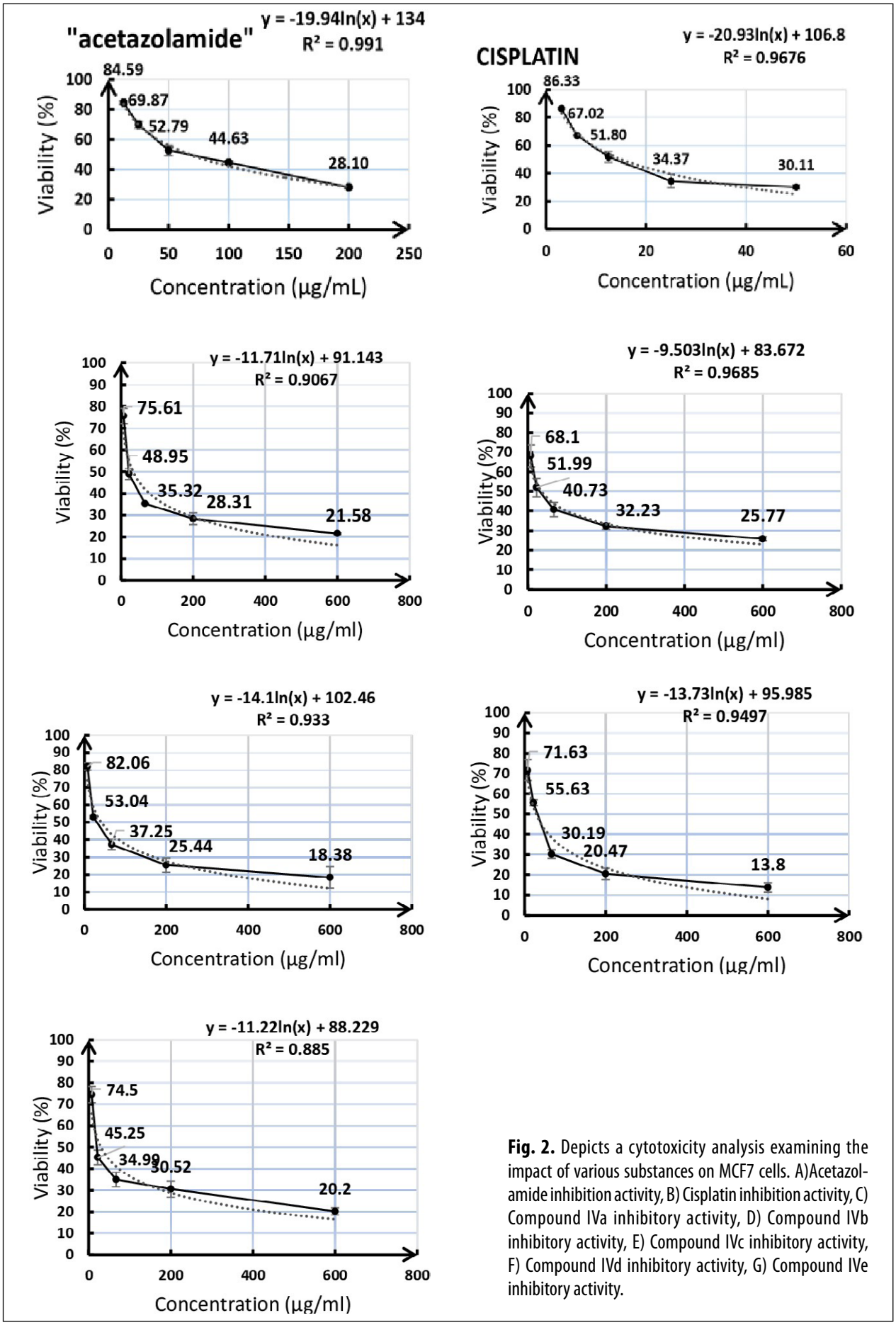

Fig. 2. Depicts a cytotoxicity analysis examining the impact of various substances on MCF7 cells. A)Acetazolamide inhibition activity, B) Cisplatin inhibition activity, C) Compound IVa inhibitory activity, D) Compound IVb inhibitory activity, E) Compound IVc inhibitory activity, F) Compound IVd inhibitory activity, G) Compound IVe inhibitory activity.

