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In silico study, Synthesis, Characterization and Preliminary Evaluation of Antimicrobial activity of new sulfonamide – 1,2,4-triazole derivatives

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Abstract

A new series of sulfonamide derivatives (6a-c), containing 1,2,4-triazole-3-thiol (5a-c) ring system, were synthesized and their structures were identified by using ATR-FT-IR and ^{1}HNMR spectroscopic analytical techniques. Bacteria *H. pylori* is the most common etiology of gastric ulcers, and gastric cancer in humans. The carbonic anhydrase enzyme regulates the acid-base balance and enables survival in the stomach's highly acidic environment. This study utilized computational techniques to screen potential carbonic anhydrase inhibitors that target *H. pylori* as a therapeutic approach. The synthesized compounds interact with the zinc-binding domain of the *H. pylori* carbonic anhydrase (PDB: 4YGF) enzyme, causing disturbance of the acid-base equilibrium and affecting the ability to adapt to the stomach's acidic environment. This, in turn, affects the pathogenicity and virulence of the *H. pylori*. The synthesized compounds undergo in vitro evaluation to assess their antimicrobial activity against specific strains of Gram-positive, and Gram-negative bacteria, and fungi species and compare them with standard antimicrobial drugs such as sulfamethoxazole, sulfadiazine, and fluconazole. The results indicate successful synthesis of the target compounds, which exhibit a high activity against, Gram-positive bacteria (*S. aureus, S. pneumonia, and B. subtilis*), moderate to a high activity against Gram-negative bacteria (*P. aeruginosa, E. coli, N. gonorrhoeae and H. pylori*), and highly active against fungi species (*C. albicans*).

Keywords: Sulfonamide, 1,2,4-triazole-3-thiol, carbonic anhydrase, computational techniques, zinc-binding domain.

Introduction

The main cause of drug resistance in the healthcare setting is the excessive and incorrect consumption of different antimicrobial agents. Many strategies have been taken to find novel antibacterial compounds capable of fighting resistant organisms [(1)]. These approaches include improving existing chemical classes or discovering new chemical scaffolds that target known or previously identified antibacterial targets [(2, 3)].

Sulfonamides are an important class in the area of medicinal chemistry [(4)] due to their wide variety of pharmacological activities, including anti-inflammatory [(5)], anti-cancer [(6, 7)], anti-glaucoma [(8)], anti-diabetic [(9)], and anti-microbial properties [(10, 11)]. Sulfonamide moieties act as carbonic anhydrase inhibitors and antimicrobial agents [(12, 13)]. All classes of carbonic anhydrases (CAs) are metalloenzymes, meaning that their catalytic sites contain a crucial metal ion cofactor necessary for catalysis [(14, 15)]. Only the α-class of CAs is found in mammals; α-, β-, γ-, δ-, and θ-classes are found in plants and algae; α- and β-CAs are encoded by fungi; α-, β-, and η-CAs are encoded by protozoa [(14)]. Several species of human pathogens, including *H. pylori* [(16)], *Vibrio cholera* [(17)], *E. coli* [(18)], *P. aeruginosa* [(19)], and *Burkholderia pseudomallei* [(20)], rely on CAs for their survival, pathogenicity, and virulence. A lot of biologically active compounds originate from heterocyclic compounds [(21)]. Many promising medicinal drug candidates, including those with anti-inflammatory [(22)], anti-viral [(23)], anti-cancer [(24, 25)], and antimicrobial properties [(26, 27)], have 1,2,4-triazole ring system [(21, 28)]. To combat the rapidly developing resistance to drugs, new agents should possess chemical characteristics that are distinct from existing agents [(29)] Among these characteristics, the inclusion of a sulfonamide-containing heterocyclic moiety has proven to be particularly significant in the field of medicinal chemistry [(30, 31)].

Materials and methods

Molecular docking

The process was initiated by obtaining the crystal structure of *H. pylori* (PDB code: 4ygf) from the Protein Data Bank (PDB). To narrow our attention to the region where the ligand interacts, we eliminated water molecules more than 5 Å away from the binding site. To replicate physiological settings, we conducted protein preparation at a pH of 7. This procedure entailed the optimization of hydrogen atoms and the assignment of bond ordering.

Afterward, we optimized the protein structure using the OPLS4 force field, a widely recognized molecular mechanics force field for biomolecular simulations. Furthermore, the ligand was prepared by applying the OPLS4 force field at a pH of 7 ± 2. This was done to ensure that the ligand molecule's shape and net charge were consistent with physiological conditions. As well as we considered the addition of a metal binding state of the ligands.
The grid-based method was used to define the specific area of interest for the binding site for conducting docking simulations. The process entailed creating a grid using the co-crystallized ligand, which yielded crucial information on the suitable binding pocket for the new ligand. Positioned the indigenous ligand at the focal point of the grid and constructed a cuboid with dimensions of 20 Å surrounding it. This delineated the area in which the upcoming docking simulation would occur.
Glide, an advanced docking software program developed by Schrödinger (version 2023) for the docking simulation, was employed. Typical precision docking with flexible sampling of the ligand to thoroughly investigate the interactions between the ligand and protein inside the specific binding pocket was used. By employing this method, we evaluated candidate binding positions and anticipated the binding configurations that would yield the highest energy stability between the ligand and the protein target [32, 33].
Docking validation was conducted by redocking the reference ligand and comparing the docked conformation with the original conformation (the cocrystallized) [34].

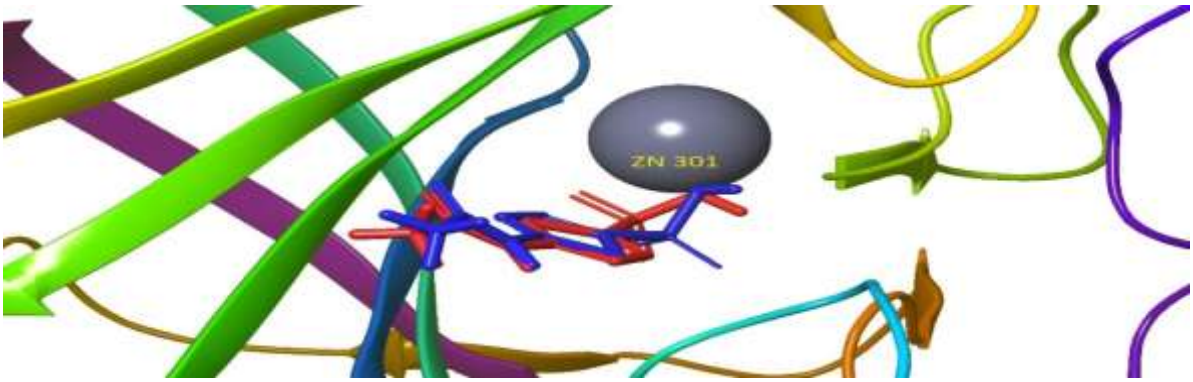


Figure 1. Docking validation by redocking the co-crystallized ligand. The molecule in red is the reference ligand from the crystal structure, while the molecule in blue is the docked ligand. The low differences in the conformation between the red and blue ligands indicate a good docking protocol validation. RMS value is 3.5 Å.

Chemical synthesis
Materials

The solvents and chemicals utilized in the synthesis were analytical grades obtained from private sources and employed without additional purification. The Thin Layer Chromatography (TLC) technique was utilized to confirm the reaction's success and assess the product's purity. The Stuart SMP30 was utilized to measure the uncorrected melting points using the capillary tube and electronic melting point apparatus. The University of Baghdad/College of Pharmacy acquired the infrared spectra using an ATR FT-IR spectrophotometer manufactured by Shimadzu, Japan. ^{1}HNMR spectra were acquired using a BRUKER model Ultra Shield 300 MHz spectrophotometer with DMSO-d6 as the solvent at the University of Mashhad in the Islamic Republic of Iran.

