



Synthesis, Spectral Characterization, and Molecular docking of Novel Schiff bases with Potential Lung Cancer Activity

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ABSTRACT

In this study, eight important compounds of new Schiff bases linked to tetrazoles and triazoles were synthesized, which may have importance in cancer treatment according to the molecular docking study conducted in this paper by the reaction of tetrazole acid with thiocarbonylhydrazide to produce an amino triazole thione compound, which was subsequently reacted with eight aromatic aldehyde derivatives. Tetrazole acid was prepared from the reaction of 4-aminobenzoic acid with sodium azide and trimethyl orthoformate with the existence of acetic acid. Thiocarbonylhydrazide was prepared by the drop wise addition of carbon disulfide to hydrazine hydrate. All synthesized compounds, their structure were confirmed by spectral (FT-IR, ¹HNMR) data. Matrix metalloproteinases (MMPs) play a critical role in cancer progression by degrading the extracellular matrix (ECM), thereby facilitating tumor cell invasion and metastasis. Additionally, MMPs contribute to tumor angiogenesis by promoting the formation of new blood vessels that sustain tumor growth. Therefore, inhibition of MMPs represents a promising therapeutic strategy in cancer treatment, as it may reduce tumor spread and enhance the efficacy of other anticancer therapies. Therefore, molecular docking studies against MMPs protein receptors showed strong binding affinities (ΔG values up to -6.4 kcal/mol), indicating promising anticancer potential.

Keywords: Tetrazole, Aminotriazole thione, Schiff base, Thiocarbonylhydrazide, Molecular docking, Matrix metalloproteinases (MMPs), and Lung cancer.

INTRODUCTION

Heterocyclic compounds containing nitrogen atoms, such as tetrazoles and triazoles, represent a prominent research area in organic and medicinal chemistry due to their broad applications in pharmaceutical and industrial fields.¹ These compounds perform a crucial role in the progress of antimicrobial, anti-inflammatory, and anticancer agents.² In

this context, the reaction of tetrazole acid with thiocarbonylhydrazide has gained growing attention, as it leads to the formation of an amino triazole thione compound reactive intermediate with significant potential for synthesizing novel compounds with enhanced biological activities.³ The introduction of an amino group into the triazole ring is a key step that enables the compound to react with aldehydes⁴, allowing for the preparation of Schiff bases various hybrid



molecules that have been shown to possess antibacterial and antifungal activities.⁵ The synthesized compounds demonstrate notable biological properties, such as anti-inflammatory, antimicrobial.⁶ An amino triazole compound was synthesized through the direct reaction of tetrazole acid with thiocarbohydrazide, followed by a condensation reaction with eight different aldehyde derivatives to obtain new derivatives that may exhibit novel or improved biological properties.⁷ Previous studies have indicated that nitrogen-containing heterocyclic compounds may display significant biological activities, including antioxidant, anticancer, and enzyme inhibition effects⁸. Such activity is attributed to the existence of multiple functional groups on the core ring, enhancing their interaction with various biological targets. Additionally, the use of thiocarbohydrazide introduces sulfur atoms into the molecular structure, expanding the structural diversity and potential functionalities of these hybrid molecules.⁹ The 1,2,4-triazole-3-thiol compounds are well known for their ability to form biologically active molecules, and the incorporation of a tetrazole ring further enhances their biological potential, making them strong candidates for pharmaceutical applications.¹⁰ The key compound, 5-(4-(1H-tetrazol-1-yl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol, was synthesized making use of advanced organic synthesis techniques. It was then reacted with various benzaldehyde derivatives such as 4-methoxybenzaldehyde, 4-nitrobenzaldehyde, and 4-methylbenzaldehyde in the existence of glacial acetic acid as a catalyst and ethanol as a solvent under moderate heating conditions.