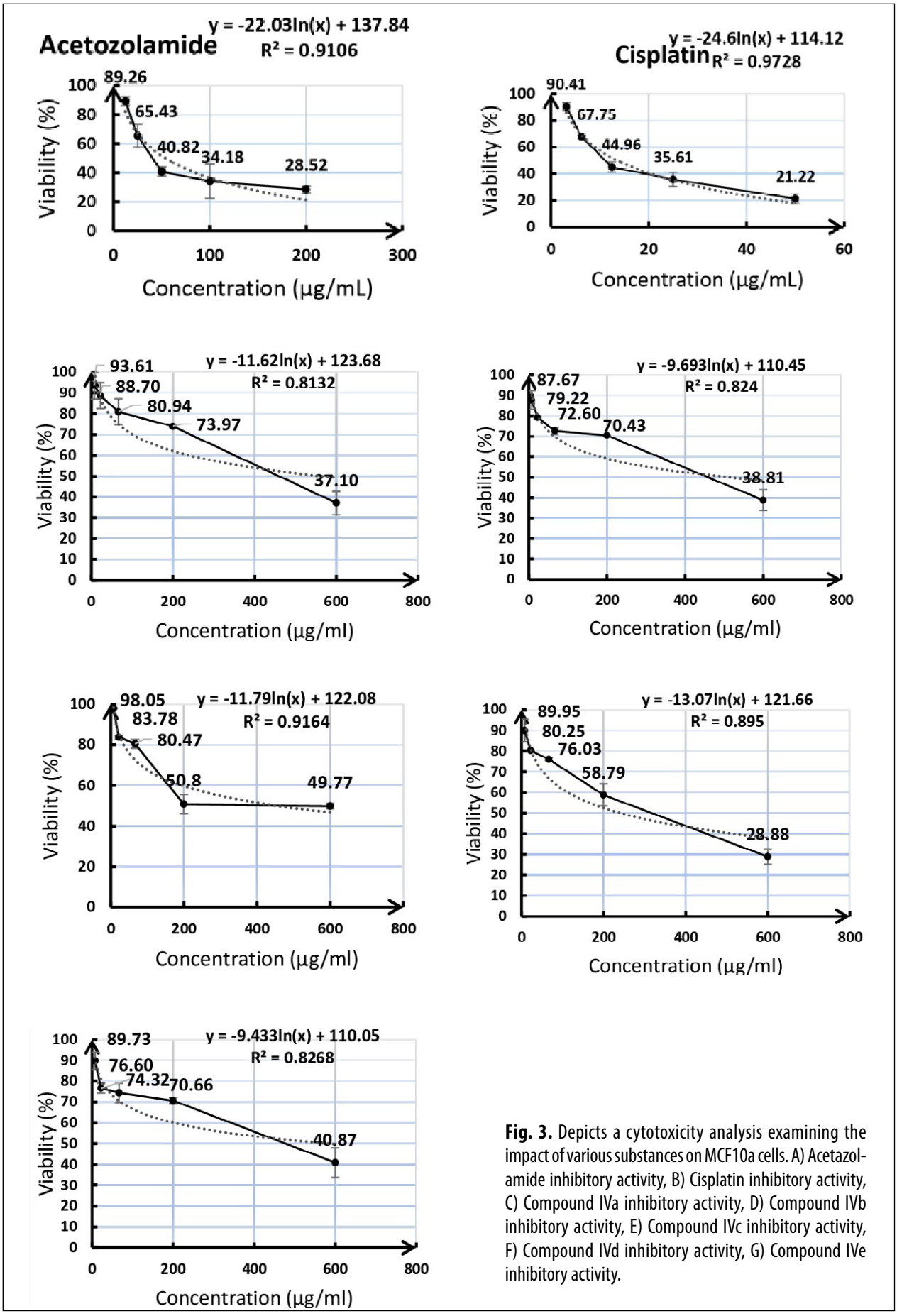

Fig. 3. Depicts a cytotoxicity analysis examining the impact of various substances on MCF10a cells. A) Acetazolamide inhibitory activity, B) Cisplatin inhibitory activity, C) Compound IVa inhibitory activity, D) Compound IVb inhibitory activity, E) Compound IVc inhibitory activity, F) Compound IVd inhibitory activity, G) Compound IVe inhibitory activity.