Synthesis of 4-acetamidobenzene sulfonyl chloride (compound1)[35]

The dry conical flask that contains (2.7 g, 20 mmol) of acetanilide is immersed in a cold water bath. Chlorosulfonic acid (8 ml, 120 mmol) was added from the dropping funnel all at once and immediately connected to the conical flask, which contained some amount of water to trap the liberated gas. The solution is rapidly stirred, keeping the temperature below 20 °C. Then the flask was heated in a hot water bath at 70–80 °C for 20 min, to complete the reaction after the acetanilide had mostly dissolved and the initial exothermic reaction had subsided. Once the flask had cooled to room temperature, it was transferred into a 250 ml beaker holding 100 g of crushed ice, and stirred by glass road to prevent the formation of large lumps. The precipitate was collected by vacuum filtration and washed with cold distilled water till the filtrate was neutral to PH paper. After the product was dried, washed with toluene, and recrystallized from chloroform to give a white to

cream-beige solid, yield 75%, melting point 143–145 °C.

General procedure for the synthesis of methyl esters (2a-c)[36]

In a round bottom flask, carboxylic acids 1.22, 1.57, and 1.67 grams of benzoic acid, 4-chlorobenzoic acid, and 4-nitrobenzoic acid respectively equivalent to (10 mmol) were dissolved in 20 ml of methanol. The flask was down-cooled in the ice bath, and thionyl chloride 1ml equivalent to (15 mmol) was added dropwise over 10 min. The mixture was refluxed for 3h. The solvent was evaporated, and the solid ester was redissolved in dichloromethane and extracted in a separatory funnel with D.W., then with a 10% saturated solution of sodium bicarbonate to remove excess unreacted carboxylic acid. The drying agent (Na_2SO_4) and evaporation of dichloromethane gave the ester in pure form.

Methyl-4-nitrobenzoate, compound (2a)

Off-white crystals, yield 90%; m.p. 90-92 °C. FT-IR (υ= cm^{-1}): 3113 aromatic (C-H) stretching, 2958 and 2854 asymmetric and symmetric stretching respectively of CH_3, 1716 (C=O) stretching of ester, 1608 and 1590 aromatic (C=C) stretching, 1519 and 1346 asymmetric and symmetric stretching respectively of NO_2, 1269 (C-O) stretching of ester, 875 (C-N) stretching of nitroaromatic.

Methyl-4-chlorobenzoate, compound (2b)

White-crystals, yield 85%, m.p. 39-40 °C. FT-IR (υ= cm^{-1}): 3039 aromatic (C-H) stretching, 2954 and 2850 asymmetric and symmetric stretching respectively of CH_3, 1716 (C=O) stretching of ester, 1593 and 1489 aromatic (C=C) stretching, 1273 (C-O) stretching of ester, 1087 (C-Cl) stretching of chlorobenzene.

Methyl benzoate, compound (2c)

Yellow oil liquid, yield 70%, B.p. 196-198 °C. FT-IR (υ= cm^{-1}): 3032 aromatic (C-H) stretching, 2951 and 2850 asymmetric and symmetric stretching respectively of CH_3, 1716 (C=O) stretching of ester, 1600 and 1492 aromatic (C=C) stretching, 1273 (C-O) stretching of ester.

General procedure for the synthesis of hydrazide (3a-c)[37, 38]

Aroyl esters (10 mmol) were added to a round-bottom flask that contained 25 ml of absolute ethanol. Hydrazine hydrate (80%, 50 mmol) was added gradually with shaking. The mixture was refluxed, and the progress of the reaction was monitored by thin-layer chromatography (Ethyl acetate: Hexane (2:1)) as a mobile phase. After completion of the reaction, the volume of ethanol was reduced by evaporation, and then cold D.W. was added. The formed solid precipitate was collected by filtration, washed with cold D.W. several times, and recrystallized from ethanol.

4-Nitrobenzohydrazide, compound (3a)

Yellow crystals, yield 85%, m.p. 195-198 °C. FT-IR (υ= cm^{-1}): 3329 and 3255 asymmetric and symmetric stretching respectively of NH_2, 3109 aromatic (C-H) stretching, 1647 (C=O) amide **I** band, 1620 (N-H) amide **II** band, 1593 and 1489 aromatic (C=C) stretching, 1508 and 1338 asymmetric and symmetric stretching respectively of NO_2.

4-Chlorobenzohydrazide, compound (3b)

White needle-like crystals, yield 80%, m.p. 153-155 °C. FT-IR (υ= cm^{-1}): 3309 and 3213 asymmetric and symmetric stretching respectively of NH_2, 3012 aromatic (C-H) stretching, 1658 (C=O) amide **I** band, 1612 (N-H) amide **II** band, 1554 and 1481 aromatic (C=C) stretching, 1091 (C-Cl) stretching of chlorobenzene.

Benzohydrazide, compound (3c)

White needle-like crystals, yield 75%, m.p. 110-112 °C. FT-IR (υ= cm^{-1}): 3298 and 3197 asymmetric and symmetric stretching respectively of NH_2, 3012 aromatic (C-H) stretching, 1658 (C=O) amide **I** band, 1612 (N-H) amide **II** band,1554 and 1485 aromatic (C=C) stretching.

General procedure for the synthesis of potassium dithiocarbamate salt (4a-c)[37]

A solution was created by dissolving (20 mmol) of potassium hydroxide in 30 ml of dry ethanol. The solution was then cooled, and (10 mmol) of hydrazide (3a-c) was added. While continuously stirring, (30 mmol) of carbon disulfide was slowly added to the clear, cold solution. The mixture was left to stir at room temperature (25 °C) for 18 h. Once complete, anhydrous diethyl ether was added, resulting in the formation of a solid precipitate. This precipitate was filtered through Whatman filter paper, and the resulting salt was used in the next step without further purification.

Potassium 2-(4-nitrobenzoyl)hydrazine-1-carbodithioate, compound (4a)

Orange crystals, yield 75%, m.p. 280-282 °C decomposed. FT-IR (υ= cm^{-1}): 3402 and 3197 (N-H) stretching of secondary amide and thioamide respectively, 3101 aromatic (C-H) stretching, 1651 (C=O) amide **I** band, 1597 (N-H) amide **II** band, 1508 and 1342 asymmetric and symmetric stretching respectively of NO_2, 1465 aromatic (C=C) stretching, 1060 (C=S) stretching, 651 (C-S) stretching.

Potassium 2-(4-chlorobenzoyl)hydrazine-1-carbodithioate, compound (4b)

Yellow crystals, yield 50%, m.p. 252-255 °C decomposed. FT-IR (υ= cm^{-1}): 3367 and 3236 (N-H) stretching of secondary amide and thioamide respectively, 3089 aromatic (C-H) stretching, 1620 (C=O) amide **I** band, 1600 (N-H) amide **II** band, 1519 and 1469 aromatic (C=C) stretching, 1091

(C-Cl) stretching of chlorobenzene, 1064 (C=S) stretching, 663 (C-S) stretching.

Potassium 2-benzoylhydrazine-1-carbodithioate, compound (4c)

off white crystals, yield 70%, m.p. 250-252 °C decomposed. FT-IR (υ= cm^{-1}): 3302 and 3248 (N-H) stretching of secondary amide and thioamide, 3109 aromatic (C-H) stretching, 1620 (C=O) amide **I** band, 1573 (N-H) amide **II** band, 1508 and 1481 aromatic (C=C) stretching, 1068 (C=S) stretching, 640 (C-S) stretching.

