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Synthesis and Molecular Modeling of 1,2,4 Triazole-Based Sulfanilamide Derivatives Using Dual Tail Strategy as Possible CAIs with their Cytotoxic Evaluation

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Abstract

Objective: The aim of the study was to design and synthesize a novel series of 1,2,4 triazole-based sulfanilamide derivatives targeting carbonic anhydrase IX (CA IX), a promising therapeutic target in cancer, using dual tail approach, and to assess their cytotoxic effect against breast and colon cancer cell lines.

Methods: The final compounds P1-P6 were synthesized starting from sulfanilamide. Characterization of their structure was performed using FT-IR, NMR and ESI-MS spectroscopies. Cytotoxic evaluation of the six derivatives was performed against breast and colon cancer cell lines compared to acetazolamide (AZM). In addition, molecular docking was conducted with CA II, CA IX and CA XII to compare fitting of the derivatives with zinc ion.

Results: showed that compound P5 had a potential cytotoxic effect against cancerous cell lines. Performing molecular docking revealed that P5 had good zinc binding interaction with CA IX compared with CA II and CAXII, indicating possible selectivity for CA IX.

Conclusion: According to the molecular docking and cytotoxic evaluation, compound P5 could be a promising CA IX inhibitor using the dual tail approach to improve selectivity against this isoform.

Keywords: Carbonic anhydrases inhibitors, triazole-based derivatives, dual tail approach, sulfanilamide

Introduction

CAs are enzymes responsible for catalyzing the hydration of carbon dioxide into bicarbonate that occurs during many physiological processes in all living organisms. They are classified as metalloenzymes due to the presence of a zinc ion that is tightly coordinated with the enzymes in their active sites.[1] Notably, human carbonic anhydrases (hCAs) that are part of the α-class family have sixteen diverse isoforms.[2]

CA IX plays an important role in the physiology of tumors by controlling the pH and influencing other processes in the cell microenvironment that facilitate cell rapid growth, invasion, or metastasis. CA IX is notably overexpressed via the hypoxia-inducible factor-1 (HIF-1) transcription factor and hence will lead to a pH difference in tumor tissue. Consequently, CA IX can be considered a promising target for hypoxic tumor therapy.[3]

Designing effective carbonic anhydrase inhibitors (CAIs) requires three necessary structural elements: first, a zinc-binding group that is capable of interacting with both the catalytic zinc ion and with the two residues, Thr199 and Glu106 that are preserved in all α-CAs active sites. Second, an organic scaffold that is a hydrophobic moiety, which could be an aromatic or a heteroaromatic moeity to which the third element (tail) is directly linked (Figure 1). Both hydrophilic and lipophilic moieties are regarded as tail units, which are capable of binding with hydrophobic or hydrophilic halves in the active site.[4]

The primary sulphonamides and related bioisosteres are the most significant class of CAIs; examples are AZM and furosemide, which are in clinical use; other compounds under investigation include indisulam and SLC-0111 (Figure 2), an ureido-based benzenesulfonamide with selective hCA IX inhibiting. It is presently undergoing Phase I/II clinical studies to treat advanced hypoxic tumors.[5]

The ring and the tail approaches are the two methods that have been extensively used in developing CAIs.[6] Furthermore, dual-tail approaches have been used to account for ligand interactions at the exterior borders of both hydrophobic and hydrophilic domains in order to investigate isoform-selective inhibitory patterns. Glycosidic and phenyl moieties that are attached to a sulfonamide scaffold were previously created by Tanpure et al. to create dual-tail CAIs.[7]

In light of what was mentioned above, the goal of the present work was to synthesize novel 1,2,4-triazole-based sulfanilamide derivatives (P1-P6) as potential CAIs and their anti-proliferative activities were evaluated *in-vitro* against two cancerous cell lines, MCF7 (a human breast cancer cell line) and HCT116 (a human colorectal carcinoma cell line), in addition to a non-cancerous cell line, HFF (a human foreskin fibroblast cell line).

Materials and Methods

The chemicals used for the synthesis were purchased from HyperChem (China). The FT-IR spectroscopy was accomplished by means of The Shimadzu IRAffinity-1 spectrometer (Shimadzu, Japan) at The University of Baghdad College of Pharmacy. The Bruker Avance III 400 MHz spectrometer at the College of Science (Basrah) was used to perform the ^{1}H-NMR and ^{13}C-NMR characterizations at 400 MHz and 100 MHz, respectively, with d6-DMSO as the solvent. The derivatives' cytotoxic effect together with Mass spectroscopy (ESI-MS) was performed at The Mashhad University of Medical Science, Iran.

