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New Design and Synthesis of a Variety of 1,2,4-Triazole Thiol Derivatives: Studying their Antimicrobial and Potential Cytotoxic Effect

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Abstract

1,2,4-Triazole-3-thiols have recently garnered sufficient concern due to their cytotoxic and antimicrobial properties, rendering them valuable in aspects of medicine and pharmacology. The interaction of phenyl hydrazine and benzoyl isothiocyanate **(1)** yielded 1,5-diphenyl-1H-1,2,4-triazole-3-thiol **(2)**, which upon treatment with hydrazine monohydrate after exposure to ethyl-chloroacetate, acetohydrazide **(4)** was achieved**,** that was taken into consideration as a raw substance for creating new derivatives **(5–9)** by interacting with carbon disulfide, isatin, glucose, phthalic anhydride, and various aldehydes. The spectroscopic analysis encompassing (IR, Mass spectrum, ^{1}H-NMR, and ^{13}C-NMR) for those newly synthesized compounds and their behavior against bacteria, fungi, and breast cancer cell lines are covered in this study. Moreover, molecular docking investigation of the antimicrobial action and the structure-activity relationships SAR based on cytotoxic impact are mentioned and discussed.

*Keywords***:** oxadiazole, Schiff-base, antimicrobial assay, antitumor, molecular docking.

1. Introduction

Recent decades have witnessed a notable increase in attention towards organic compounds bearing heterocyclic rings that are composed of one or more hetero-atom, such as oxygen, nitrogen, or sulfur, owing to their biological properties and assessments against multiple diseases, as well as existence in numerous substantial biological molecules including hemoglobin, vitamins, DNA, RNA, hormones, etc. [1, 2]. Nitrogen-based composites were regarded as the most intriguing among heterocyclic compounds as a consequence of their diverse biological features, such as analgesic, antidepressant, antipsychotic, antihistaminic, antimuscarinic, anticancer, and antitumor [3, 4]. Also, they have abundant applications in scientific chemistry and are valuable in the advancement of organic synthesis procedures, in addition to the physiological and pharmacological activities they possess [5, 6].

Triazoles are heterocyclic moieties constructed from nitrogen involving five-constituents, 2C and 3N atoms represented in two isomeric arrangements referring to the placement of nitrogen within the ring: 1,2,3- and 1,2,4-triazoles. The wide diversity of bioactivities they possess has attracted considerable curiosity in therapeutic chemistry and drug discovery [7, 8]. 1,2,3- and 1,2,4-triazoles can both receive an extensive array of substituents (nucleophiles and electrophiles) around their main structures, paving the path to establishing vast varieties of distinct bioactive compounds [9, 10]. They are able to interact with a wide series of biological targets by making non-covalent bonds including the forces of London Dispersion, hydrogen bonding, hydrophobic, and dipole-dipole interactions [11]. Besides that, triazoles comprising sulfur in the format of thiols (mercapto) and thione substitutions exhibit further efficacy[4, 12].

Similarly, 1,2,4-Triazoles and their derivatives have acquired a sufficient value for being included into many anti-cancer drugs such as vorozole[13], letrozole[14], and anastrozole[15], which are valuable against advanced breast cancer cells. They also turned out to be efficacious in pharmaceutical and medicinal fields [16, 17], due to their presence in various therapeutically significant drugs utilized in clinical therapy including itraconazole[18], posaconazole[19],

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and voriconazole[20] as antifungal[21], ribavirin[22] as antiviral **Fig.(1)**. In addition to, antibacterial[23, 24], anticonvulsant[25], anti-inflammatory[23, 26], and antidepressant[27] characteristics. Depending on these views, we designed a new series of 1,2,4-Triazole derivatives specifically 1,2,4-Triazole-3-thiols, providing a route for many syntheses which proved that they are not only promising for further research in cancer treatment but also have potential in antimicrobial applications.