The primary function of MMPs is to break down extracellular matrix proteins, which is crucial for normal physiological processes; MMPs are involved in developmental processes, tissue repair, and remodeling and they help in the formation of new blood vessels by degrading the ECM, providing a pathway for endothelial cells. MMPs facilitate the migration of immune cells by modifying the vascular and interstitial barriers and they are implicated in a range of pathological conditions, including arthritis, cardiovascular diseases, and particularly cancer. These enzymes contribute significantly to cancer progression by

promoting tumor growth, invasion, and metastasis and they can degrade basement membranes and other structures of the ECM which facilitating the escape of cancer cells from primary tumors and their spread to distant organs. By degrading ECM components, MMPs release vascular endothelial growth factor (VEGF) and other factors, enhancing angiogenesis and supplying tumors with necessary nutrients and oxygen. MMPs alter the tumor microenvironment, influencing inflammation, immune surveillance, and cancer cell behavior.¹¹

As a result of the significant biological activity of the mentioned compounds, we present newly synthesized compounds that show potential as lung cancer treatments, as demonstrated by our molecular docking studies and their biological activities. This research represents an extension of our dedicated efforts in designing and developing a flexible protocol for the synthesis of these crucial compounds, including novel hybrid molecules of Schiff bases linked tetrazoles and triazoles derivatives.

EXPERIMENTAL

MATERIALS AND METHODS

Melting points (uncorrected) were measured by electro-thermal SMP30- Stuart melting point apparatus. ¹H-NMR spectra were recorded using Bruker Bio Spin GmbH Spectrophotometer (400 MHz using TMS as internal standard and using DMSO-d₆ as a solvent) [(s) singlet, (d) doublet, (m) multiply]. FT-IR spectra were recorded using a Japanese-made device (Bruker FT-IR-ATR) in a region confined between 400 and 4000 cm⁻¹. TLC aluminum sheets silica gel 60 F254 were used to monitor the progress of all reactions and the homogeneity of the produced compound. As for the used mobile phase, it consisted of a mixture of ethyl acetate and n-hexane in a ratio of (3:7).

Preparation of thiocarbohydrazide by two different methods¹²

First methods

Carbon disulphide (0.22 mole, 13 mL) was added drop wise to vigorously stirred solution

of $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (80%) (0.44 mole, 24 mL) in (15 mL) dis. water during (30 minutes). Then the temperature of the reaction was raised to 100-110°C and the reaction mixture was refluxed until the emission of H_2S gas was stopped [(identified by using paper saturated with lead acetate (~3 h)], then the mixture cooled at room temperature. The precipitated thiocarbohydrazide was filtered off, washed with dis. water and then air dried. m.p.: 168- 170°C, yield 35%, color: Pale Yellow Powder. FT-IR ν (cm^{-1}): 3304 and 3267 (primary NH_2), 3165 (N-H), 1279 (C=S).

Second methods

Carbon disulphide (0.20 mole, 12 mL) was added drop wise to vigorously stirred solution of $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (80%) (0.22 mole, 12 mL) during 30 minutes. Then the temperature of the reaction was raised to 100-110°C and the reaction mixture was refluxed for (6 h), then the mixture cooled at room temperature. The precipitated thiocarbohydrazide was filtered off, washed with dis. water and then air dried. m.p.: 168-170°C, yield 65%, color pale yellow powder. FT-IR ν (cm^{-1}): 3304 and 3267 (primary NH_2), 3165 (N-H), 1279 (C=S).

Synthesis of 4-tetrazol-1-yl-benzoic acid (S1)¹³

A mixture of 4-aminobenzoic acid (0.005 mole, 0.685 g) with (0.005 mole, 0.325 g) of sodium azid and (0.005 mole, 0.635 g) of trimethyl orthoformate in (40 mL) of acetic acid. The reaction was reflux for 32 h, followed by TLC chromatography. Cooled the reaction mixture to room temperature and then pour it into crushed ice., filtered, washed with water, dried, and recrystallized by ethanol to produce

a glossy white precipitate with a yield of 92%, m.p. = 257-259°C, R_f = 0.62. FT-IR (ν cm^{-1}): 3360 (OH), 3041 (= CH), 1633 (C = O), 1598 (C = N), 1130 (C = S). $^1\text{H-NMR}$ (DMSO-d_6 , 400 MHz), δ (ppm): 7.68-7.70, 7.87 -7.89 [d-d, 4H, Ar-H], 10.27 [s, 1H, CH=N], 12.71 [br, 1H, OH], Scheme 1.