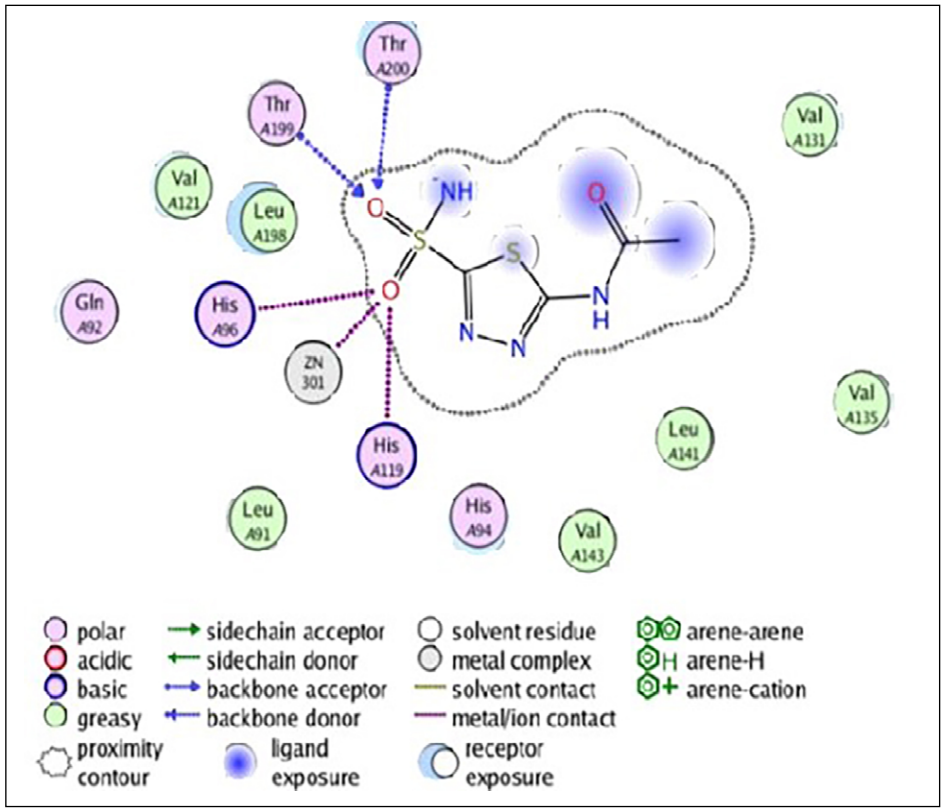

Fig. 4. Acetazolamide in combination with HCA IX (PDB code: 4M2V) (2D).

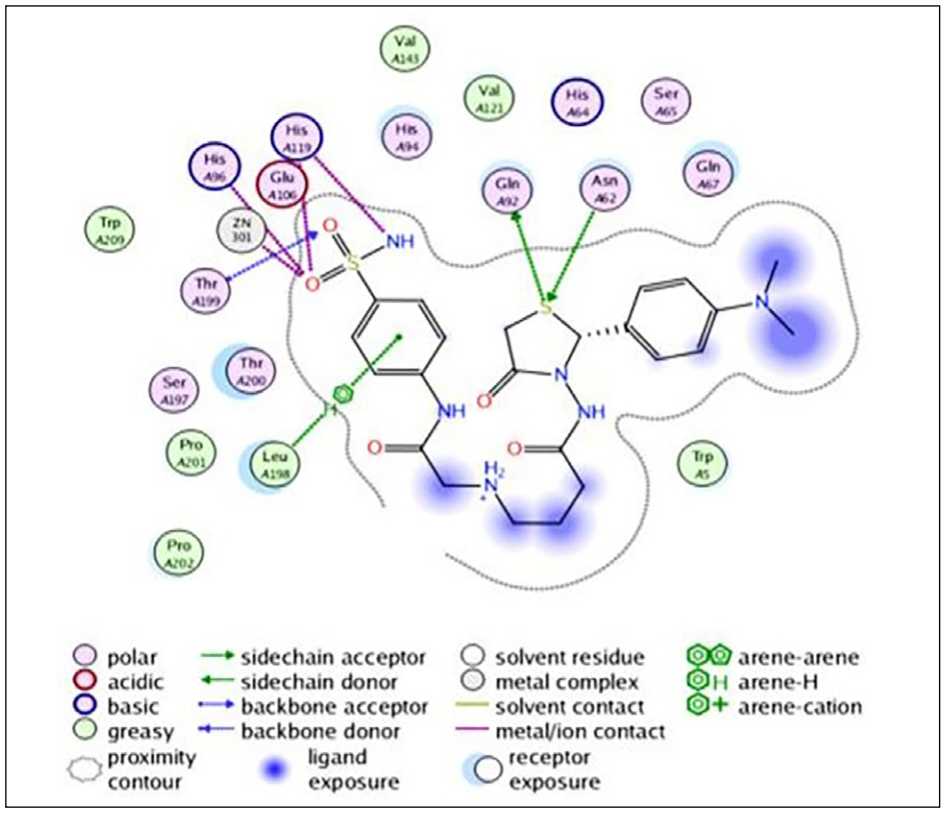

Fig. 5. IVa in combination with HCA IX (PDB code: 4M2V) (2D).