Chemical Synthesis

Ethyl 2-((4-sulfamoylphenyl) amino) acetate synthesis (A)

Sulfanilamide (0.75 g, 0.0043 mol) was dissolved in 7 ml dried (dimethyl formamide) DMF, then ethylbromoacetate

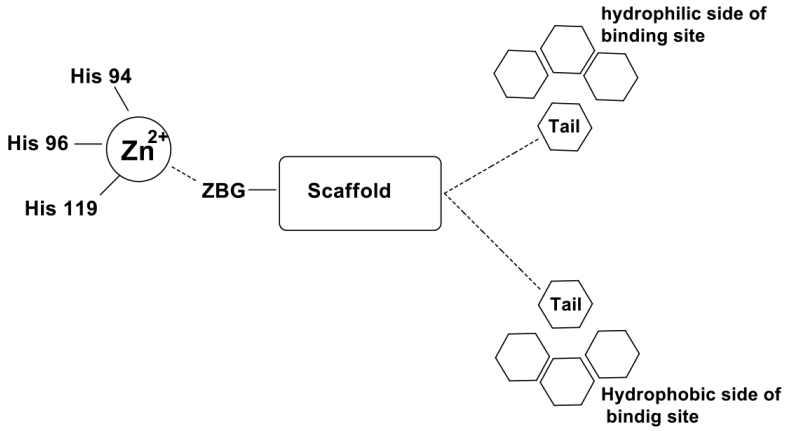


Fig. 1 **Scheme represents the general structure of carbonic anhydrase inhibitors.**

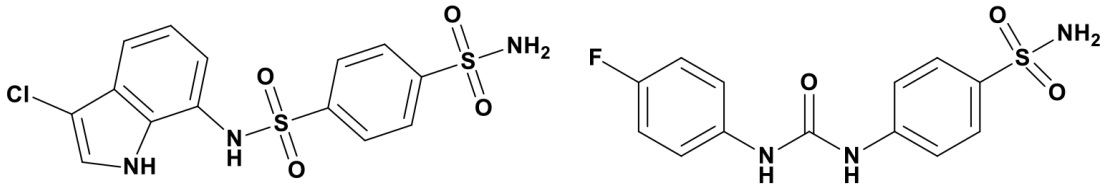


Fig. 2 **Structures of some CAIs.**

(0.72 ml, 0.0065 mol) was added dropwise, and the mixture was refluxed for 3 hours. The reaction was followed using TLC. The mixture was poured into a beaker, to which cold water was added, and kept in the fridge overnight. The solid product was collected by filtration, washed several times with water, and crystallized using DMF/water.[8]

Shiny white flakes, yield 47%, m.p. 138-141°C, R_f 0.5(ethylacetate: hexane;3:2). FT-IR (υ=cm^{-1}): 3356 (NH_2SO_2), 3267 (ArNH), 3074 (aromatic C-H), 2997 (CH_3), 2904 (CH_2), 1732 (ester C=O), 1600 (aromatic C-C), 1296&1138 (asym. & sym. SO_2), 1269 (C-O). ^{1}H NMR (400 MHz, DMSO) δ 7.53 (d, 2H, ArH), 6.97 (s, 2H, NH_2SO_2), 6.74 (t, 1H, N<u>H</u>CH_2), 6.63 (d, 2H ArH), 4.13 (q, 2H, OCH_2), 3.98 (d, 2H, NHCH_2), 1.21 (t, 3H, CH_3).

4-((2-hydrazinyl-2-oxoethyl) amino) benzenesulfonamide synthesis (B)

Compound A (0.5 g, 0.0019 mol) was suspended in 20 ml of absolute ethanol, then hydrazine hydrate (1.5 ml, 0.031 mol) was added, and a clear solution was obtained. The mixture was reflexed for 12 hours, and the reaction was followed using TLC. The mixture was poured into a beaker and kept in the fridge overnight. The solid product was filtered and washed with cold ethanol twice and crystallized from water.[9]

White needles, yield 85%, m.p. 176-178°C, R_f 0.5(Ethanol:toluene;2:3). FT-IR (υ=cm^{-1}): 3332&3290 (asym. & sym. NH_2), 3032 (aromatic C-H), 2900 (CH_2), 1658 (amide C=O), 1612&1508 (aromatic C-C), 1315&1138 (asym. & sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 9.18 (s, 1H, N<u>H</u>NH_2), 7.51 (d, 2H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.64 (m, 2H, ArH), 6.60 (s, 1H, N<u>H</u>CH_2), 4.26 (s, 2H, NH_2), 3.70 (d, 2H, CH_2).