Synthesis of 5-(4-(1H-tetrazol-1-yl)phenyl)-4-amino-4H-1,2,4-triazole-3-thiol (S2)¹²

A mixture of 4-tetrazol-1-yl-benzoic acid (S1) (0.005 mole, 1.0 g) with (0.005 mole, 0.55 g) of thiocarbohydrazide in (30 mL) of absolute ethanol. The reaction was reflux for 9 h, followed by TLC chromatography. The solution is concentrated to half, filtered, washed with ethanol, dried, and recrystallized by ethanol to produce a bright yellow precipitate with a yield of 91% , m.p. = 240-242°C. FT-IR (ν cm^{-1}): 3339, 3419 (NH_2 , NH), 3046 (=CH), 1633, 1680 (C=N), 1594-1474 (C=C aromatic), 1103 (C=S), 1162-1246 (O- CH_3). $^1\text{H-NMR}$ (DMSO-d_6 , 400 MHz), δ (ppm): 2.8 [s, 2H, NH_2], 7.68-7.70, 7.78-7.89 [d-d, 4H, J = 8HZ, Ar-H], 10.26 [s, 1H, CH=N], 11.68 [br, 1H, NH], Scheme 1.

Synthesis of Schiff bases derivatives (S3-S10)^{12,14}

A mixture of aminotriazole-thione (S2) (0.001 mole, 0.5 g) with (0.001 mole) of different substituted aromatic aldehydes in (30 mL) of absolute ethanol and 7 drops of glacial acetic acid as catalyst. Monitored by thin-layer chromatography, was refluxed for 7-9 hours. After that, the mixture was cooled at room temperature, the formed precipitate was filtered, washed with ethanol, dried and recrystallized from ethanol. Table 1, 2, Scheme 1.

Table 1: Physical Properties and FT-IR data of Schiff Bases Derivatives (S3-S10)

| Compounds No | X | m.p.,°C | Yield% | Color | R_f | FT-IR, ν (cm^{-1}) | | | | | |
|--------------|------------------------------|------------|--------|---------------|-------|-----------------------------------|------|------|---------------|------|---------------------------------------|
| | | | | | | NH | =CH | C=N | C=C(aromatic) | C=S | Others |
| S3 | 2-OH | 150-152 | 66 | Glossy yellow | 0.52 | 3315 | 3020 | 1610 | 1513-1535 | 1077 | 3160 (OH) |
| S4 | 3- OCH_3 | Decomposed | 74 | Brown | 0.52 | 3200 | 3033 | 1597 | 1540-1597 | 1177 | 1228-1246 (OCH_3) |
| S5 | 3-OH | Decomposed | 71 | Yellow | 0.67 | 3300 | 3049 | 1668 | 1519-1593 | 1010 | 3230 (OH) |
| S6 | 4- $\text{N}(\text{CH}_3)_2$ | 226-228 | 70 | Orange | 0.63 | 3304 | 3095 | 1673 | 1583-1525 | 1162 | |
| S7 | H | 233-235 | 95 | Glossy yellow | 0.63 | 3195 | 3067 | 1677 | 1569-1594 | 1238 | |
| S8 | 4- OCH_3 | 215-217 | 72 | Yellow | 0.61 | 3300 | 3093 | 1666 | 1593-1642 | 1178 | 2836-2962 (OCH_3) |
| S9 | 3- OCH_3 , 4-OH | 140-142 | 73 | Dark brown | 0.66 | 3340 | 2934 | 1668 | 1515-1591 | 1284 | 3304 (OH) 2857-2934(OCH_3) |
| S10 | 3- NO_2 | 150-152 | 77 | Light yellow | 0.65 | 3377 | 2977 | 1654 | 1477-1545 | 1178 | 1300-1343 (NO_2) |

Table 2: The ¹H-NMR spectral data of Schiff bases derivatives (S3-S10)