CH_2 of GABA); 3.88-3.98 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.24 (1H, S, CH of thiazolidinone); 6.53 (2H, S, NH_2 of sulfonamide); 7.34-7.41 (8H, M, Aromatic CH); 9.29 (1H, S, NH of amide); 10.21 (1H, S, NH of amide). Compound IVc ($C_{21}H_{24}N_6O_7S_2$) FT-IR (cm^{-1}) 3471 and 3435 (N-H_2 of sulfonamide), 3265 (N-H of amide), 1714 (C=O of thiazolidinone), 1676 (C=O of amide), 1519-1390 (asymmetric and symmetric of NO2), 1207 (C-S stretching). ^{1}H NMR (ppm): 1.04(2H, M, CH_2 of GABA); 3.22 (2H, S, CH_2-C=O); 3.29(1H, S, NH of amine);

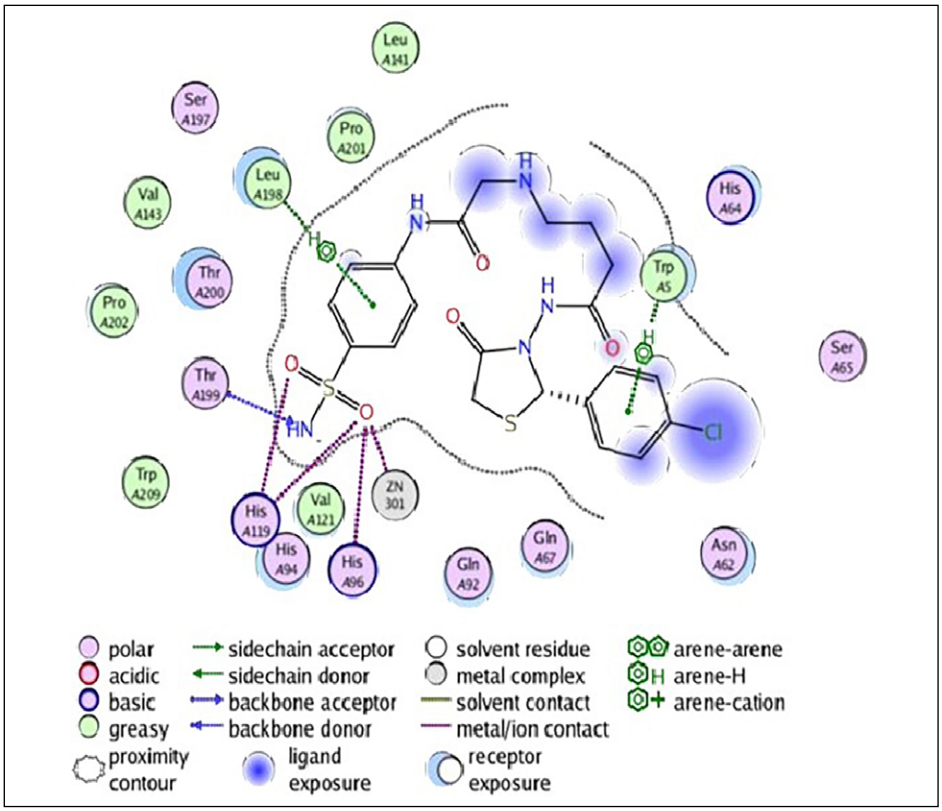

Fig. 6. IVb in combination with HCA IX (PDB code: 4M2V) (2D).