General synthesis of 2-(2-((4-sulfamoylphenyl)amino) acetyl) hydrazine carbothioamide derivatives (C1-C4)

Compound B (0.0012 mol, 0.3 g) was suspended in 25 mL of dry methanol, then added separately to the mixture: 1-chloro-4-isothiocyanatobenzene (0.0012 mol, 0.2 g), 1-bromo-4-isothiocyanatobenzene (0.0012 mol, 0.25 g), 1-isothiocyanato-4-nitrobenzene (0.0012 mol, 0.22 g), 4-isothiocyanatobenzene (0.0012 mol, 0.147 ml). The reaction mixture was refluxed for 6 h., and then kept stirring overnight. The mixture was filtered, washed twice with cold ethanol, and then washed with water several times, crystallized from DMSO/water.[10]

N-(4-chlorophenyl)-2-(2-((4-sulfamoylphenyl)amino) acetyl) hydrazine carbothioamide **C1**: white powder, yield 80%, m.p. 205-207°C, R_f 0.5(Ethanol:toluene;2:3). FT-IR (**υ=cm**$^{-1}$): 3344 (NH_2SO_2), 3294 (*sec.* amide NH), 3259 (thioamide NH), 3097 (aromatic C-H), 1705 (amide C=O), 1600 (aromatic C-C), 1300&1145 (asym. &sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H, CONH), 9.78 (s, 1H, ArNH), 9.66 (s, 1H, NHN<u>H</u>CS), 7.56 – 7.38 (m, 6H, ArH), 6.98 (s, 2H, NH_2SO_2), 6.68 (d, 3H, ArH & N<u>H</u>CH_2), 3.89 (d, 2H, CH_2).

N-(4-bromophenyl)-2-(2-((4-sulfamoylphenyl) amino) acetyl) hydrazine carbothioamide **C2**: white powder, yield 80%, m.p. 198-200°C, R_f 0.5 (Ethanol:toluene;2:3). FT-IR (υ=cm^{-1}): 3348 (NH_2SO_2), 3298 (*sec.* amide NH), 3244 (thioamide NH), 3097(aromatic C-H), 1705 (amide C=O), 1600 (aromatic C-C), 1300&1145 (asym. & sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H, CONH), 9.79 (s, 1H, ArNH), 9.65 (s, 1H, NHN<u>H</u>CS), 7.54 (m, 4H, ArH), 7.42 (d, 2H, ArH), 6.98 (s, 2H, NH_2SO_2), 6.68 (t, 3H, ArH & N<u>H</u>CH_2), 3.89 (d, 2H, CH_2).

N-(4-nitrophenyl)-2-(2-((4-sulfamoylphenyl) amino) acetyl) hydrazine carbothioamide **C3**: yellow powder, yield 85%, m.p.211-212°C, R_f 0.75(Ethanol:toluene;2:3). FT-IR (**υ=cm**$^{-1}$): 3367 (NH_2SO_2), 3329 (*sec.* amide NH), 3278 (thioamide NH), 1705 (amide C=O), 1597&1546 (aromatic C-C), 1504&1334 (asym. &sym. NO_2), 1296&1145 (asym. &sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 10.26 (s, 1H, CONH), 10.09 (s, 1H, ArNH), 9.93 (s, 1H, NHN<u>H</u>CS), 8.24 (d, 2H,

ArH), 7.89(m, 2H, ArH), 7.53 (d, 2H, ArH), 6.98 (s, 2H, NH_2SO_2), 6.69 (d, 3H, ArH & $NHCH_2$), 3.91 (d, 2H, CH_2).

N-phenyl-2-(2-((4-sulfamoylphenyl) amino) acetyl hydrazine carbothioamide **C4**: white powder, yield 75%, m.p.204–206°C, R_f 0.5 (Ethanol:toluene;2:3). FT-IR (υ=cm^{-1}): 3348 (NH_2SO_2), 3282 (*sec*. amide NH), 3251 (thioamide NH), 3062 (aromatic C-H), 1701 (amide C=O), 1597 (aromatic C-C), 1300&1145 (asym. & sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 10.17 (s, 1H, CONH), 9.67 (s, 1H, ArNH), 9.62 (s, 1H, NHNHCS), 7.52 (d, 2H, ArH), 7.42 (s, 2H, ArH), 7.35 (t, 2H, ArH), 7.19 (s, 1H, ArH), 6.97 (s, 2H, NH_2SO_2), 6.68 (m, 2H, ArH), 6.66 (s, 1H, $NHCH_2$), 3.89 (d, 2H, CH_2).

General synthesis of 4-(((5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide derivatives (D1-D4)

Compounds C1-C4 (0.0007 mol) were separately added to a 15-ml D.W. containing NaOH (0.0035 mol), then stirred at room temperature for fifteen minutes until a clear solution was obtained. The mixture was then reflexed for 4 hours and then cooled to room temperature and neutralized using dilute HCl 10%. The precipitate was filtered and washed with water several times and crystallized from DMSO/water.[11]

4-(((4-(4-chlorophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide D1: white powder, yield 87%, m.p. 220–222°C, R_f 0.4 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3394 (NH_2SO_2), 3232 (NH), 3074 (aromatic C-H), 2893 (CH_2), 1593 (C=N),1489 (aromatic C-C) 1323&1149 (asym. &sym. SO_2).^{1}H NMR (400 MHz, DMSO) δ 13.91 (s, 1H, NH), 7.63 (m, 2H, ArH), 7.49 (m, 4H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.69 – 6.56 (m, 3H, ArH & $NHCH_2$), 4.21 (d, 2H, CH_2).