| Compounds No | X | ¹ H-NMR (DMSO-d ₆ , 400 MHz), δ (ppm) |
|--------------|------------------------------------|---|
| S3 | 2-OH | 6.80-7.22 [m, 4H, Ar1-H], 7.57-8.00 [d-d, 4H, Ar2-H], 8.97 [s, 1H, CH=N for Schiff base], 9.20 [s, 1H, CH=N for tetrazole ring], 9.88 [s, 1H, OH], 11.38 [s, 1H, NH] |
| S4 | 3-OCH ₃ | 3.92 [s, 3H, OCH ₃], 7.07-7.71 [m, 9H, Ar-H & CH=N], 8.81 [s, 1H, CH=N], 10.37 [s, 1H, NH] |
| S5 | 3-OH | 7.09-7.44 [m, 4H, Ar1-H], 7.87-7.89, 7.68-7.70 [d-d, 4H, J = 8HZ, Ar2-H], 9.92 [s, 1H, CH=N for Schiff base], 9.97 [s, 1H, CH=N for tetrazole ring], 12.71 [br, 1H, NH], 10.26 [s, 1H, OH] |
| S6 | 4-N(CH ₃) ₂ | 3.03 [s, 6H, -N(CH ₃) ₂], 7.27-6.79 [d-d, 4H, Ar1-H], 7.68-7.96 [m, 5H, Ar2-H & CH=N], 8.45 [s, 1H, CH=N for tetrazole ring], 12.73 [s, 1H, NH] |
| S7 | H | 7.53-8.01 [m, 6H, Ar1-H & CH=N], 7.68-7.70, 7.78-7.89 [d-d, 4H, J = 8HZ, Ar2-H], 10.26 [s, 1H, CH=N for tetrazole ring], 12.65 [br, 1H, NH] |
| S8 | 4-OCH ₃ | 3.87 [s, 3H, OCH ₃], 6.98-7.98 [m, 8H, Ar-H], 9.91 [s, 1H, CH=N for Schiff base], 10.26 [s, 1H, CH=N for tetrazole ring], 10.53 [s, 1H, NH] |
| S9 | 3-OCH ₃ , 4-OH | 3.84 [s, 3H, OCH ₃], 6.88-7.46 [m, 3H, Ar1-H], 7.6-8.0 [d-d, 4H, Ar2-H], 9.78 [s, 1H, CH=N for Schiff base], 10.01 [s, 1H, CH=N for tetrazole ring], 10.26 [s, 1H, OH], 12.64 [s, 1H, NH] |
| S10 | 3-NO ₂ | 7.57-8.30 [m, 8H, Ar-H], 8.76 [s, 1H, CH=N for Schiff base], 9.18 [s, 1H, CH=N for tetrazole ring], 11.64 [s, 1H, NH] |

Molecular docking

Molecular docking programs are advanced computational tools that enable scientists and researchers to analyze the nature of interactions between chemical molecules and specific proteins or biological targets within the human body. The importance of these programs lies in their fundamental role in pharmaceutical research and drug development, as they are used to predict the binding mechanisms of potential drug compounds with target proteins and assess their biological interaction capabilities.¹⁵

MCULE docking and BIOVIA Discovery Studio 2024 software were used to simulate the binding orientation and interaction of the strong antitumor derivatives (S3-S10), as depicted in figures (1–7), with anticancer regulating proteins, specifically Matrix Metalloproteinases (MMPs). Additionally, the active site property of the enzyme was taken from Swiss Target Prediction, the protein and ligands were added to molecular docking, and the chosen three-dimensional (3D) protein was downloaded from the Protein Data Bank (PDB) website as 4wnx, which mutated in lung cancer. By estimating the ideal location, orientation, and confirmation of a small molecule (drug candidate) when it binds to a protein, molecular docking makes it much simpler to optimize the medications in the future. By determining the interaction's G value (free energy),¹⁶ as indicated in Table (3), MCULE evaluates the strength of the binding between the chemical molecule and enzyme. The strength of the contact and its possible effect on the enzyme are indicated by the ΔG value; a greater negative value

indicates spontaneous and preferential binding.¹⁷ As illustrated in Fig. 1-7, molecule docking (discovery studio) provides 2D and 3D representations of the interaction between the enzyme and produced chemical compounds.

In this study, Matrix Metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in extracellular matrix remodeling which are closely associated with pathological processes such as inflammation, cancer progression, and metastasis. In this study, molecular docking was achieved to evaluate the blend affinity and interaction profile of the synthesized compound S3-S10 with MMPs. The docking results revealed that compounds (S3-S10) fits well into the active site of the MMPs enzyme, forming multiple stabilizing interactions, including conventional hydrogen bonding with HIS306, Pi-Sulfur interaction with TRP316, Pi-Alkyl with CYS307, and van der Waals forces with PRO309, ASN312, and PHE343. The compounds also exhibited a Pi-Pi T-shaped interaction with GLN305, indicating favorable π-π stacking.