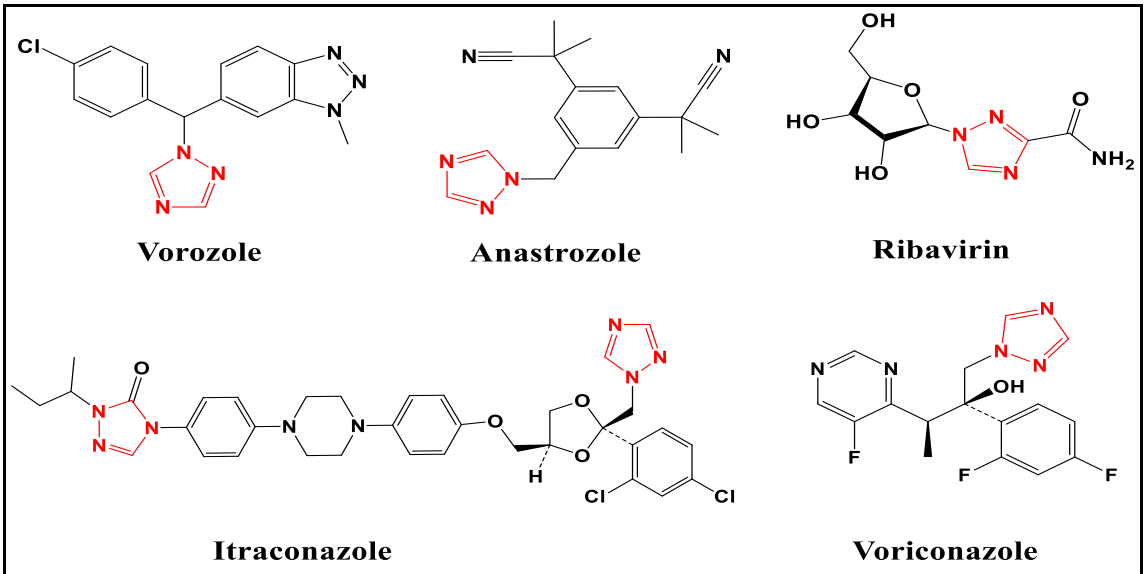


Fig. (1): particular examples of drugs based on 1,2,4-triazoles

2. Materials and Methods

2.1. Chemistry

All chemical supplies required during this work have been procured from (Alfa, Sigma-Aldrich, Fluka, and Merck). All fusion points were estimated and uncorrected via an electrothermal apparatus (Gallenkamp). The infrared (cm^{-1}) spectrum was proceeded using (KBr) disks on a "Fourier transform infrared spectrometer (FTIR 7600)". ^{1}H and ^{13}C NMR spectroscopy were analyzed in DMSO-d6 at 300 MHz and 75 MHz on a "Bruker Biospin AG spectrometer" at (the Microanalytical Center of Cairo University), chemical shifts δ were specified in ppm utilizing TMS (tetramethylsilane) as the inward reference. The mass spectrum was measured on "Shimadzu GC-MS spectrometer" (70 eV) at (the Regional Centre for Mycology and Biotechnology of Al-Azhar University). Elemental analyses have been implemented at (the Microanalytical Center of Cairo University). whole reaction progressions have been tracked using thin layer chromatography (silica gel, Merck). The antimicrobial activities were studied at (The Microanalytical Center of Cairo University). The anticancer efficacy of the products underwent investigation at (the National Cancer Institute, Cairo University).

2.1.1. Synthesis of 1,5-Diphenyl-1H-1,2,4-triazole-3-thiol (2)

Phenyl hydrazine (0.003 mol) was incorporated dropwise into a combination of benzoyl isothiocyanate **(1)** (0.003 mol) in acetonitrile 30 ml. The mixture was then stirred for 1 h at room temperature then refluxed for 3 hrs. Once cooling the product was gathered and crystallized with EtOH giving compound **(2)** as colorless needles; yield 76%; m.p 243-245 °C; FT-IR (KBr, cm^{-1}): 3064, 3018 ($CH_{Arom.}$), 2513 (SH), 1595 (C=N), 687 (C-S); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 7.40-8.05 (m, 10H, Ar-H), 14.27 (s, 1H, SH exchangeable); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 125.78, 126.09, 127.93, 128.77, 129.24, 129.65, 131.21, 149.31, 166.32; MS m/z (%): 253 (25), 103 (39), 91 (100), 77 (61), 64 (35); Anal. Calcd for: $C_{14}H_{11}N_3S$ (253.32): C, 66.38; H, 4.38; N, 16.59; S, 12.66; Found: C, 66.35; H, 4.40; N, 16.63; S, 12.60.

2.1.2. Synthesis of Ethyl 2-((1,5-diphenyl-1H-1,2,4-triazol-3-yl)thio)acetate (3)

An equivalent amount of (0.1 mol) ethyl chloroacetate and compound **(2)** was added to a stirred mixture of ethanolic KOH (0.1 mol in 25 ml ethanol). After a five-hour reflux, the solution was poured onto ice cold water. The resulting solid was collected, washed with water, and crystallized from ethanol to afford compound (**3**) as white crystals; yield 70%; m.p 48-50

°C; FT-IR (KBr, cm^{-1}): 3059 (CH_{Ar}), 2989, 2938 ($CH_{Aliph.}$), 1732 (C=O), 1177 (C-O); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 1.20 (t, *J=6 Hz*, 3H, CH_3), 4.17 (q, *J=6 Hz*, 2H, CH_2), 4.23 (s, 2H, CH_2), 7.43-8.03 (m, 10H, Ar-H); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 14.10, 35.05, 61.38, 123.79, 125.91, 128.90, 129.14, 129.80, 130.03, 136.57, 152.45, 161.08, 168.22; MS m/z (%): 339 (58), 266 (51), 103 (31), 91 (100), 77 (60); Anal. Calcd for: $C_{18}H_{17}N_3O_2S$ (339.41): C, 63.70; H, 5.05; N, 12.38; S, 9.45; Found: C, 63.68; H, 5.08; N, 12.30; S, 9.50.

2.1.3. Synthesis of 2-((1,5-Diphenyl-1H-1,2,4-triazol-3-yl)thio)acetohydrazide (4)

A mixture of hydrazine hydrate (1 ml) with compound **(3)** (0.01 mol) in ethanol (10 ml) was left overnight at room temperature. The precipitate was gathered and crystallized with ethanol as colorless crystals; yield 86%; m.p 127-129°C; FT-IR (KBr, cm^{-1}): 3423 (NH), 3295, 3206 (NH_2), 1653 (C=O); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.06 (s, 2H, CH_2), 4.32 (s, 2H, NH_2), 7.44- 8.07 (m, 10H, Ar-H), 9.39 (s, 1H, NH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 35.20, 124.00, 125.99, 128.86, 129.10, 129.68, 130.06, 136.59, 152.71, 161.05, 166.07; MS m/z (%): 325 (6), 278 (75), 248 (45), 180 (80), 104 (62), 88 (100); Anal. Calcd for: $C_{16}H_{15}N_5OS$ (325.39): C, 59.06; H, 4.65; N, 21.52; S, 9.85; Found: C, 59.01; H, 4.60; N, 21.58; S, 9.90.