4-(((4-(4-bromophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide D2: white powder, yield 87%, m.p. 240-242°C, R_f 0.4 (Ethanol:Toluene;1:3) FT-IR (υ=cm^{-1}): 3394 (NH_2SO_2), 3232 (NH), 3074 (aromatic C-H), 2893 (CH_2), 1593 (C=N),1489 (aromatic C-C) 1323&1149 (asym. &sym. SO_2).^{1}H NMR (400 MHz, DMSO) δ 13.91 (s, 1H, NH), 7.77 (m, 2H, ArH), 7.50 (m, 2H, ArH), 7.43 (m, 2H, ArH), 6.95 (s, 2H, NH_2SO_2), 6.69 – 6.56 (m, 3H, ArH & $NHCH_2$), 4.21 (d, 2H, CH_2).

4-(((4-(4-nitrophenyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide D3: yellow powder, yield 85%, m.p. 250–252°C, R_f 0.3 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3371 (NH_2SO_2), 3232 (NH), 3082 (aromatic C-H), 2893 (CH_2), 1597 (C=N),1492 (aromatic C-C), 1523&1346 (asym. &sym. NO_2), 1307&1141 (asym. &sym. SO_2).^{1}H NMR (400 MHz, DMSO) δ 14.02 (s, 1H, NH), 8.41 (m, 2H, ArH), 7.78 (m, 2H, ArH), 7.49 (d, 2H, ArH), 6.95 (s, 2H, NH_2SO_2), 6.58 (m, 3H, ArH & $NHCH_2$), 4.29 (d, 2H, CH_2).

4-(((4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide D4: white powder, yield 80%, m.p. 236-238°C, R_f0.3 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3383 (NH_2SO_2), 3248 (NH), 3089 (aromatic C-H), 2843 (CH_2), 1593 (C=N),1481 (aromatic C-C) 1300&1145 (asym. &sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 13.87 (s, 1H, NH), 7.60 – 7.42 (m, 7H, aromatic), 6.95 (s, 2H, NH_2SO_2), 6.66 (t, 1H, $NHCH_2$), 6.57 (d, 2H, ArH), 4.18 (d, 2H, CH_2).

General synthesis of alkyl thiol derivatives (P1-P6)

Compounds (D1-D4) (0.00036 mol) were added separately to 10 ml of dry methanol, and then triethylamine (0.00054 mol, 0.07 ml) was added. After stirring for ten minutes, substituted benzylbromides: 1-(bromomethyl)-3-methoxybenzene (0.00036 mol, 0.05 ml), 1-(bromomethyl)-3-methylbenzene (0.00036 mol, 0.05 ml), 1-(bromomethyl) -3-nitrobenzene (0.0036 mol, 0.077 g) was added separately, and the mixture was stirred for 12 hours. The precipitate was filtered and washed with cold methanol twice; the product was crystallized from acetone/water.[12]

4-(((4-(4-chlorophenyl)-5-((3-methoxybenzyl)thio)-4H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide P1: white powder, yield 70%, m.p. 179–181°C, R_f0.6 (Ethanol:Toluene;1:3). FT-IR (**υ=cm**$^{-1}$): 3363 (NH2SO2), 3309 (NH), 3078 (aromatic C-H), 2935 (CH_3), 2831 (CH_2), 1597 (C=N),1492 (aromatic C-C), 1296 &1153 (asym. &sym. SO_2), 1234&1037 (asym. & sym. C-O-C OCH_3).^{1}H NMR (400 MHz, DMSO) δ 7.6 (m, 2H, ArH), 7.48 (m, 2H, ArH), 7.33 (m, 2H, ArH), 7.17 (t, *J* = 7.8 Hz, 1H), 6.96 (s, 2H, NH_2SO_2), 6.83 (m, 3H, ArH), 6.66 (d, 1H, NH), 6.59 (m, 2H, ArH), 4.30 (d, 4H, $NHCH_2$&SCH_2), 3.69 (s, 3H, CH_3).^{13}C NMR (101 MHz, DMSO) δ 159.68(C-O), 153.74(C-S, triazole ring), 150.89(C-NH, ring A), 150.86 (C-CH_2, triazole ring), 138.89, 135.11(C-N, ring C), 132.08 (C-Cl), 131.60 (C-SO_2), 130.26, 129.98, 129.48, 127.62, 121.52, 114.93, 113.53, 111.65 (*o*-C*2, ring A), 55.46 (CH_3), 38.20 ($NHCH_2$), 36.99 (S-CH_2). ESI-MS of compound P1, calculated m/z 515.09 $(m+1)^+$, found 515.512.