The binding energy ranged between -6.1 and -6.4 kcal/mol, Table 3, suggesting a moderate but significant binding affinity. These outcomes support the potentiality of compounds (S3-S10) as auspicious candidates for further investigation as a selective MMPs inhibitor. This could contribute to the development of novel therapeutic agents targeting MMP-mediated diseases, particularly in cancer and chronic inflammatory disorders including lung cancer.

Table 3: Binding energies of the potent anticancer derivatives (Schiff bases) with examined protein MMPs

| Ligands | S3 | S4 | S6 | S7 | S8 | S9 | S10 |
|--------------------------|------|------|------|------|------|------|------|
| MMPs ΔG Kcal/mol | -6.3 | -6.3 | -6.4 | -6.3 | -6.4 | -6.1 | -6.3 |

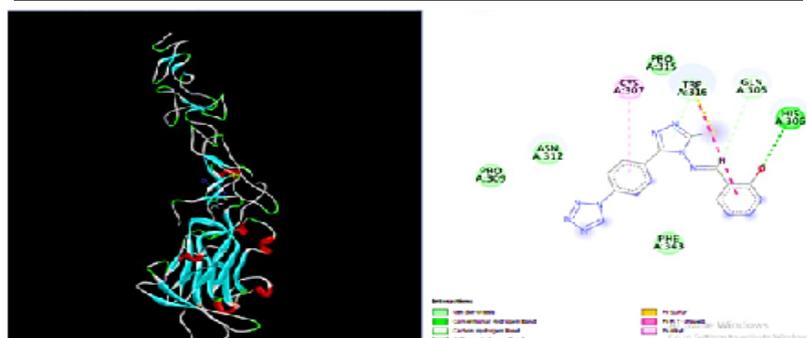


Fig. 1. 3D & 2D are illustrated for a possible interaction of compound S3 with the MMPs protein

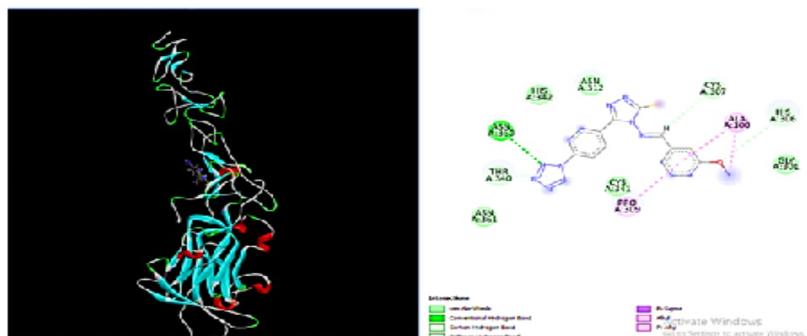


Fig. 2. 3D & 2D are illustrated for a possible interaction of compound S4 with the MMPs protein

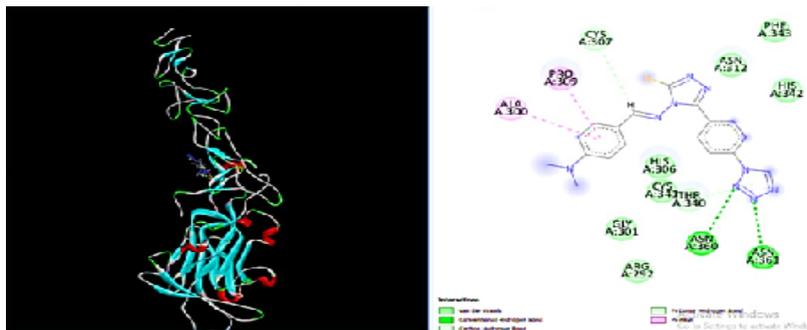


Fig. 3. 3D & 2D are illustrated for a possible interaction of compound S6 with the MMPs protein