General procedure for the synthesis of 1,2,4-triazole-3-thiol (5a-c)[37]

Potassium dithiocarbamate salt (5 mmol) was dissolved in a little portion of D.W. in a round-bottom flask equipped with a condenser and an outlet. Hydrazine hydrate (10 mmol) was added to it. The mixture was refluxed for 10-12h, and the progress of the reaction was monitored by the evolution of hydrogen sulfide (H_2S) gas that was detected on lead acetate paper put on the outlet. During reflux time, the color of the solution changed to light green, and finally, the homogenous solution resulted. A few drops of 35% hydrochloric acid were added to liberate the solid residue, filtered, and recrystallized from ethanol.

4-amino-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol, compound (5a)

Yellow powder, yield 70%, m.p. 187-190 °C decomposed. FT-IR (υ= cm^{-1}): 3448 and 3352 asymmetric and symmetric stretching respectively of NH_2, 3086 aromatic (C-H) stretching, 2769 (S-H) stretching, 1604 (C=N) stretching, 1577 and 1485 aromatic (C=C) stretching, 1172 (C=S) stretching.

4-amino-5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol, compound (5b)

White powder, yield 60%, m.p. 212-214 °C decomposed. FT-IR (υ= cm^{-1}): 3356 and 3251 asymmetric and symmetric stretching respectively of NH_2, 3147 aromatic (C-H) stretching, 2758 (S-H) stretching, 1608 (C=N) stretching, 1566 and 1477 aromatic (C=C) stretching, 1068 (C=S) stretching.

4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol, compound (5c)

White powder, yield 65%, m.p. 204-206 °C decomposed. FT-IR (υ= cm^{-1}): 3290 and 3240 asymmetric and symmetric stretching respectively of NH_2; 3109 aromatic (C-H) stretching, 2758 (S-H) stretching, 1612 (C=N) stretching, 1573 and 1481 aromatic (C=C) stretching, 1064 (C=S) stretching.

General procedure for the synthesis of sulfonamide derivatives (6a-c)[39]

To the stirred ice-cold solution of compounds (5a-c) equivalent to (1 mmol) in THF (8ml) and pyridine (1,5 mmol), compound (1) equivalent to (1 mmol) was added gradually. Then the solution was left to be stirred at room temperature, under argon gas for 24 h, the precipitate was formed, filtered out, and recrystallized from ethanol.

N-(4-((3-mercapto-5-(4-nitrophenyl)-4H-1,2,4-triazole-4-yl)sulfonyl)phenyl)acetamide, compound (6a)

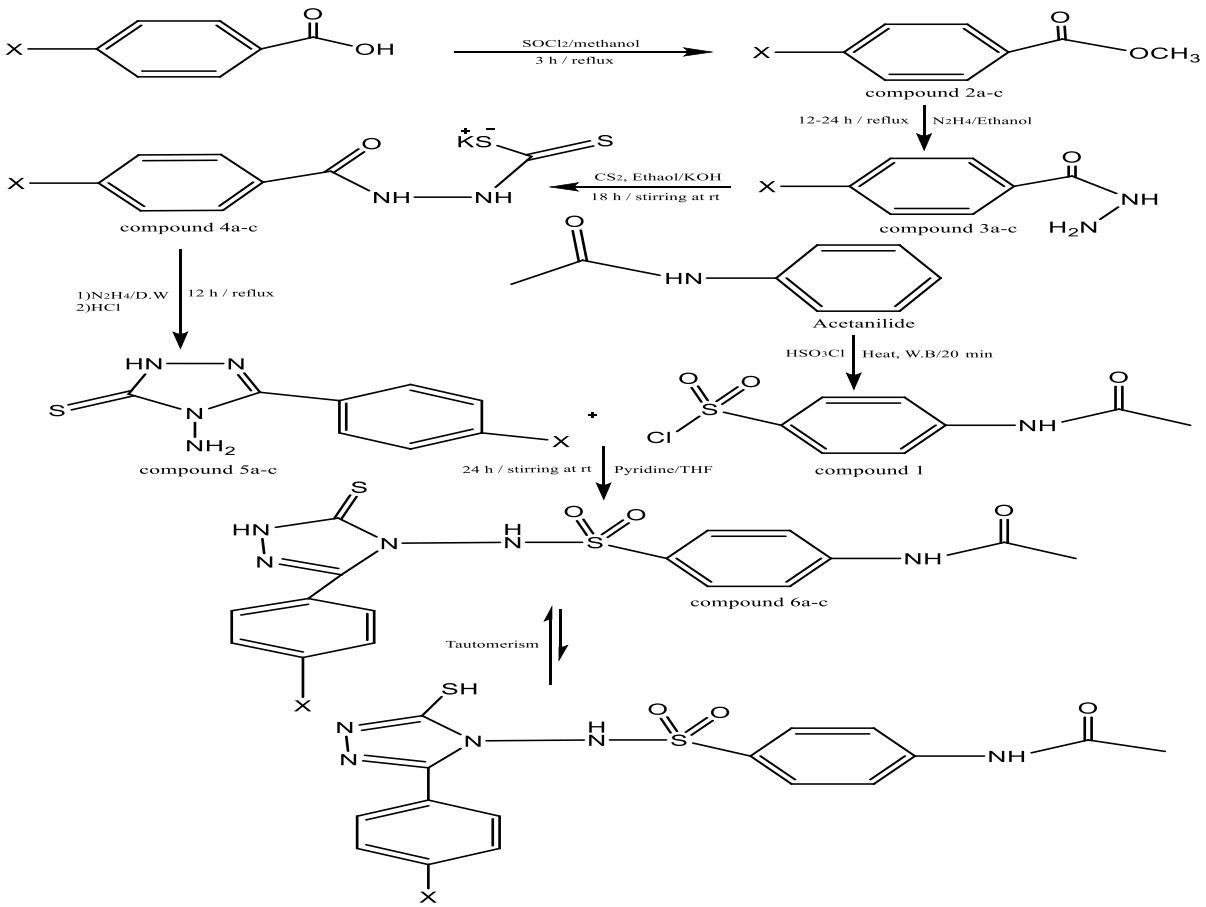
Pale yellow powder, yield 50%, m.p. 118-120 °C. FT-IR (υ= cm^{-1}): 3379 (N-H) stretching of sulfonamide, 3109 aromatic (C-H) stretching, 1693 (C=O) amide **I** band, 1635 (N-H) bending of sulfonamide, 1589 (N-H) amide **II** band, 1527 and 1489 aromatic (C=C) stretching, 1315 and 1157 asymmetrical and symmetrical stretching respectively of (O=S=O), 1257 (C-N) stretching of amide. ^{1}HNMR: (300 MHz, DMSO-d =ppm): 10.01(s, 1H, NH of triazole), 8.95(s, 1H, -NH-SO_2), 8.61(s, 1H, -NH-C=O), 7.53-8.60(m, 8H, Ar-), 2.06(s, 3H, CH_3-).

N-(4-(N-(3-(4-chlorophenyl)-5-mercapto-4H-1,2,4-triazole-4-yl)sulfamoyl)phenyl)acetamide, compound (6b)

White powder, yield 60%, m.p. 116-119 °C. FT-IR (υ= cm^{-1}): 3379 (N-H) stretching of sulfonamide, 3109 aromatic (C-H) stretching, 1693 (C=O) amide **I** band, 1635 (N-H) bending of sulfonamide, 1589 (N-H) amide **II** band, 1527 and 1489 aromatic (C=C) stretching, 1315 and 1157 asymmetric and symmetric stretching respectively of (O=S=O), 1257 (C-N) stretching of amide. ^{1}HNMR: 10.01(s, 1H, NH of triazole), 8.92(s, 1H, -NH-SO_2), 8.57(s, 1H, -NH-C=O), 7.52-8.55(m, 8H, Ar-), 2.06(s, 3H, CH_3-).