4-(((4-(4-bromophenyl)-5-((3-methoxybenzyl)thio)-4H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide P2: white powder, yield 70%, m.p. 198-200°C, R_f0.6 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3363 (NH_2SO_2), 3209 (NH), 3074 (aromatic C-H), 2935 (CH_3), 2831 (CH_2), 1597 (C=N),1489 (aromatic C-C), 1296&1149 (asym. & sym. SO_2), 1234&1037 (asym. & sym. C-O-C OCH_3).^{1}H NMR (400 MHz, DMSO) δ 7.73 (d, 2H, ArH), 7.48 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.16 (t, *J* = 7.9 Hz, 1H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.83 (m, 3H, ArH), 6.67 (t, *J* = 5.6 Hz, 1H, NH), 6.59 (d, 2H, ArH), 4.29 (m, 4H, $NHCH_2$&SCH_2), 3.69 (s, 3H, CH_3).^{13}C NMR (101 MHz, DMSO) δ 159.67 (C-O), 153.69 (C-S, triazole ring), 150.86 (C-NH), 150.83 (C-CH_2, triazole ring), 138.88, 133.21 (C-N, ring C), 132.49 (*m*-C*2, ring C), 131.59 (C-SO_2), 129.98, 129.73, 127.61, 123.76, 121.52 (C-Br), 114.91, 113.53, 111.66 (*o*-C*2, ring A), 55.46 (CH_3), 38.19 ($NHCH_2$), 36.99 (S-CH_2). ESI-MS of compound P2, calculated m/z 561.03 $(m+1)^+$, found 560.9.

4-(((5-((3-methoxybenzyl) thio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide P3: yellow powder, yield 80%, m.p. 202–204°C, R_f0.6 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3379 (NH_2SO_2), 3236 (NH), 3078 (aromatic C-H), 2943 (CH_3), 2835 (CH_2), 1597 (C=N),1492 (aromatic C-C),1523 &1346 (asym. &sym. NO_2), 1307&1145 (asym. &sym. SO_2), 1234&1037 (asym. &sym. C-O-C OCH_3).^{1}H NMR (400 MHz, DMSO) δ 8.34 (m, 2H, ArH), 7.57 (m, 2H, ArH), 7.47 (m, 2H, ArH), 7.15 (m, 1H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.81 (m, 3H, ArH), 6.61 (t, 1H, NH), 6.56 (m, 2H, ArH), 4.38 (d, 2H, $NHCH_2$), 4.28 (s, 2H, SCH_2), 3.68 (s, 3H, CH_3).^{13}C NMR (101 MHz, DMSO) δ 159.66 (C-O), 153.78 (C-S, triazole ring), 150.70 (C-NH, ring A), 150.52 (C-CH_2, triazole ring), 148.34 (C-NO_2), 138.78 (C-N, ring C), 138.66, 131.70 (C-SO_2), 130.00, 129.11, 127.60, 125.41, 121.49, 114.90, 113.53, 111.65 (o-C*2, ring A), 55.43 (CH_3), 38.25 ($NHCH_2$), 37.35 (S-CH_2). ESI-MS of compound P3, calculated m/z 526.11 $(m+1)^+$, found 528.7.

4-(((5-((3-methoxybenzyl) thio)-4-phenyl-4H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide P4: white powder, yield 65%, m.p. 150–152°C, R_f 0.6 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3367 (NH_2SO_2), 3244 (NH), 3059 (aromatic C-H), 2993 (CH_3), 2835 (CH_2), 1597 (C=N),1492 (aromatic C-C), 1319&1149 (asym. &sym. SO_2), 1211&1037 (asym. &sym. C-O-C OCH_3). ^{1}H NMR (400 MHz, DMSO) δ 7.52 (m, 5H, ArH), 7.32 (dd, 2H, ArH), 7.18 (t, 1H, ArH), 6.97 (s, 2H, NH_2SO_2), 6.86(m,3H, ArH), 6.69 (t, 1H, NH), 6.59 (d, 2H, ArH), 4.30 (m, 4H, $NHCH_2$&SCH_2), 3.70 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 159.68 (C-O), 153.71 (C-S, triazole ring), 150.96 (C-NH), 150.94 (C-CH_2, triazole ring), 138.94, 133.15 (C-N, ring C), 131.50 (C-SO_2), 130.44, 130.25, 129.99, 127.61, 127.52, 121.57, 114.98, 113.48, 111.63(*o*-C*2, ring A), 55.47 (CH_3), 38.18 ($NHCH_2$), 36.72(S-CH_2). ESI-MS of compound P4, calculated m/z 481.12 $(m+1)^+$, found 481.682.