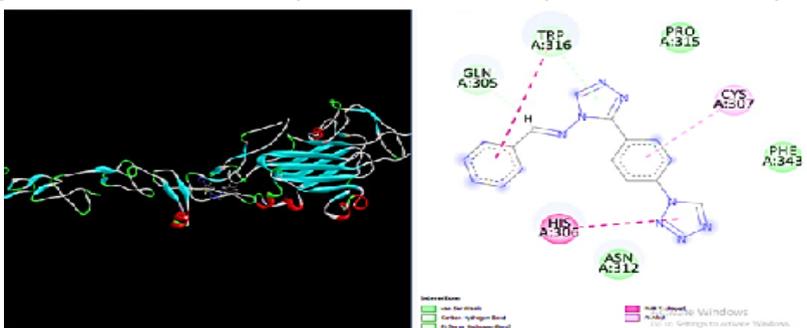


Fig. 4. 3D & 2D are illustrated for a possible interaction of compound S7 with the MMPs protein

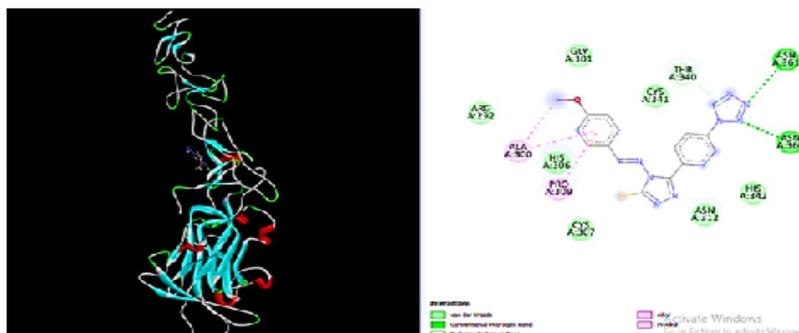


Fig. 5. 3D & 2D are illustrated for a possible interaction of compound S8 with the MMPs protein

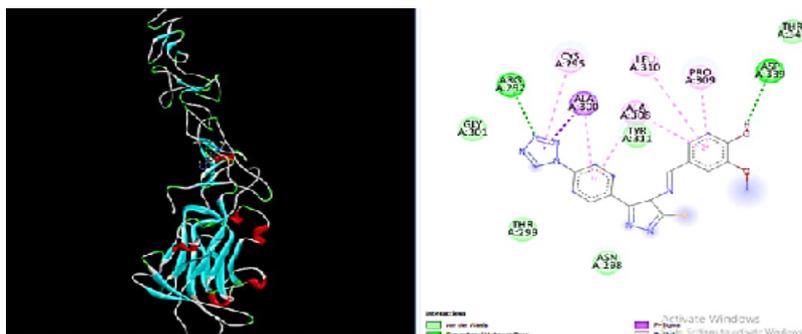


Fig. 6. 3D & 2D are illustrated for a possible interaction of compound S9 with the MMPs protein

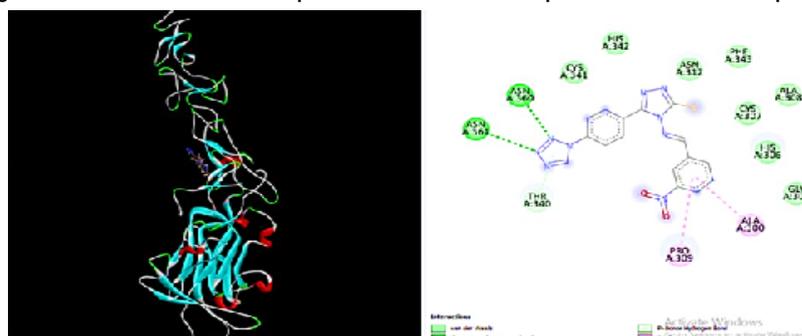


Fig. 7. 3D & 2D are illustrated for a possible interaction of compound S10 with the MMPs protein