N-(4-(N-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)sulfamoyl)phenyl)acetamide, compound (6c)

White powder, yield 70%, m.p. 120-122 °C. FT-IR (υ= cm^{-1}): 3375 (N-H) stretching of sulfonamide, 3109 aromatic (C-H) stretching, 1693 (C=O) amide **I** band, 1635 (N-H) bending sulfonamide, 1589 (N-H) amide **II** band, 1527 and 1489 aromatic (C=C) stretching, 1315 and 1157 asymmetric and symmetric stretching respectively of (O=S=O), 1257 (C-N) stretching of amide. ^{1}HNMR: 10.02(s, 1H, NH of triazole), 8.94(s, 1H, -NH-SO_2), 8.56(s, 1H, -NH-C=O), 7.53-8.07(m, 9H, Ar-), 2.06(s, 3H, CH_3-).



X= a (NO_2), X= b (Cl), X= c (H)

Scheme 1. Synthesis of the titled compounds.

In vitro antimicrobial study [40-42]

The minimum inhibitory concentration (MIC), alongside the agar diffusion method, was used to assess the antimicrobial activity of the target derivatives against various microorganisms, including Gram-positive bacteria (*S. aureus, S. pneumonia, and B. subtilis*), Gram-negative bacteria (*P. aeruginosa, E. coli, N. gonorrhoeae, and H. pylori*), and fungal species (*C. albicans*). At concentrations ranging from 10-1000 mcg/ml, several diluted solutions were created from a stock solution (10 mg/ml) of each derivative. These solutions were prepared on a microtiter plate. The diluent used was Mueller-Hinton broth. Each well was inoculated with 20 µl of a bacterial suspension equivalent to McFarland standard no. 0.5 (1.5×108CFU/ml), except for the negative control wells. After that, the microtiter plates were incubated for 18 to 20 hours at 37°C. Following the incubation period, 20 µl of resazurin dye was introduced into each well. The samples then underwent incubation for 2 hours to observe color changes. In the resazurin broth assay, the sub-MIC concentrations were ascertained visually in broth micro dilutions as the lowest concentrations at which the color changed from blue to pink. Sulfadiazine, sulfamethoxazole, and fluconazole were utilized as reference antibiotics.

Statistical analysis

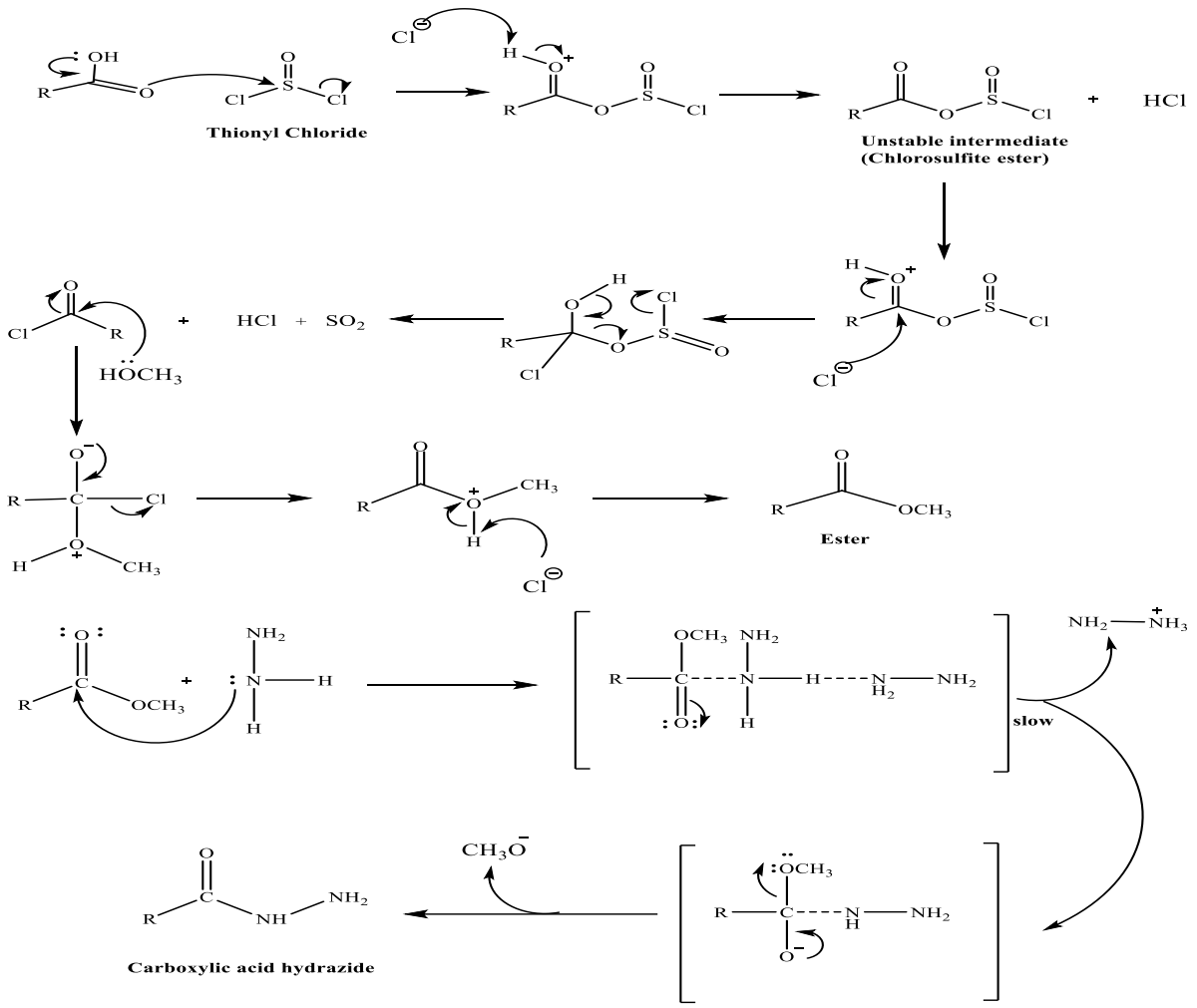
All the experiments were performed and reported in triplicate. The average mean values were reported along with standard deviation values. The T-test was conducted after verifying the normality and homogeneity of the data to assess its significance and compare the means (* <0.05). The software used for statistical analysis is (R Studio 4.5 was used for the correlations).