2.1.4. Synthesis of 5-(((1,5-Diphenyl-1H-1,2,4-triazol-3-yl)thio)methyl)-1,3,4-oxadiazole-2-thiol (5)

A solution of hydrazide **(4)** (0.01 mol) in ethanolic KOH (0.015mol) was stirred until solution turned clear. then, CS_2 (20 ml) was dropped wisely and the mixture was refluxed for 8 h with H_2S evolution throughout the reaction. The resulted product was filtered and crystallized from ethanol after being concentrated, cooled, and acidified with concentrated HCl to obtain compound **(5)** as yellow crystals; yield 68.2%; m.p 172-174°C; FT-IR (KBr, cm^{-1}): 3057 (CH_{Ar}), 2993 ($CH_{Aliph.}$), 2561 (SH), 1590 (C=N), 1170 (C-O); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.70 (s, 2H, CH_2), 7.46-8.06 (m, 10H, Ar-H), 14.60 (s, 1H, SH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 27.10, 124.12, 126.01, 128.92, 129.31, 129.72, 129.86, 136.40, 150.97, 160.66, 161.30, 177.92; MS m/z (%): 367 (10), 346 (100), 338 (40), 290 (78), 220 (50), 101 (15); Anal. Calcd for: $C_{17}H_{13}N_5OS_2$ (367.45): C, 55.57; H, 3.57; N, 19.06; S, 17.45; Found: C, 55.53; H, 3.54; N, 19.11; S, 17.40.

2.1.5. Synthesis of 2-((1,5-Diphenyl-1H-1,2,4-triazol-3-yl)thio)-N'-(2-oxoindolin-3-ylidene)-acetohydrazide (6)

An equimolar mixture of compounds **(4)** (0.01mol) and isatin (0.01mol) was refluxed for an hour with glacial acetic acid (0.5 ml) in ethanol (25 ml). A yellow precipitate was collected, washed with hot ethanol, and crystallized using EtOH and DMF to obtain the expected compound **(6)** as yellow ppt; yield 80%; m.p 260-262°C; FT-IR (KBr, cm^{-1}): 3235, 3153 (NH), 3066 ($CH_{Ar.}$), 2965 ($CH_{Aliph.}$), 1730, 1695 (C=O), 1604 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.78 (s, 2H, CH_2), 7.04- 8.14 (m, 14H, Ar-H), 10.81 (s, 1H, NH isatin), 11.28 (s, 1H, NH hydrazone); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 38.71, 115.26, 120.95, 121.77, 122.75, 123.95, 125.93, 126.28, 128.80, 129.19, 129.79, 129.97, 131.92, 136.57, 142.62, 151.01, 161.02, 162.55, 170.00; MS m/z (%): 454 (46), 349 (77), 158 (100), 140 (83), 70 (75); Anal. Calcd for: $C_{24}H_{18}N_6O_2S$ (454.51): C, 63.42; H, 3.99; N, 18.49; S, 7.05; Found: C, 63.48; H, 4.09; N, 18.43; S, 7.01.

2.1.6. Synthesis of 2-((1,5-Diphenyl-1H-1,2,4-triazol-3-yl)thio)-N'-(2,3,4,5,6-pentahydroxy-hexylidene)acetohydrazide (7)

A mixture of hydrazide **(4)** (0.01 mol) and glucose (0.01 mol) was refluxed for 4 h in EtOH (30 ml). The afforded product was gathered and crystallized from EtOH to produce compound **(7)** as white ppt; yield 83%; m.p 70-72°C; FT-IR (KBr, cm^{-1}): 3390-3284 (OH, NH), 2924 ($CH_{Aliph.}$), 1673 (C=O), 1594 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 3.09-3.13 (m, 4H, 4CH), 3.60-3.98 (m, 2H, CH_2 glucose), 4.08 (s, 2H, CH_2), 4.38-4.93 (s, 5H, 5OH), 7.47- 8.05 (m, 10H, Ar-H), 8.07 (s, 1H, HC=N), 9.95 (s, 1H, NH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 35.24, 61.90, 63.54, 70.07, 71.05, 74.90, 123.99, 126.06, 128.90, 129.14, 129.73, 130.09, 136.63, 152.76, 153.44, 161.11, 170.99; MS m/z (%): 487 (45), 448 (100), 367 (57), 341 (83), 166 (91); Anal. Calcd for: $C_{22}H_{25}N_5O_6S$ (487.53): C, 54.20; H, 5.17; N, 14.37; S, 6.58; Found: C, 54.15; H, 5.20; N, 14.33; S, 6.52.

2.1.7. Synthesis of N-(1,3-Dioxoisoindolin-2-yl)-2-((1,5-diphenyl-1H-1,2,4-triazol-3-yl)thio)-acetamide (8)

A mixture of hydrazide **(4)** (0.01 mol), phthalic anhydride (0.01 mol), and glacial acetic acid (20 ml) was refluxed for 8 h. then cooled to room temperature, poured onto ice cold water, and crystallized from hexane to afford compound **(8)** as colorless crystals; yield 77%; m.p 181-183°C; FT-IR (KBr, cm^{-1}): 3255 (NH), 3065, 3003 (CH_{Ar}), 2931 ($CH_{Aliph.}$), 1749-1679 (C=O), 1593 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.43 (s, 2H, CH_2), 7.45-8.17 (m, 14H, Ar-H), 11.16 (s, 1H, NH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 34.63, 123.80, 123.87, 126.22, 128.79, 129.10, 129.44, 129.73, 130.09, 135.30, 136.56, 152.23, 161.21, 164.89, 166.57; MS m/z (%): 455 (15), 352 (33), 325 (70), 58 (100); Anal. Calcd for: $C_{24}H_{17}N_5O_3S$

(455.49): C, 63.29; H, 3.76; N, 15.38; S, 7.04; Found: C, 63.33; H, 3.80; N, 15.29; S, 7.02.

2.1.8. General procedure for synthesis of Schiff Base hydrazones (9a, b)

An equivalent quantity of acetohydrazide **(4)** (0.01 mol) and various aldehydes in ethanol (25 ml) was heated till the mixture turned clear. Glacial acetic acid was then added in a few drops and the reaction mixture was refluxed for 4 h. The product was gathered upon cooling and crystallized from the appropriate solvent.