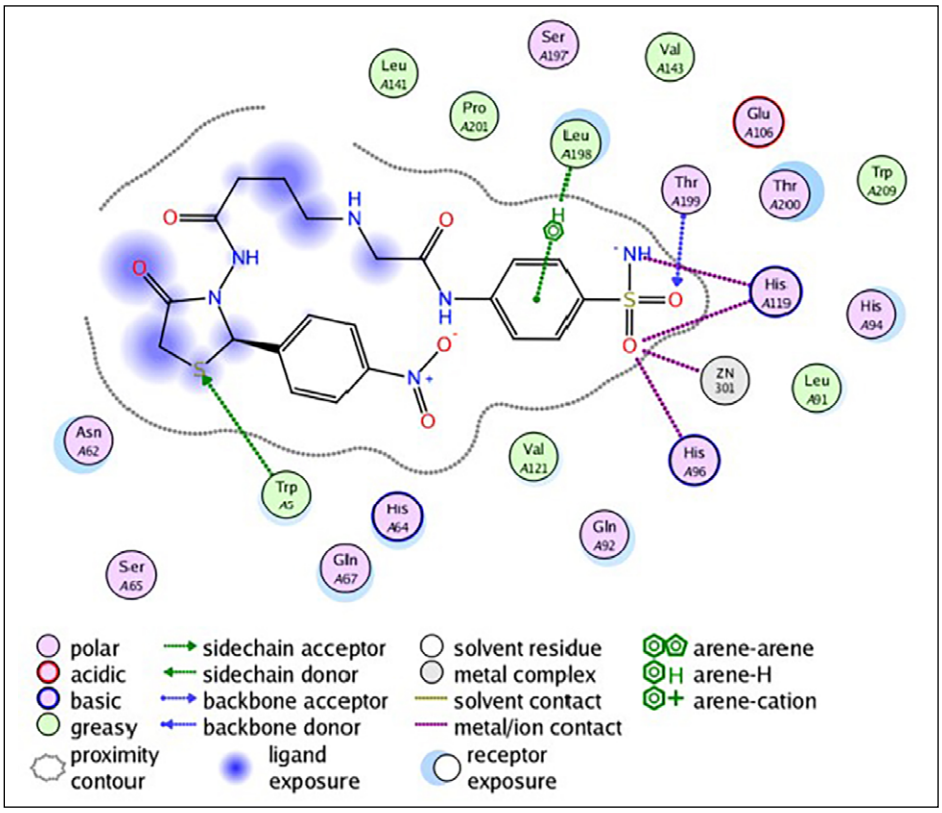

Fig. 7. IVc in combination with HCA IX (PDB code: 4M2V) (2D).

3.61-3.64 (4H, M, CH_2 of GABA); 3.67-3.95 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.41 (1H, S, CH of thiazolidinone); 6.60 (2H, S, NH_2 of sulfonamide); 7.64-7.28 (8H, M, Aromatic CH); 10.37 (1H, S, NH of amide); 10.51 (1H, S, NH of amide). Compound IVd ($C_{21}H_{24}BrN_5O_5S_2$) FT-IR (cm^{-1}) 3439-3392 (N-H_2 of sulfonamide), 3201 (N-H of amide), 1730 (C=O of thiazolidinone), 1668 (C=O of amide), 1286 (C-S stretching), 644 (stretching of C-Br). ^{1}H NMR (ppm): 1.28 (2H, M, CH_2 of GABA); 3.24 (2H, S, CH_2-C=O); 3.42 (1H, S, NH of amine); 3.64-3.73 (4H, M,