Results and Discussion

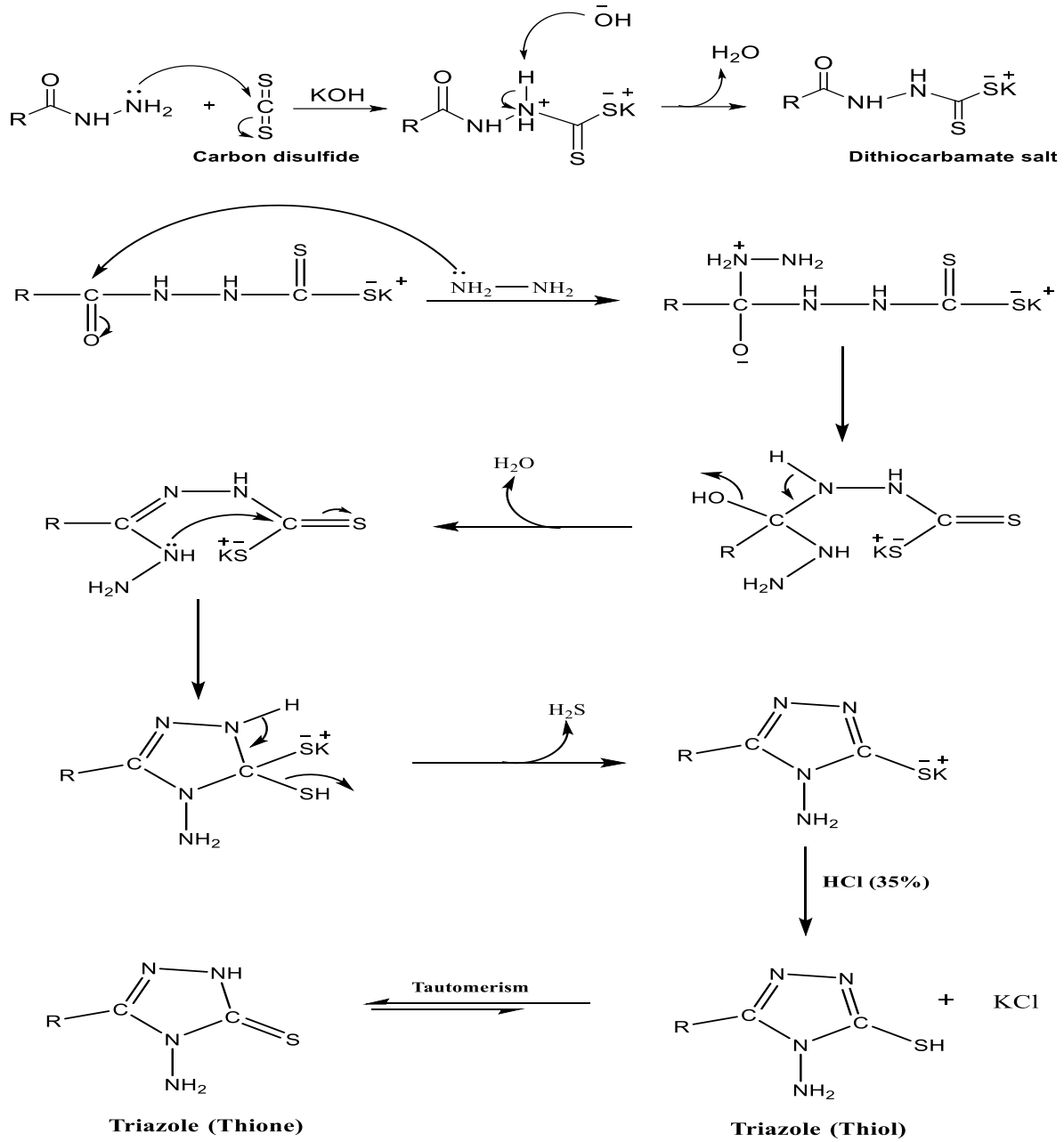
1-Chemistry

New sulfonamide derivatives, compounds (6a-c), were synthesized by directly substituting an aryl sulfonyl chloride with 1,2,4-Triazole-3-thiol, as illustrated in Scheme 1. To synthesize compounds (2a-c), aromatic carboxylic acids were converted into their corresponding methyl ester compounds through a reaction with thionyl chloride in the presence of methanol as a solvent and reactant. Subsequently, compounds (3a-c) were prepared by refluxing compounds (2a-c) with hydrazine hydrate ($NH_2NH_2.H_2O$), as shown in Scheme 2[43]. The resulting hydrazides were treated with carbon disulfide and ethanolic potassium hydroxide to form their dithiocarbamate salt compounds (4a–c), as shown in Scheme 3 [44, 45]. These salts were then subjected to a reaction with

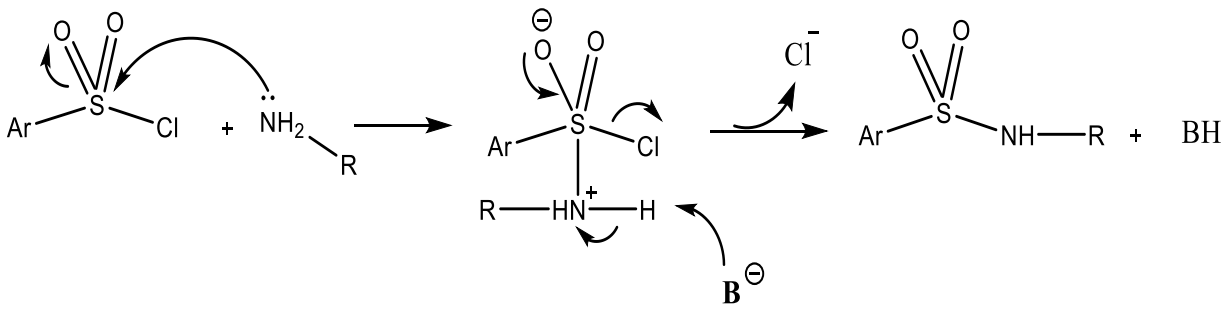
hydrazine to produce compounds (5a–c). Finally, compounds (6a–c) were created by stirring compounds (5a–c) with 4-acetamidobenzene sulfonyl chloride under argon gas in the presence of THF as a solvent and pyridine as a catalyst, the reaction proceeded through the S_N-2 type mechanism, Scheme 4 [46]. The newly synthesized compounds were characterized and identified using FTIR and ^{1}HNMR spectrum studies. The identification of methyl ester compounds (2a–c) relies on a prominent, well-defined peak at 1716 cm^{-1}. The carbonyl bands exhibit a shift towards a lower frequency, specifically around 1650 cm^{-1}. Additionally, two new bands appeared around 3300–3200 cm^{-1}, representing the primary amine's asymmetrical and symmetrical stretching vibrations, respectively, indicating the synthesis of hydrazide compounds (3a–c). The presence of two additional bands around 1060 cm^{-1} and 600 cm^{-1}, which correspond to the stretching of (C=S) and (C-S) bonds, respectively, provides evidence for the successful synthesis of dithiocarbamate salts, (compounds 4a–c). The disappearance of the carbonyl band, the distinct appearance of two bands of primary amine around 3400–3200 cm^{-1} (asymmetrical and symmetrical stretching respectively of NH_2), and the rise of a new band around 1600 cm^{-1} for (C=N) stretching indicate the formation of the 1,2,4-triazole-3-thiol ring system. Identifying a single band at approximately 3370 cm^{-1}, corresponding to the (N-H) stretching vibrations of sulfonamide groups, assures the successful synthesis of the final compounds. The successful chemical synthesis of the final sulfonamide derivatives is supported by ^{1}HNMR spectra. All spectra show characteristic singlet peaks around 10 ppm and 9 ppm for the (NH) of triazole ring systems and sulfonamide groups, respectively. Additionally, singlet peaks around 8 ppm for the (NH) of the amide group and around 2 ppm for (CH_3) indicate the synthesis of new classes of sulfonamide derivatives containing 1,2,4-triazole-3-thiol ring system.



Scheme 2. Mechanism of reactions for the synthesis of carboxylic acid hydrazides from carboxylic acids.



Scheme 3. Mechanism of reactions for the synthesis of the 5-substituted-1,2,4-triazole-3-thiol ring from carboxylic acid hydrazides.



Where Ar= phenyl with or without substitutions, B= base

Scheme 4. S_N-2 mechanism in synthesizing sulfonamide derivatives.