2.1.8.1. N'-(4-Chlorobenzylidene)-2-((1,5-diphenyl -1H-1,2,4-triazol-3-yl)thio)acetohydrazide (9a)

Colorless crystals (benzene); yield 84%; m.p 210-212°C; FT-IR (KBr, cm^{-1}): 3174 (NH), 3065 (CH_{Ar}), 2971, 2938 ($CH_{Aliph.}$), 1684 (C=O), 1596 (C=N); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.66 (s, 2H, CH_2), 7.36-8.08 (m, 14H, Ar-H), 8.20 (s, 1H, CH), 11.78 (s, 1H, NH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 35.15, 125.94, 128.40, 128.61, 128.88, 128.99, 129.74, 129.79, 130.08, 133.01, 134.47, 136.69, 145.76, 152.94, 160.99, 168.82; MS m/z (%): 447.5 (7), 411 (50), 409 (44), 229 (25), 218 (35), 40 (100); Anal. Calcd for: $C_{23}H_{18}ClN_5OS$ (447.94): C, 61.67; H, 4.05; Cl, 7.91; N, 15.63; S, 7.16; Found: C, 61.72; H, 4.02; Cl, 7.88; N, 15.69; S, 7.20.

2.1.8.2. 2-((1,5-Diphenyl-1H-1,2,4-triazol-3-yl) thio)-N'-(4-methoxybenzylidene)acetohydrazide (9b)

Colorless crystals (acetic acid); yield 74%; m.p 188-190°C; FT-IR (KBr, cm^{-1}): 3319 (NH), 3066 (CH_{Ar}), 2933 ($CH_{Aliph.}$), 1672 (C=O), 1604 (C=N), 1251 (C-O); ^{1}H-NMR (DMSO-d6, 300 MHz) δ (ppm): 4.25 (s, 3H, CH_3), 4.67 (s, 2H, CH_2), 6.93-8.08 (m, 14H, Ar-H), 8.20 (s, 1H, CH), 11.55 (s, 1H, NH); ^{13}C-NMR (DMSO-d6, 75 MHz) δ (ppm): 35.28, 55.32, 114.33, 125.99, 126.65, 128.54, 128.83, 129.05, 129.68, 129.73, 130.11, 130.15, 143.76, 153.02, 161.11, 163.16, 168.47; MS m/z (%): 443 (20), 364 (62), 335 (21), 277 (7), 120 (68), 77 (21), 55 (100); Anal. Calcd for: $C_{24}H_{21}N_5O_2S$ (443.53): C, 64.99; H, 4.77; N, 15.79; S, 7.23; Found: C, 65.10; H, 4.71; N, 15.82; S, 7.18.

2.2. Antimicrobial Assay

The derived substances were examined *in vitro* for their antibacterial efficacy towards *Staphylococcus Aureus* (Gram-positive bacteria), and *Escherichia Coli* (Gram-negative bacteria) through nutritional agar media. The fungicidal action of the inspected derivatives was carried out against *Candida Albicans and Aspergillus Niger* via Sabouraud dextrose agar medium. The examination of the investigated derivatives proceeded at 15 mg/ml concentration with DMSO as a solvent of negative control. Derivatives were examined by decantation of the sterilized media in the sterilized petri plates (20-25 ml per every petri plates), enabling them to harden at 25°C. A microbial suspension was set in sterile saline comparable to "McFarland 0.5 standard solution (1.5x 10^5 CFU ml^{-1})" with adjustment of the turbidness at optical density of 0.13 utilizing a spectrophotometer at 625 nm. After 15 min, a swab of cotton was immersed into the prepared suspension, flowed on the dry surface of agar, and left to be dried while covered for another 15 minutes. A sterile borer was helpful for establishing wells having a diameter of (6 mm) in the hardened media. 100 µl of the examined solution was extracted by using a micropipette then added to the wells. In instance of bacterial evaluation, the plates underwent incubation overnight at 37°C. This examination was triplicated using (mm) as the zones of inhibition scale [28].

2.3. Cytotoxicity and Antitumor Activity

The compounds undergoing investigation were screened in opposition to MCF-7 (human breast cancer cell lines) attained from "the American Type Culture Collection (ATCC, Minisota, U.S.A.)". they are preserved at the National Cancer Institute, Cairo, Egypt, using serial subculturing technique. For sample preparation, a stock solution was dissolved by a ratio of 1:1 in DMSO and stored at -20°C using variable drug concentrations (µg/ml). The Sulforhodamine-B (SRB) is the method used for cytotoxicity assessment [29, 30]. SRB is an aminoxanthrene dye that possesses two sulphonic groups, which acts as a protein by binding itself to the amino groups of the protein cells in slightly acidic media in order to offer a precise indication of the protein amount within cells. Cells were implanted in about 96 well µl plates at inception concentration of (4x10^3 cell/well) in a fresh medium of (200 µl) and kept overnight to be fixed into the plates. Subsequently, variant concentrations of the drug were utilized (3 wells per each one), The plates were subsequently put into incubation for 2 days. Fixation of the cells was carried out for 1 h at 4^0C using (10 µl of 10% frigid trichloroacetic acid). After that, the plates have been rinsed with deionised water with the assistance of (an automatic washer Tecan, Germany), and underwent staining by (50 µl of 0.2% SRB) dissolving into acetic acid 1% at ambient temperature for ½ h in a dark area, followed by washing via acetic acid 1% and air drying. The dissolving of the dye has been done with 200 µl/well of 10 M tris base (pH 10.5) and the optical density O.D. of every well was recorded at 570 nm spectrophotometrically with an ELISA microplate reader (Sunrise Tecan reader, Germany). The mean value for every drug concentration in addition to the

mean background absorbance were calculated and monitored clearly. The IC_{50} values are the drug concentrations necessary to inhibit 50% of carcinoma cell growth. They were calculated via prism version 5, and the % of cell survival has been computed using the following expression:

$$\text{Surviving fraction} = \frac{\text{O. D. of cells underwent treatment}}{\text{O. D. of control cells}}$$