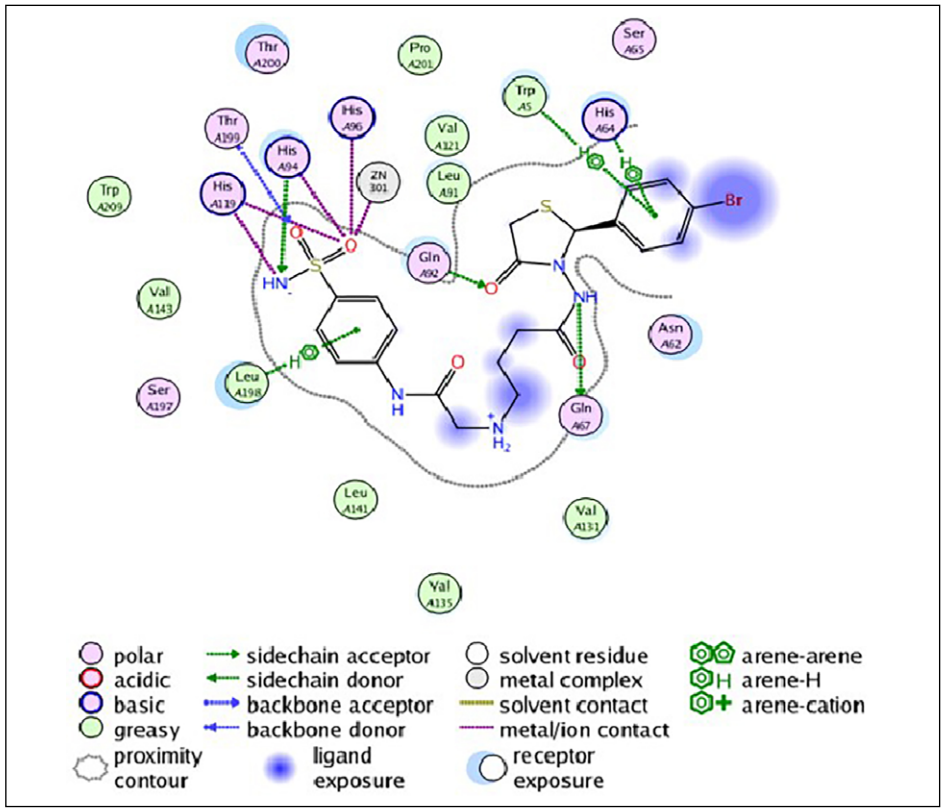

Fig. 8. IVd in combination with HCA IX (PDB code: 4M2V) (2D).

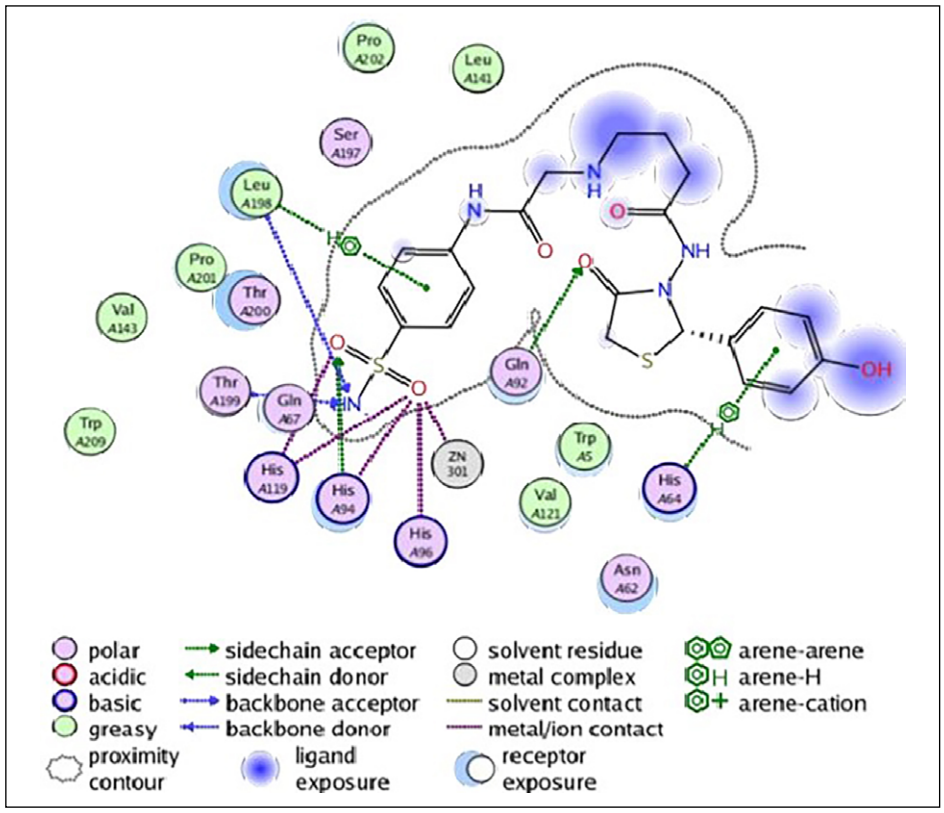

Fig. 9. IVc in combination with HCA IX (PDB code: 4M2V) (2D).

CH_2 of GABA); 3.90-4.03 (2H, Doublet of doublet, CH_2 of thiazolidinone); 5.29 (1H, S, CH of thiazolidinone); 6.68 (2H, S, NH_2 of sulfonamide); 7.35-7.59 (8H, M, Aromatic CH); 10.31 (1H, S, NH of amide). Compound IVe ($C_{21}H_{25}N_5O_6S_2$) FT-IR (cm^{-1}) 3433 (O-H stretching), 3342-3217 (N-H_2 of sulfonamide), 3142 (N-H of amide), 1718 (C=O of Thiazolidinone), 1641 (C=O of amide), 1292 (C-S stretching), 1156 (alcoholic C-O). ^{1}H NMR (ppm): 1.22 (2H, M, CH_2 of GABA); 3.21 (2H, S, CH_2-C=O); 3.31 (1H, S, NH of amine); 3.58-3.65 (4H, M, CH_2 of GABA); 3.95-4.02

(2H, Doublet of doublet, CH_2 of thiazolidinone); 5.16 (1H, S, CH of thiazolidinone); 6.63 (2H, S, NH_2 of sulfonamide); 7.17-7.79 (8H, M, Aromatic CH); 9.67 (1H, S, alcoholic OH); 10.37 (1H, S, NH of amide).