2-In silico study

This section will emphasize the analysis of the binding interactions and affinities of different compounds with carbonic anhydrase, focusing on the critical interactions within the active site of the enzyme and their impact on the efficiency of binding. Acetazolamide has shown high binding affinity, reflected by a docking score of -6.5 kcal/mol. This high binding is supported by several key interactions, including hydrogen bonds between its amide group and the Asn 108, the sulfonamide group and Thr 191, and an extended coordination between the sulfonamide group and the zinc ion Zn301. These interactions stabilize the ligand within the enzyme's active site and enhance its inhibitory effect. The docking score for compound 6a is slightly lower, -5.5, reflecting its interactions within the active site. This compound forms hydrogen bonds with Pro 193 via an amide group, Asn 108 via a sulfonamide group, and Lys 88 via its nitro group. In addition, compound 6a binds to the zinc ions via the sulfur substituent in its triazole ring, which helps to explain why it has a moderate affinity for binding. The docking score of compound 6b was -4.6; it forms two π-π stacking interactions with His 110 and His 84. It also establishes a hydrogen bond with Trp 23 via its amide group and coordinates via the sulfur substituent of the triazole ring with Zn301. Similar to compound 6b, compound 6c also showed a docking score of -4.7, exhibiting the same interaction pattern, including π-π stacking with His 110 and His 84, a hydrogen bond with Trp 23, and coordination with Zn301 via the sulfur substituent of the triazole ring.

Table 1. Docking score in (kcal/mol)

Compounds	Docking score (kcal/mol)
Compound 6a	-5.5
Compound 6b	-4.6
Compound 6c	-4.7
Acetazolamide	-6.2
Sulfamethoxazole	-3.83
Sulfadiazine	-2.5

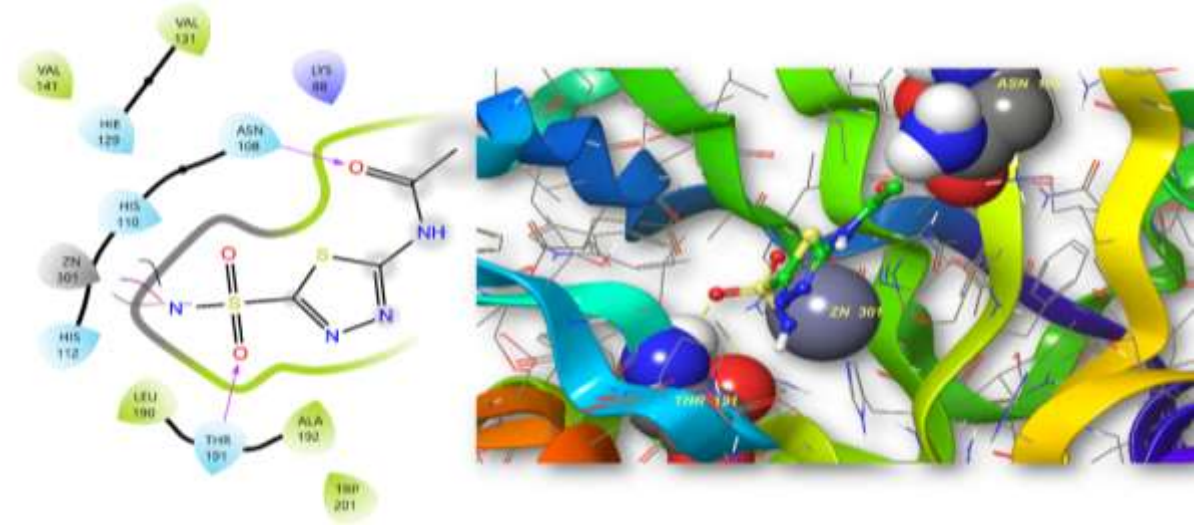


Figure 2. This figure illustrates the interaction of acetazolamide (AZM) with carbonic anhydrase, highlighting the key binding interactions within the enzyme's active site.

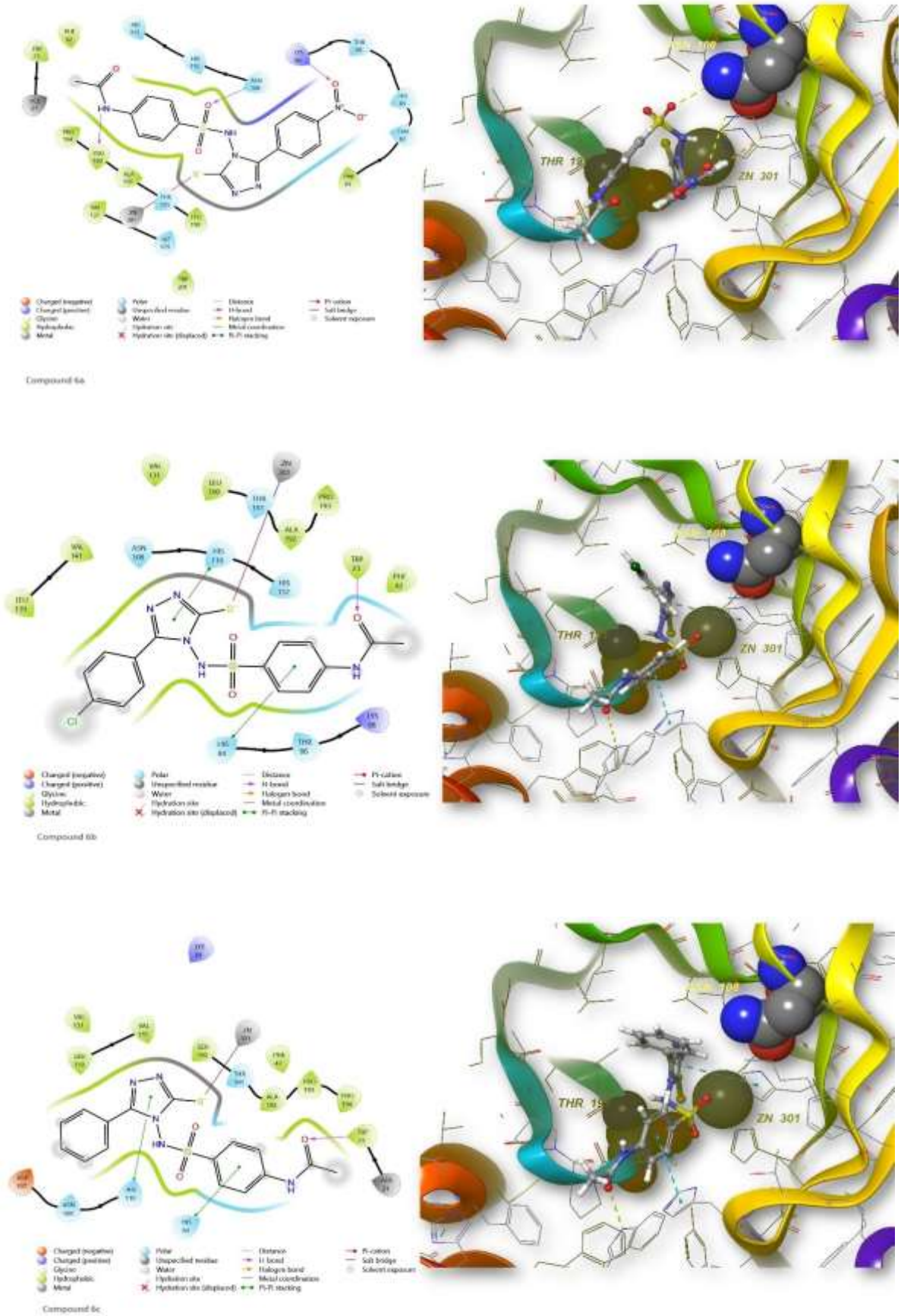


Figure 3. Molecular docking of CAs isolation from (PDB ID 4YGF) with compounds (6a,6b, and 6c).