4-(((4-(4-bromophenyl)-5- ((3-methylbenzyl) thio)-4H-1,2,4-triazol-3-yl)methyl)amino)benzenesulfonamide P5: white powder, yield 70%, m.p. 172–174°C, R_f 0.6 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3363 (NH_2SO_2), 3240 (NH), 3066 (aromatic C-H), 2931 (CH_3), 2850 (CH2), 1597 (C=N),1492 (aromatic C-C), 1319&1149 (asym. &sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 7.73 (m, 2H, ArH), 7.48 (d, 2H, ArH), 7.24 (d, 2H, ArH), 7.13 (t, 1H, ArH), 7.05 (d, 3H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.67 (t, 1H, NH), 6.59 (d, 2H, ArH), 4.29 (d, 4H, $NHCH_2$&SCH_2), 2.23 (s, 3H, CH_3).^{13}C NMR (101 MHz, DMSO) δ 153.69 (C-S, triazole ring), 150.88 (C-NH), 138.14 (C-CH_2, triazole ring), 137.20 (C-CH_3, ring B), 133.19 (C-CH_2, ring B), 132.57 (C-N, ring C), 131.75 (*m*-C*2,ring C), 129.93(C-SO_2), 129.75, 128.81, 128.61, 127.63, 126.40, 123.71 (C-Br), 111.71 (*o*-C*2,ring A), 38.26($NHCH_2$), 37.25 (S-CH_2), 21.32 (CH_3). ESI-MS of compound P5, calculated m/z 545.04 $(m+1)^+$, found 543.368.

4-(((5- ((3-methylbenzyl) thio)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl) methyl)amino)benzenesulfonamide P6: yellow powder, yield 70%, m.p. 200-203°C, R_f 0.6 (Ethanol:Toluene;1:3). FT-IR (υ=cm^{-1}): 3387 (NH_2SO_2), 3309 (NH), 3082 (aromatic C-H), 2978 (CH_3), 2916 (CH_2), 1597 (C=N), 1523&1342(asym. &sym. NO_2), 1492 (aromatic C-C), 1307&1145 (asym. & sym. SO_2). ^{1}H NMR (400 MHz, DMSO) δ 8.34 (m, 2H, ArH), 7.56 (m, 2H ArH,), 7.47 (d, 2H, ArH), 7.06 (m, 4H, ArH), 6.96 (s, 2H, NH_2SO_2), 6.59 (m, 3H, aromatic &NH), 4.38 (d, 2H, $NHCH_2$), 4.28 (s, 2H, SCH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 153.75 (C-S, triazole ring), 150.71 (C-NH, ring A), 150.58 (C-CH_2, triazole ring), 148.37 (C-NO_2), 138.67 (C-N, ring C), 138.15 (C-CH_3, ring B), 137.10 (C-CH_2, ring B), 131.71 (C-SO_2), 129.94, 129.11, 128.85, 128.66, 127.61, 126.42, 125.42, 111.65 (*o*-C*2,ring A), 38.25 ($NHCH_2$), 37.44 (S-CH_2), 21.31 (CH_3). ESI-MS of compound P6, calculated m/z 510.11 $(m+1)^+$, found 509.6.

In Vitro Cytotoxicity Study

Cytotoxicity Assay

Cell growth and viability was assessed using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (Sigma-Aldrich) test on the final compounds to evaluate their cytotoxic effect on breast and colon cancer cell lines. Using an ELISA reader and a 570 nm absorbance measurement, the vitality of the cells were assessed.[13,14]

Molecular docking

The Docking study was accomplished using a licensed Glide program embedded within the maestro software from Schrodinger's modeling suite (version 13.013).[15] The crystal structure of CAII (PDB ID: 4HS3),[16] CA IX (PDB ID: 5FL4)[17] and CAXII (PDB ID: 8CO3)[18,19] proteins was downloaded from the protein data bank (PDB). The protein preparation involved the removal of water molecules and non-essential atoms, then adding the missed atoms in protein residue and hydrogen atoms. All ligands in the workspace were imported from files drawn by the ChemBioDraw Ultra 12.0 program.[20] AZM served as the standard substance. The extra precision (XP) docking mode was used to conduct the molecular docking investigate for the prepared ligands and the constructed target protein.[21]