DOCKING STUDY

Molecular docking analysis, including protein and ligand structure preparation, was carried out by the study using the MOE (Molecular Operating Environment) software version 2015.10. We used ChemDraw Professional 12.0 to draw the ligand structures precisely. Afterward, we protonated the ligands in their 3D structure in MOE and applied partial charges. Then, we performed energy minimization. The structure of carbonic anhydrase IX (PDB code: 4M2V) was obtained from the Protein Data Bank database and imported into MOE for analysis. Isolating the target protein included identifying and eliminating non-functional chain sequences and small molecules. Hydrogen bonds were introduced, and the atomic potentials of the protein were modified. After identifying the active site, the next step is to import the already prepared ligands into MOE, and the docking process is carried out.

CYTOTOXIC STUDY

In the cytotoxicity study, we used two types of breast cells: MCF7 (malignant) and MCF10a (benign), both sourced from the National Cell Bank of Iran. Cell cultures were maintained in RPMI-1640 and DMEM media, each supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin at 100 U/mL and streptomycin at 100µg/mL). The cells were incubated at 37°C in a controlled atmosphere with 5% CO2 and appropriate humidity. Trypsin/EDTA and phosphate-buffered saline (PBS) were used for cell passage. The same culture conditions and media were used for 3D colony formation and regular monolayer cell cultures. The MTT assay was employed to assess cell growth and viability. For the monolayer culture, cells were trypsinized, counted, and seeded into 96-well plates at a density of 1.4×10^4 cells per well in 200 µL of fresh media. After forming a monolayer, the cells were exposed to various concentrations of compounds (ranging from 600µg/mL to 7.4µg/mL) for 24 hours at 37°C with 5% CO2. Post-treatment, the media were removed, and cells were incubated with MTT solution (0.5 mg/mL in PBS) for an additional 4 hours at 37°C. Subsequently, the MTT solution was replaced with 100 µL of dimethyl sulfoxide (DMSO) per well, and the cells were agitated at 37°C until complete dissolution of the formazan crystals. Cell viability was measured using an ELISA reader at an absorbance of 570 nm. The IC50 values, representing the concentration of compounds causing 50% cell death, were estimated by analyzing the dose-response curves.

RESULT AND DISCUSSION

CHEMISTRY

The appearance of a sharp band at 1689 cm^{-1} for the amide group signifies a nucleophilic attack by the electron pairs on the nitrogen atom of the primary amine in sulfonamide. This interaction with CAC leads to the formation of the 2-chloroacetamide derivative. Using methanol and thionyl chloride, Gamma-aminobutyric acid was esterified to produce its methyl ester. The presence of a 1730 cm^{-1} carbonyl band (for ester) indicates that GABA has been converted to methyl 4-aminobutanoate and the disappearance of the broad OH group of the carboxylic acid. The coupling of GABA ester with compound A in which the CABA ester amino group undergoes nucleophilic attack on the electrophilic carbon atom of chloroacetamide to give compound I, and it is formation confirmed by the absence of a 690 cm^{-1} peak corresponding to the stretching vibration of C-Cl, and the emergence of a 3292 cm^{-1} peak indicating the presence of a secondary amine. Hydrazide was obtained by reacting the resulting ester with 99% hydrated hydrazine. The ester's carbonyl group disappeared, while the amide band at 1668 cm^{-1} was unaffected. Additionally, at 3294-3190 cm^{-1}, the NH2 sulfonamide group overlapped with the primary amine group. The compounds (IIIa-e) were synthesized through Schiff base formation by reacting compound (II) with appropriate aldehydes in the presence of an acid catalyst. The formation of the imine bond was confirmed by FT-IR spectroscopy, indicated by absorption in the range of 1600-1660 cm^{-1}. Additionally, the absence of the aldehyde carbonyl bond and the disappearance of the characteristic aldehydic C-H stretching in the 1750-1850 cm^{-1} range further verified the reaction. The final cyclization and formation of the 4-thiazolidine ring, as depicted in Scheme 1, were achieved through a solvent-free synthesis by reacting compounds (IIIa-e) with thioglycolic acid, resulting in the formation of compounds (IVa-e). The structures of these compounds were confirmed by the appearance of new characteristic peaks corresponding to the carbonyl group of thiazolidinone in the range of 1718-1730 cm^{-1} and the disappearance of the thioglycolic acid broad OH peak.