3-Anti-microbial study

According to the minimum inhibitory concentration results, compound (6a) can inhibit various strains of Gram-positive bacteria, Gram-negative bacteria, and fungal species Candida albicans with low concentration as compared to another compound (6b and 6c).

Table 2. Statistical test between the minimum inhibitory concentration (MIC) and isolations.

MIC in mcg/ml									
Compounds	*S. aureus*	*S. pneumonia*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*N. gonorrhoeae*	*H. pylori*	*C. albicans*	Control positive
6a	125	250	125	250	250	250	500	125	-
6b	500	1000	500	500	1000	1000	1000	500	-
6c	250	250	500	500	500	500	500	250	-
Sulfadiazine	125	125	125	125	250	125	250	-	-
Sulfamethoxazole	125	250	125	125	250	125	125	-	-
Fluconazole	-	-	-	-	-	-	-	125	-
Mean ±SD	187.5± 172	312.5 ±351	229±21 5	250±20 9	375±34 4	333± 367	396±35 7	167± 188	-
SEM	70	143	88	85	141	150	145	77	-
P-value	0.04*	0.08 NS	0.04*	0.03*	0.04*	0.07 NS	0.04*	0.08 NS	-
NS: No Significant Value (* <0.05)									

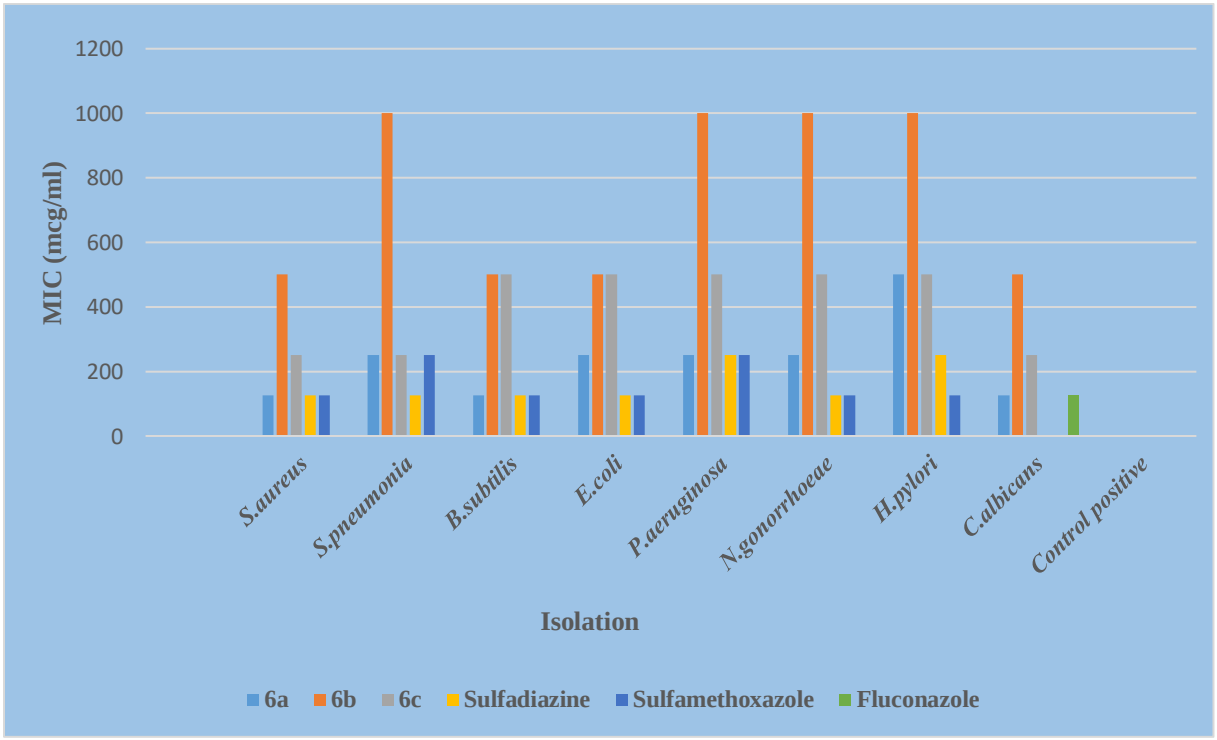


Figure 4. The MIC of new sulfonamide derivatives (6a,6b, and 6c) with reference compounds (Sulfadiazine, sulfamethoxazole, and fluconazole) on different isolation.

Table 3. The significance between the zone of inhibition and isolations.

The zone of inhibition in (mm) depends on MIC for each compound in (mcg/ml)									
Compounds	*S. aureus*	*S. pneumonia*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*N. gonorrhoeae*	*H. pylori*	*C. albicans*	Control negative
6a	32	32	21	8	20	-	33	32	-
6b	27	32	19	18	21	8	29	29	-
6c	30	31	22	20	23	6	28	31	-
Sulfadiazine	33	32	24	20	29	17	37	-	-
Sulfamethoxazole	29	35	21	19	28	18	36	-	-
Fluconazole								31	
DMSO	-	-	-	-	-	-	-	-	-
Mean± SD	30.2±2.38	32.4±1.51	21.4±1.81	17±5.09	24.2±4.08	12.25±6.13	32.6±4.03	30.75±1.25	--
SEM	1.06	0.67	0.81	2.28	1.82	3.06	1.805	0.62	--
p-value	9.29657E-6*	1.14873E-6*	1.23431E-5*	0.00173*	1.87953E-4*	0.02	5.53206E-5*	1.88604E-5*	--
NS: No Significant Value * (P<0.05)									

(-) indicates no activity, slightly active (inhibition zone between 5-10 mm), moderately active (inhibition zone between 10-15 mm), and highly active (inhibition zone greater than 15 mm).

The results presented in Table 2, we conducted statistical tests to examine the significance between the minimum inhibitory concentration (MIC) and various microbial isolates. The T-test results revealed significant between the MIC values and the isolates (*S. aureus, B. subtilis, E. coli, P. aeruginosa, and H. pylori*), with p-values lower than 0.05. Specifically, the strongest significance was observed for (*P. aeruginosa and H. pylori*) Mean ± SD: 375 ± 344;396 ± 357 mcg/ml) respectively, indicating that these inhibitors are particularly effective against these pathogens.

In contrast, the T-test results indicated no significant between the MIC values of the (6a, 6b, 6c, Sulfadiazine, Sulfamethoxazole, and Fluconazole) and the isolates *S. pneumonia, N. gonorrhoeae, and C. albicans*. Additionally, there was no observed significance for Gram-negative isolates with the tested inhibitors, suggesting that these inhibitors may not be effective against Gram-negative bacteria, which typically have different resistance mechanisms compared to Gram-positive bacteria.

Overall, the significant correlations between MIC values and the isolates (*S. aureus, B. subtilis, E. coli, P. aeruginosa, and H. pylori*) highlight the potential effectiveness of the tested inhibitors against these pathogens. The particularly strong significance for (*P. aeruginosa and H. pylori*) suggests that these inhibitors could be promising candidates for developing treatments targeting these microorganisms. On the other hand, the lack of significance for *S. pneumonia, N. gonorrhoeae, and C. albicans*, as well as for Gram-negative isolates, indicates alternative treatments.