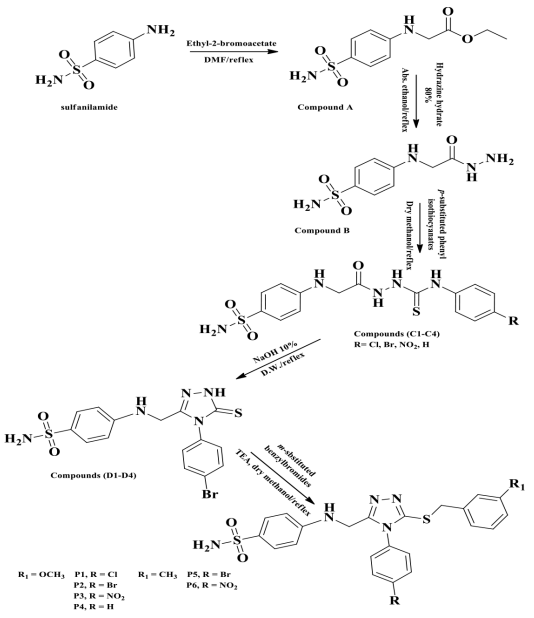
Results

Chemical Synthesis

The reaction of sulfanilamide with ethylbromoacetate led to the formation of compound A. Compound A underwent hydrazinolysis with excess hydrazine hydrate in absolute ethanol and resulted in compound B. The reaction of (B) with different substituted phenyl isothiocyanates was performed in dry methanol and provided thiosemicarbazide derivatives (C1-C4). Compounds (D1-D4) were prepared by cyclization in basic medium using thiosemicarbazides (C1-C4). Compounds P1–P6 were formed by treating compounds (D1–D4) using different substituted benzylbromides. Scheme 1 summarizes the general routes for the synthesis of the desired compounds.

Cytotoxicity Study

Considering the reported overexpression of hCAIX in cases of colorectal and breast cancers,[22] the *in vitro* cytotoxic activity of the synthesized derivatives P1-P6 was estimated on MCF7,



Scheme 1. **Synthesis pathways used to prepare the target compounds.**

HCT116, and HFF, at concentrations of 6.25, 12.5, 25, 50, and 100 µM. AZM was used as a reference drug, and the concentration of the derivatives that resulted in 50% inhibition of cell growth (IC_{50}) was measured as in (Figure 3).[23]

Molecular Docking

The docking scores of P1-P6 and AZM on three CA isoforms (CA II, CA IX, and CAXII) are demonstrated in Table 1, and the two-dimensional (2D) interactions of compound P5 with CA IX are in Supplementary File 2.

Discussion

Chemical Synthesis

The IR spectrum (Supplementary File 1) of compound A demonstrated an easily distinguishable band at ύ= 1732 cm^{-1} assigned for (C=O, ester). Compound B was identified by the appearance of two bands in its IR spectrum at ύ= 3332 cm^{-1} and ύ=3290 cm^{-1} and were attributed to the primary amine of hydrazide, as well as a marked band at ύ = 1658 cm^{-1} pounds C1-C4 displayed in their IR spectra the following typical absorption bands: ύ= (3282–3329) cm^{-1} attributed to (NH) stretching of secondary amide; ύ= (3128–3248) cm^{-1} owing to (NH) stretching of thioamide; ύ= (1701–1705) cm^{-1} for (C=O) stretching of the amide I band; and ύ= (1211–1219) cm^{-1} correlated to (C=S) stretching. The appearance of distinguishing absorption bands at ύ= (1593–1597) cm^{-1}, that were attributed to C=N stretching in the IR spectra of compounds (D1-D4) confirmed the successful cyclization into triazoles. Moreover, the appearance of a weak band at 2762 cm^{-1} was correlated to (SH) stretching and a typical absorption band at (1226–1232) cm^{-1} for (C=S) stretching were observed. The IR spectra of the target compounds P1-P6 were characterized by the disappearance of SH stretching, proving the successful SH-alkylation with m-substituted benzylbromides.[24]

The ^{1}H NMR spectrum (Supplementary File 1) of compound A revealed a quartet and a triplet signals at δ = 4.13 ppm and δ = 1.21 ppm owing to the aliphatic (CH_2) and CH_3 respectively. Compound B exhibited a distinctive signals at δ = 9.18 and 4.26 ppm for NH and NH_2 of the hydrazide, respectively. The thiosemicarbazide derivatives C1-C4 revealed a distinctive singlet at δ = (9.62–9.93) ppm, attributed to the (NH-amide), and a signal at δ = (9.67–10.09) ppm for (NH-thioamide). The successful synthesis of compounds (D1-D4) was illustrated by the appearance of a recognizable signal at δ= (13.87–14.02) ppm, assigned to (NH-triazole). Notably, the target compounds P1-P4 showed a distinctive signal at (δ = 3.7-3.96) ppm, attributed to (CH_3), while compounds P5 and P6 showed a distinctive signal at (δ = 2.2) ppm, attributed to methyl group.[25]