2.4. In Silico Molecular Docking Analysis

The potential binding profile of derivatives **5** and **6** with four target proteins, namely enoyl-[acyl carrier protein] reductase (FabI) of *Escherichia Coli* (PDB ID: 4D46), enoyl-[acyl carrier protein] reductase (FabI) of *Staphylococcus Aureus* (PDB ID: 4CUZ), cytochrome P-450-dependent enzyme lanosterol 14-α-demethylase (CYP51) from *Candida Albicans* (PDB ID: 5V5Z), and the catalytic domain of glucoamylase from *Aspergillus Niger* (PDB ID: 3EQA) were explored by molecular docking studies. The 3D crystal structures of different target proteins utilized during this investigation were obtained from the Protein Data Bank (PDB). The geometrical structures of compounds **5** and **6** were fully optimized at the B3LYP level [31–33] using the 6-311G (d, p) basis set [34] employing the Gaussian 16 software package [35]. The initial PDB structures of various ligands were constructed using UCSF Chimera software [36]. UCSF Chimera was utilized to eliminate any nonstandard residues and solvent molecules from the original PDB structure of the target proteins [36]. Furthermore, the proteins underwent additional optimization, involving the incorporation of polar hydrogens, adjustment of the grid box configuration to align with the most appropriate active site amino acid residues, and the creation of pdbqt inputs using Autodock Tools (ADT) v1.5.6. [37–41]. Different ligands were subjected to optimization and PDBQT generation using ADT. The docking simulations were performed with the use of the AutoDock Vina software and the ADT v1.5.6. software package[42].

3. Results and Discussion

3.1. Chemistry

1,2,4-triazole-3-thiols were regarded as the basis for creation of new derivatives **(3, 4)**, and have been previously synthesized according to the procedures described in [43, 44]. Firstly, benzoyl isothiocyanate **(1)** [45] was produced by treating ammonium thiocyanate with benzoyl chloride in a solution of acetonitrile. Then, the isothiocyanate **(1)** was interacted with phenyl hydrazine to give thiosemicarbazide which supposed to undergo an intramolecular cyclization to afford **(2)** with elimination of H_2O molecule as shown in **(Scheme 1)**. The predicted structure was validated by the infrared spectra, which revealed that the carbonyl group of isothiocyanate vanished, and a new absorption band of SH appeared at 2513 cm^{-1}. Likewise, SH group was detected as a singlet signal at 14.27 ppm as indicated by ^{1}H-NMR. Meanwhile, signals of ^{13}C-NMR were attained at 149.31 and 166.32 ppm associated with Ph-C=N and S-C=N, respectively.

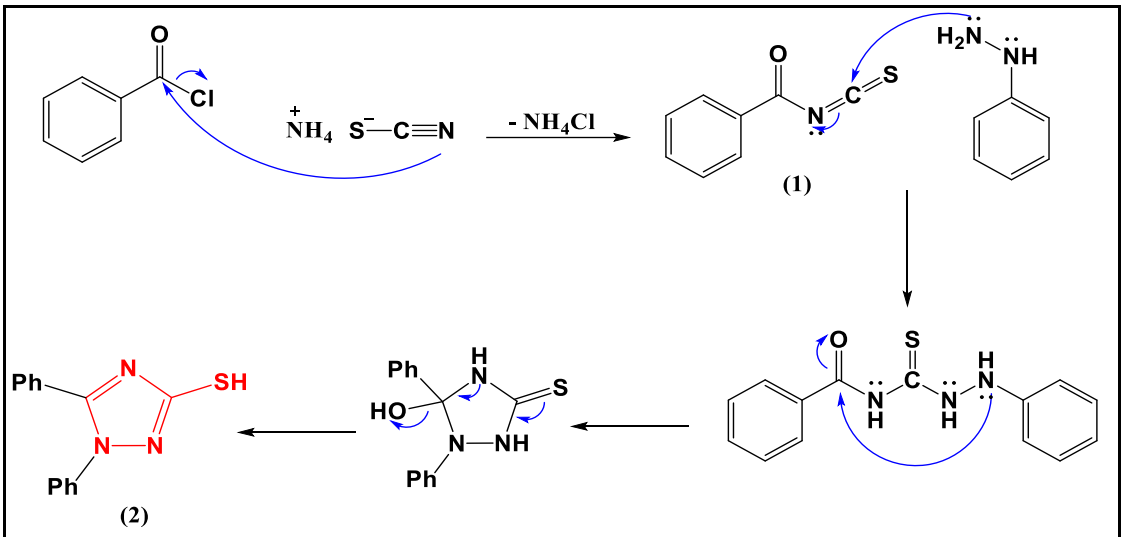
Furthermore, the interaction of **(2)** and ethyl chloroacetate into ethanolic KOH offered compound **(3)** [46]. This was identified by IR absorption value of C=O (ester) which developed at 1732 cm^{-1}. However, ^{1}H-NMR demonstrated signals for CH_3 (triblet), CH_2 (quartet), and CH_2 (singlet) at 1.20, 4.17, and 4.23 ppm, respectively. Additionally, signals pertaining to CH_3 and CH_2 groups were revealed by ^{13}C-NMR at 14.10 and 35.05, 61.38 ppm, respectively along with the C=O at 168.22 ppm.