CYTOTOXIC EVALUATION

We evaluated the cytotoxicity of substances (IVa, IVb,

IVc, IVd, and IVe) using MTT assays on cell lines. We employed two cell types, cancer cells (MCF7) and normal cells (MCF10), to assess the effects of our synthesized compounds. We determined the IC50 for each compound. The inhibitory action of each compound mentioned in this study was evaluated compared to acetazolamide, and their cytotoxic activity was compared to cisplatin, as indicated in Table 2.

The results indicate that each compound synthesized displayed inhibitory activity and possesses the potential to be developed into potent anticancer medications. The compounds showed varying levels of IC50 against the MCF7 and MCF10a cell lines, ranging from 28.55 to 41.29 μM and from 240.51 to 567.24 μM, respectively. All the compounds exhibited notable cytotoxicity variations compared to cisplatin, suggesting their lower efficacy against cancer cells. On the other hand, as compared to cisplatin, these compounds have a far lower toxicity to normal cells, suggesting that they may be more selective and have fewer side effects. Fig. 2 illustrates the correlation between the concentration of each compound and its impact on the viability of MCF7 cells, in which the inhibitory activity of acetazolamide is shown in Fig.2 A, while that of cisplatin is shown in Fig. 2B. Figures 2C, 2D, 2E, 2F, and 2G show the inhibitory actions of compounds IVa-e. Fig. 3 shows the relationship between the concentration of each compound and its effect on the viability of MCF10a cells. It depicts the inhibitory effects of various substances, including acetazolamide, in Fig. 3A, while that of cisplatin is shown in Fig. 3B. Figures 3C, 3D, 3E, 3F, and 3G show the inhibitory actions of compounds IVa-e.

DOCKING STUDY

The docking simulations identified multiple binding forms for the ligands within the 4M2V protein's binding pocket. The binding modes were identified based on specific residues in ligand-protein interactions, such as hydrogen bonding and electrostatic interactions. The results demonstrated varying binding affinities across the synthesized compounds, with greater interactions observed with the target protein than with acetazolamide. We observed a degree of similarity in the inhibition action of the produced compounds. All compounds bind to zinc, essential for inhibition activity and establishing hydrogen bonds with Th199 in the enzyme's active region. Furthermore, thiazolidinone moiety plays a vital role in binding to the enzyme's catalytic region by linking to important amino acids in substrate binding. Table 3 shows the result of the docking study.

Their S. score values were -8.8, -9.1, -8.55, -9.16, and -8.68, respectively, while their rmsd values were 1.6, 1.4, 1.9, 1.9, and 1.7, respectively.

CONCLUSIONS

This study aimed to explore the potential of newly developed sulfonamide-thiazolidinone hybrids as effective anticancer drugs, specifically targeting breast cancer. Compounds IVa-e were designed, thoroughly examined using computer simulations, chemically synthesized, and subjected to biological testing to evaluate their effectiveness against cancer. The compounds were examined for their cytotoxic effects against a cell line, resulting in IC50 values ranging from 28.55 to 41.29 μM. Among these compounds, the parabromo-derivative exhibited the highest cytotoxic activity against the tested breast cancer cell lines, with an IC50 of 28.55 μM. Furthermore, the same compound exhibited the highest s.score of 9.1 in relation to the in silico investigation, making it an excellent candidate for use as an anti-cancer drug. Furthermore, the four other compounds generated also displayed significant changes in cytotoxicity when compared to cisplatin. These compounds demonstrated promising efficacy against the enzyme carbonic anhydrase IX, as indicated by the docking results in MOE. However, these compounds demonstrate a notable variation in toxicity compared to cisplatin in normal cells, suggesting that they cause less harm to these cells. In summary, these results emphasize the capability of the produced compounds to inhibit tumor cells while minimizing harm to normal cells specifically. The data indicate that all five generated compounds, classified as sulfonamide-thiazolidinone, can potentially be employed as therapeutic agents for breast cancer. Further research is required to determine the in vivo anticancer efficacy more precisely.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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