^{13}C NMR spectra revealed that all compounds P1-P6 have signals at δ= 38.19-38.25 and δ= 36.72–37.44 ppm, due to $NHCH_2$ and SCH_2, respectively. Compounds P1-P4 $^{13}CNMR$ spectra exhibited typical signals of C-O and CH_3 at δ= 159.66-159.69 and 55.43-55.47 ppm, respectively due to the methoxy group. Compounds P5 and P6 ^{13}C NMR spectra displayed typical signals of the CH_3 group at δ= 21.34 and 21.31 ppm, respectively. The ESI-MS spectra of compounds P1-P6 (Supplementary File 1) confirmed that the target compounds were successfully synthesized.[26]

Cytotoxicity Study

The results of the MTT assay, explained that compound P5 had a better cytotoxic activity on HCT116 with IC_{50} of 42.86 µM compared to the AZM IC_{50} of 110 µM. Moreover, compound P5 had a good cell growth inhibition on MCF7 with IC_{50} of (86.44 µM) compared to the AZM with IC_{50} of (149 µM).

Molecular Docking

The binding energy of AZM against CA IX was –7.5 kcal/mol, with one hydrophobic binding site with HIS94 and two H-bonds with GLN92 and THR200. Moreover, it exhibited a bidentate interaction with the zinc ion (Figure 4). P5 binding energy against CAIX was –7.0, which were close to that of AZM. P5 showed bidenatate interaction with zinc ions in addition to hydrogen bonding with THR200 and hydrophobic interaction with HIS94 (Figure 5).

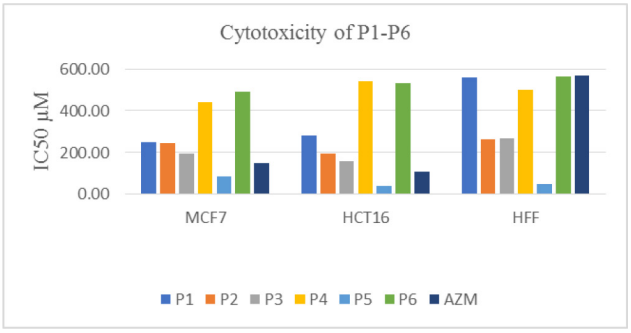


Fig. 3 **Effect of P1-P6 compounds on cell viability of MCF7, HCT116 and HFF cell lines using AZM as reference standard.**

Table 1. **The docking scores of AZM and the final compounds (P1-P6) against three isoforms of CAs**

Compound ID	Docking score (kcal/mol)		
	CA II/3HS4	CA IX/5FL4	CA XII/8C03
P1	–4.08	–7.4	–3.95
P2	–2.3	–7.24	–2.8
P3	–5.57	–3.0	–4.73
P4	–4.21	–7.66	–4.12
P5	–5.46	–7.0	–3.93
P6	–4.11	–2.72	–2.83
AZM	–8.6	–7.5	–8.0

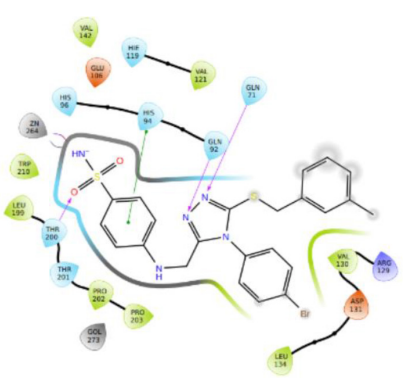


Fig. 4 **2D interaction of compound P5 with CA IX.**

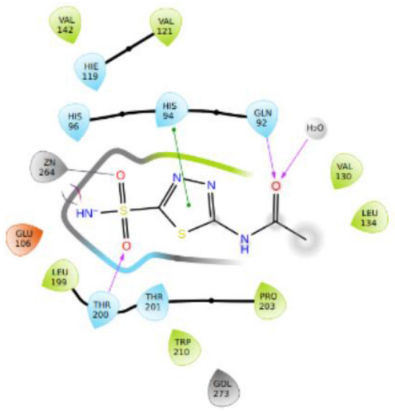


Fig. 5 **2D interaction of compound AZM with CA IX.**

Conclusions

Synthesis of the target sulfanilamide derivatives (P1-P6) was achieved successfully starting from sulfanilamide. P5 showed cytotoxic effect against cancer cell lines (MCF7 and HCT116) that was better than that of AZM. Both the docking and the cytotoxicity study results indicate that P5 could be a promising CA IX inhibitor, having sulfonamide functionality as a ZBG and a disubstituted triazole ring as a dual tail moiety.

Supplementary Materials

Supplementary File 1 illustrated the FT-IR, NMR spectroscopic analysis of the synthesized compounds. Figures that demonstrated the 2D interaction of the target compounds P1-P6 with CA II, CA IX and CA XII are illustrated in the Supplementary File 2.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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