Subsequently, addition of hydrazine hydrate 99% to compound **(3)** in ethanol [47] yielded 2-((1,5-diphenyl-1H-1,2,4-triazol-3-yl)thio)acetohydrazide **(4)** which was considered as the essential key for further syntheses **(5-9)** that would be discussed below. The structure of Compound **(4)** was verified through $NHNH_2$ emergence at 3423-3206 cm^{-1}, besides the reduced value of the C=O group that noticed clearly at 1653 cm^{-1} as detected by IR spectrum. Also, ^{1}H-NMR revealed singlet signals at 4.06, 4.32, and 9.39 ppm for CH_2, NH_2, and NH, sequentially. While, ^{13}C-NMR signals were detected for CH_2 at 35.20 ppm, and at 166.07 ppm for the carbonyl carbon. The synthetic pathways for compounds **(3, 4)** are depicted in **(Scheme 2)**, starting from compound **(2)** as the basic structure.

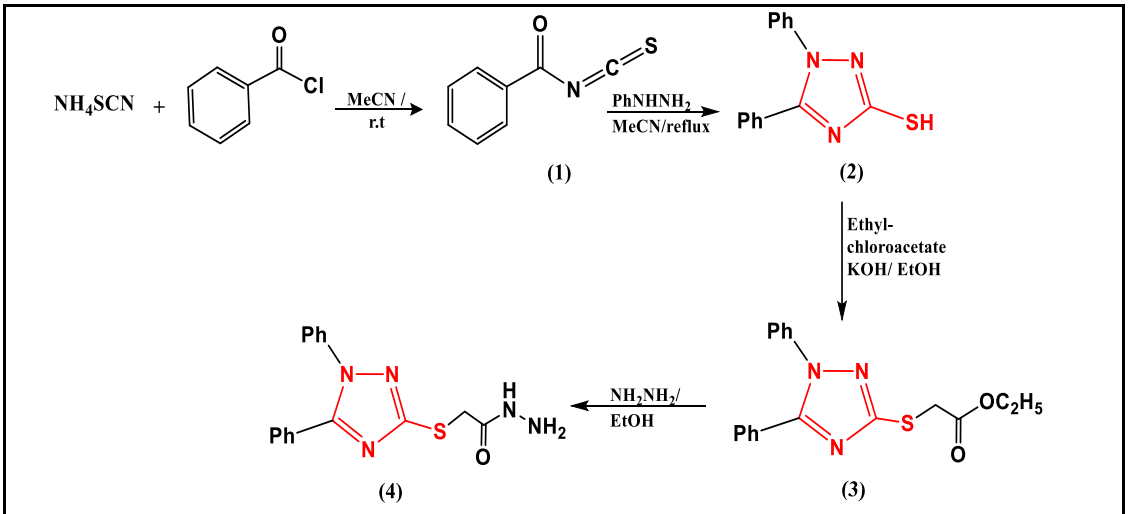
The reaction of **(4)** and CS_2 in the existence of KOH, followed by the acidification (HCl) resulted in the desired oxadiazole derivative **(5)** [48], this was clarified from the disappearance of carbonyl group and the establishment of new SH band at 2561 cm^{-1} as referred by IR spectrum. Singlet signals from ^{1}H-NMR analysis additionally disclosed the presence of the SH group at 14.60 ppm. Whereas, ^{13}C-NMR highlighted signals of C=N groups at 161.30, 177.92 ppm for the new oxadiazole ring.

The reaction mechanism for synthesis of compound **(5)** was proposed to proceed via ring closure reaction between compound **(4)** and CS_2 in a basic medium **(Scheme 3)**.

Another derivative of triazoles was introduced by the treatment of **(4)** with isatin moiety under reflux with the help of acetic acid [49] to obtain the oxindole derivative **(6)**. The structure of **(6)** was illustrated by the values of the observed N-H and C=O groups at 3235, 3153, 1730, and 1695 cm^{-1}, respectively according to the IR spectrum.



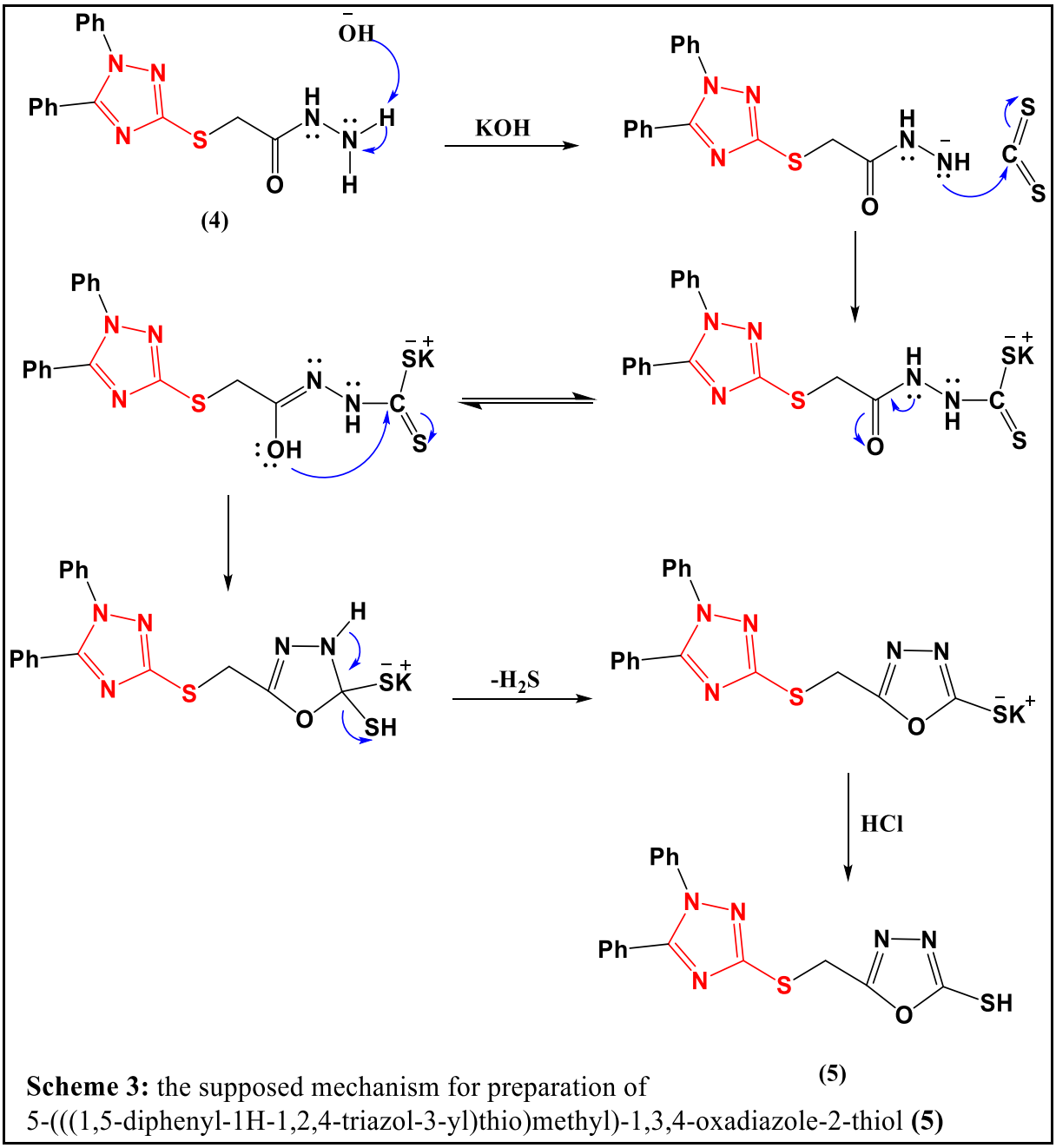
Scheme 1: the supposed mechanism for synthesis of 1,5-diphenyl-1H-1,2,4-triazole-3-thiol **(2)**