Based on the results presented in Table 3, the mean ± SD for *S. aureus* is 30.2 ± 2.38. The T-test indicates a significant between the zone of Inhibition (6a, 6b, 6c, Sulfadiazine, Sulfamethoxazole, Fluconazole, and DMSO) and *S. aureus*, with a p-value less than 0.05. Similarly, for *S. pneumonia*, the mean ± SD is 32.4 ± 1.51, and the T-test shows significance with the same set of inhibitors, with a p-value less than 0.00001. *H. pylori*, with a mean ± SD of 32.6 ± 4.03, also shows a significant with the zone of Inhibition (6a, 6b, 6c, Sulfadiazine, Sulfamethoxazole, Fluconazole, and DMSO), with a p-value less than 0.05. Lastly, *C. albicans*, with a mean ± SD of 30.75 ± 1.25, demonstrates a significance with the same set of inhibitors, indicated by a p-value less than 0.05. These results suggest a significant and strong inhibitory effect of the inhibitors on these microorganisms, highlighting their potential effectiveness in controlling infections caused by *S. pneumonia, H. pylori, and C. albicans.* Additionally, *B. subtilis, E. coli, P. aeruginosa, and N. gonorrhoeae* also show a significant with the same set of inhibitors, indicated by a p-value less than 0.05.

In summary, the T-test results across various bacteria and fungi indicate significant between the zone of inhibition provided by the inhibitors (6a, 6b, 6c, Sulfadiazine, Sulfamethoxazole, Fluconazole, and DMSO) and the inhibition of these microorganisms, with all p-values falling below significant thresholds, underscoring the effectiveness of these inhibitors.

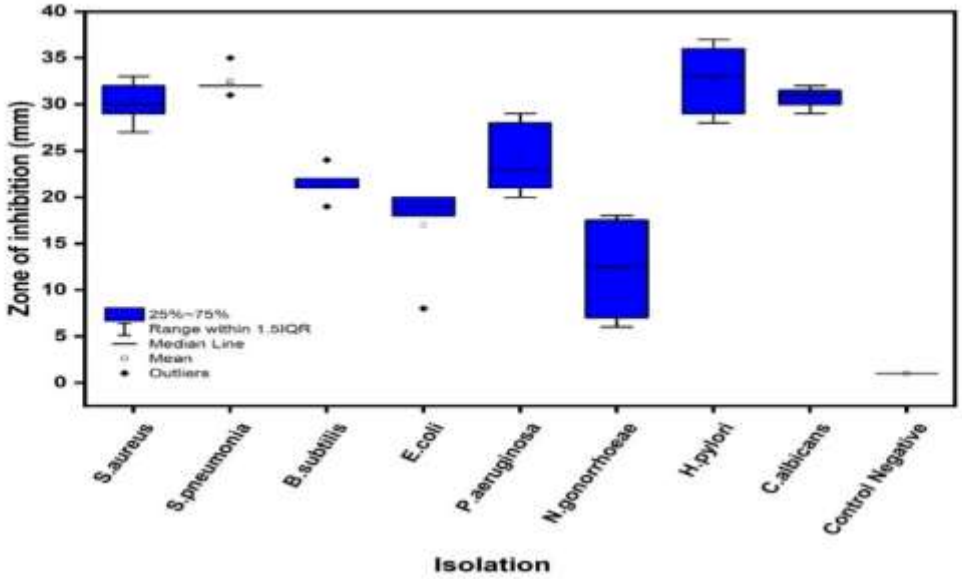


Figure 5. The boxplot of the Zone of inhibition (6a, 6b, 6c, Sulfadiazine, Sulfamethoxazole, Fluconazole, and DMSO) and the isolations.

Based on Figure 5, the highest range of the zone of inhibition was observed in *H. pylori*, which exhibited a range between 30 mm and 36 mm. In contrast, the lowest range was noted in *N. gonorrhoeae*, with the zone of inhibition measuring between 5 mm and 16 mm. The results highlight a significant variation in the efficacy of the inhibitors against different microorganisms. *H. pylori* showed the greatest susceptibility to the inhibitors, as evidenced by its high zone of inhibition. This suggests that the inhibitors are particularly effective against this bacterium, potentially due to its specific cellular structure or metabolic pathways that are more susceptible to the inhibitory compounds.

On the other hand, *N. gonorrhoeae* displayed the smallest zone of Inhibition, indicating a lower susceptibility to the inhibitors. This could be due to several factors, such as the presence of resistance mechanisms, differences in cell wall permeability, or variations in metabolic activity that reduce the inhibitors' effectiveness. The differences in the zone of inhibition among the tested microorganisms underscore the importance of understanding the specific interactions between antimicrobial agents and target organisms. These findings suggest that while some inhibitors are broadly effective, others may need to be tailored or combined to address less susceptible strains effectively.

Conclusion

The synthesis of a new series of sulfonamide derivatives was accomplished with successful results. Their chemical structure was determined using ART-FT-IR spectroscopy and ^{1}HNMR spectroscopy. All synthetic compounds can target the carbonic anhydrase enzyme of *H. pylori* (4YGF) via the sulfur atom in the triazole ring system, leading to a reduction in the pathogenicity and virulence of these bacteria. The MIC results show that compound 6a can inhibit a variety of bacterial strains at low concentrations when compared to another synthetic compound. Compared to fluconazole, compound 6a is more effective against the fungus *C. albicans*.

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Conflicts of Interest

The authors affirm that no conflicts of interest or outside funding have been received for this work.

Funding

No particular funding was received for this research.

Ethics Statements

Do not use any animals for in vitro studies. The study was done on microorganisms.

Author Contribution

Ali, as the first author, contributed to the synthesis of the final compounds, analyzed the IR and ^{1}HNMR data, assessed the antibacterial activity, and drafted the manuscript. Zainab, the second author, approved the final version after reviewing the results.

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دراسة النمذجة، تخليق، تشخيص وتقيم أولي للفعالية المضادة للميكروبات لمركبات جديدة من السلفونمايد –١،٢،٤-تريازول

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الخلاصة

تم تصنيع سلسلة جديدة من مشتقات السلفونمايد (6a-c) تحتوي على نظام حلقي ١،٢،٤ -تريازول-٣-ثيول (5a-c) وتم تشخيص المركبات المحضرة باستخدام مطياف الاشعة تحت الحمراء وجهاز الرنين النووي المغناطيسي. بكتريا (*H. pylori*) هو السبب الشائع لقرحة المعدة وسرطان المعدة. انزيم الكربونيك الانهيدراز ينظم التوازن بين الحامض والقاعدة و يمكنها من العيش بوجود بيئة حامضية عالية داخل المعدة. الدراسة استخدمت تقنيات الحاسوب لتحديد الانزيم المستهدف الموجود في هذا النوع من البكتريا. المركبات المصنعة قادرة على تثبيط الانزيم عن طريق الارتباط مع عنصر الزنك وبذلك يؤثرعلى قدرة هذه البكتريا على تحمل حامضية المعدة وقدرتها على تسبب بالمرض داخل الجهاز الهضمي. المركبات المصنعة، تم تقيم فعاليتها المضادة للميكروبات خارج الجسم الحي ضد سلالات محددة من البكتريا موجبة الصبغة، سالبة الصبغة والفطريات. تم تصنيع المركبات المستهدفة بنجاح وقد وجد انها تمتلك فعالية عالية ضد البكتريا موجبة الصبغة (*S. aureus, S. pneumonia, and B. subtilis*) فعالية عالية الى متوسطة ضد البكتريا سالبة الصبغة (*P. aeruginosa, E. coli, N. gonorrhea and H. pylori*) و فعالية عالية ضد فطريات (*C. albicans*).

الكلمات المفتاحية: سلفونمايد، مركبات حلقية ١،٢،٤ -تريازول-٣-ثايول، انزيم كاربونك الانهيدراز، نظرية تقنيات، عنصر الزنك.