RESULTS AND DISCUSSION

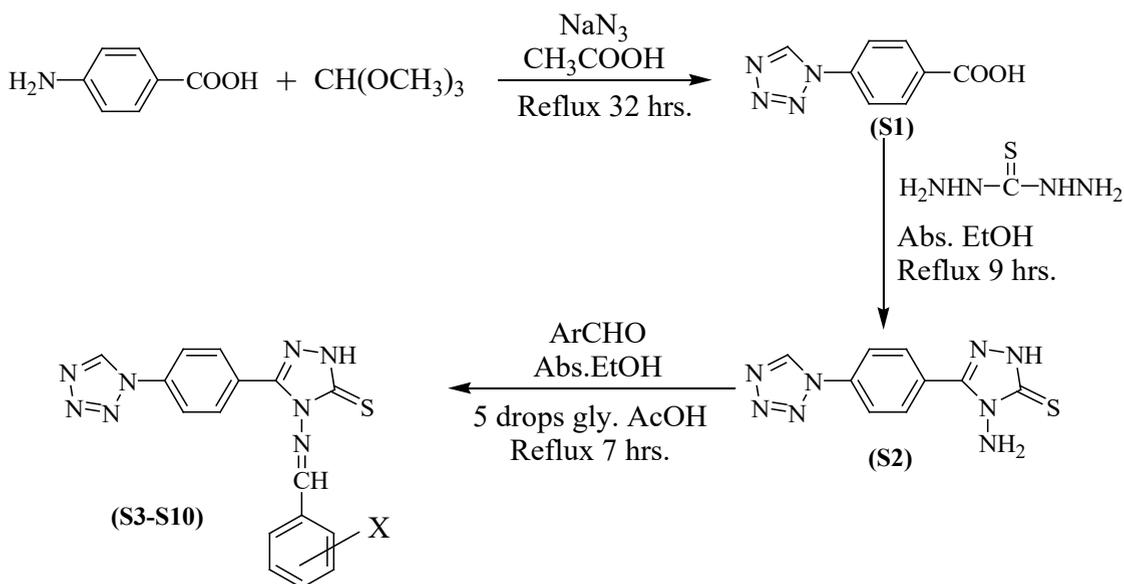
A series of 4-tetrazol-1-yl-benzoic acid (S1) and 1,2,4-triazole-3-thiol derivatives (S2) containing a tetrazole moiety were synthesized to obtain many compounds that are expected to be biologically effective as the similar compounds prepared before¹⁸⁻³⁰, and their structures were confirmed using spectroscopic techniques. The prepared Schiff base compounds (S3-S10) exhibited distinctive features in their FT-IR and ¹H-NMR spectra, Scheme 1.

For compound S3, clear signals for CH=N groups of both the Schiff base and tetrazole ring were observed, along with aromatic proton and hydroxyl signals in the NMR spectrum, confirming

the formation of the target structure. Compound S4 showed the characteristic OCH₃ group, in addition to aromatic proton and Schiff base proton (CH=N) signals. Compound S5 was distinguished by the presence of an OH signal and two separate CH=N signals (one for the Schiff base and another for the tetrazole ring). While, compound S6 contained a characteristic dimethylamino (N(CH₃)₂) group, along with clear NH signal. Compound S7 exhibited the highest yield (95%), with a distinct CH=N signal and a clear NH signal, while compound S8 displayed characteristic OCH₃ group signals along with CH=N and NH. Compound S9 contained an OH signal, an OCH₃ group, and characteristic CH=N signals. Finally, compound S10 was distinguished by the presence of a NO₃ group in IR, with clear CH=N and NH signals

in the $^1\text{H-NMR}$ spectrum. These results confirm the successful synthesis of the target compound series, with the spectra showing properties consistent with the expected chemical structures. The variations in

spectroscopic characteristics among the compounds reflect the influence of different functional groups on molecular properties, which may impact their potential applications in pharmaceutical or industrial fields.



$\text{X} = 2\text{-OH}, 3\text{-OCH}_3, 3\text{-OH}, 4\text{-N}(\text{CH}_3)_2, \text{H}, 4\text{-OCH}_3, 3\text{-OCH}_3, 4\text{-OH}, 3\text{-NO}_2$

Scheme 1. Synthesis of Tetrazole Benzoic acid (**S1**), Aminotriazole-thione (**S2**) and Schiff bases (**S3-S10**)

CONCLUSION

In this study, we successfully synthesized important derivatives of novel Schiff bases linked to tetrazoles and triazoles using straightforward procedures, mild reaction conditions, and readily available, cost-effective reagents, which are believed to have medical applications such as lung cancer, based on our study of molecular docking; therefore, our research concentrated on these derivatives. Compounds **S6** and **S8** exhibited the most favorable docking scores against MMPs, respectively, indicating strong potential as anticancer agents. The

study recommends further research to evaluate the biological activity and structure-activity relationships of these promising compounds.

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Conflict of interest

The author declare that we have no conflict of interest.

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