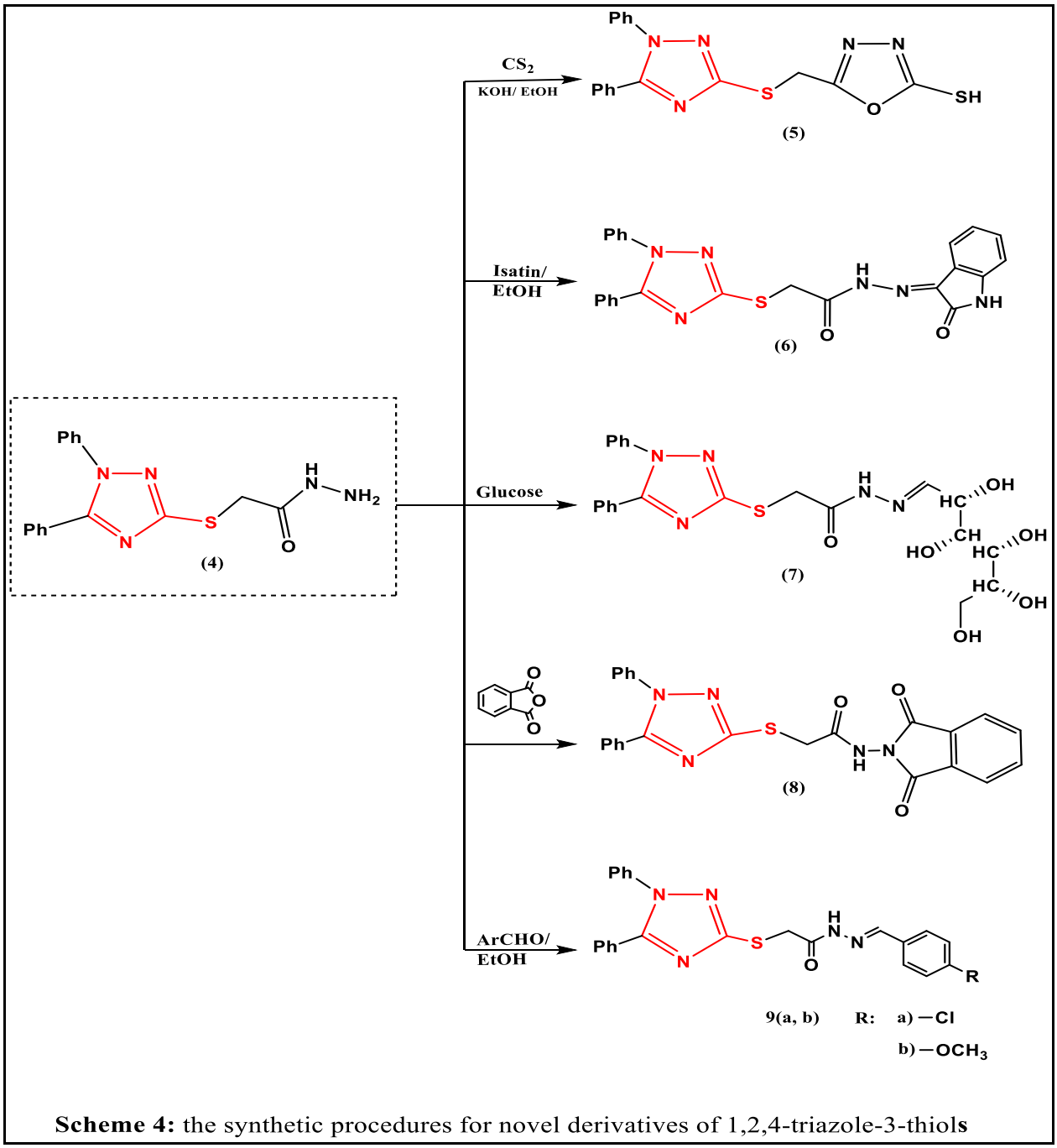
Scheme 2: the synthetic pathways for synthesis of 2-((1,5-diphenyl-1H-1,2,4-triazol-3-yl)thio)acetohydrazide **(4)**

The ^{1}H-NMR values also provided a confirmation of the expected structure by giving singlet signals characterized for NH groups at 10.81 and 11.28 ppm. As well as the values of ^{13}C-NMR which investigated the compound at 162.55 and 170.00 ppm for C=O groups. Furthermore, compound **(7)** was synthesized by treatment of the acetohydrazide **(4)** with glucose in ethanol, the elucidation of the structure was submitted by IR frequency values observed for OH and NH groups at 3390-3284 cm^{-1} as a broad band. ^{1}H-NMR showed multiplet signals at 3.09-3.13 ppm for 4CH, and at 3.60 ppm for CH_2 of the glucose molecule, besides the 5OH, and N=CH protons that respectively appeared at 4.38-4.93, and 8.07 ppm as singlet signals. The ^{13}C-NMR also supported the results by presenting the 4CH of glucose molecule at 63.54-74.90 ppm, CH_2-OH at 61.90 ppm, and N=CH at 153.44 ppm. Compound **(8)** was also attained through the condensation reaction between phthalic anhydride and acetohydrazide **(4)** in glacial acetic acid to give the desired dioxoisoindolin **(8)**. The evidences for the structure of **(8)** were provided by IR absorption band values at 3255 cm^{-1} for NH group and 1749-1679 cm^{-1} for C=O. Also, the chemical shifts of ^{1}H-NMR provided a singlet value at 11.16 ppm for NH. In addition to ^{13}C-NMR which gave signals at 164.89, 166.57 ppm for C=O. Both compounds **(7, 8)** were generated using the procedures provided in [50].

Similarly, Schiff base derivatives **(9a, b)** were produced by the condensation of hydrazide **(4)** with assorted aldehydes whilst acetic acid was involved. [51]. The resulted structures **(9a, b)** were investigated by the spectral data. The derived compound **(9a)** was proved by IR spectrum at 3174 cm^{-1} for NH and 1684 cm^{-1} for C=O. Also, ^{1}H-NMR illustrated the structure as signals showed up at 8.20 ppm for CH and 11.78 ppm for NH as singlet signals. ^{13}C-NMR also indicated the value of CH at 145.76 ppm, and 168.82 ppm for C=O. Likewise, compound **(9b)** was deduced by IR values at 3319 cm^{-1} for NH, 1672 cm^{-1} for C=O group, and 1251 cm^{-1} for C-O. Even more, ^{1}H-NMR declared CH_3 at 4.25 ppm, CH at 8.20 ppm, and NH at 11.55 ppm as singlet signals. Also, ^{13}C-NMR showed signals at 55.32 ppm for OCH_3, 143.76 ppm for CH, 163.16 ppm for C-O, and 168.47 ppm for C=O. In addition, all the discussed products were detected and proved by mass spectrum and elemental analysis (exp. section) **(Scheme 4)**.



Scheme 3: the supposed mechanism for preparation of 5-(((1,5-diphenyl-1H-1,2,4-triazol-3-yl)thio)methyl)-1,3,4-oxadiazole-2-thiol **(5)**



Scheme 4: the synthetic procedures for novel derivatives of 1,2,4-triazole-3-thiols

3.2. Antimicrobial Activity

The newly prepared substances were assessed for their *in vitro* antibacterial efficacy against Gram-positive *(Staphylococcus Aureus ATCC13565)* and Gram-negative *(Escherichia Coli ATCC:10536)* bacteria using agar well diffusion method. The reference drug used for Gram-positive bacteria was Ampicillin whereas, Gentamicin was used for Gram-negative. The fungicidal assay of the investigated moieties was evaluated against *(Candida Albicans ATCC:10231)* and *(Aspergillus Nigar ATCC:16404)* employing the medium of Sabouraud dextrose agar. The standard drug used for fungi strains was Nystatin. The zone of inhibition for the investigated compounds is expressed with standard deviation (mm) and is summarized in **Table 1**, whereas, the activity index % can be calculated from the formula provided below and are plotted in **Fig. (2)**.

$$\text{Activity index \%} = \frac{\text{inhibitory zone of tested compound (mm)}}{\text{inhibitory zone of reference drug (mm)}} \times 100$$

Table1: the antimicrobial studies of the examined compounds measured by inhibition zone (mm), and activity index %:

Microorganism	*Escherichia Coli (ATCC:10536)*		*Staphylococcus Aureus (ATCC:13565)*		*Candida Albicans (ATCC:10231)*		*Asperagillus Nigar (ATCC:16404)*	
Compound No.	the inhibitory zone's diameter (mm)	Activity Index %	the inhibitory zone's diameter (mm)	Activity Index %	the inhibitory zone's diameter (mm)	Activity Index %	the inhibitory zone's diameter (mm)	Activity Index %
2	NA	-	NA	-	14.3	67.1	NA	-
3	NA	-	NA	-	10.3	48.4	NA	-
4	NA	-	NA	-	9.7	45.5	NA	-
5	16.7	61.2	21.3	96.8	8.7	40.8	NA	-
6	NA	-	NA	-	15.3	71.8	NA	-
7	10.0	36.6	NA	-	14.7	69	NA	-
8	NA	-	9.7	44	9.7	45.5	NA	-
9a	10.0	36.6	NA	-	14.6	68.5	NA	-
9b	NA	-	9.3	42.3	8.7	40.8	NA	-
Gentamicin	27.3	100	NA	-	NA	-	NA	-
Ampicillin	NA	-	22.0	100	NA	-	NA	-
Nystatin	NA	-	NA	-	21.3	100	19.0	100

NA: no activity

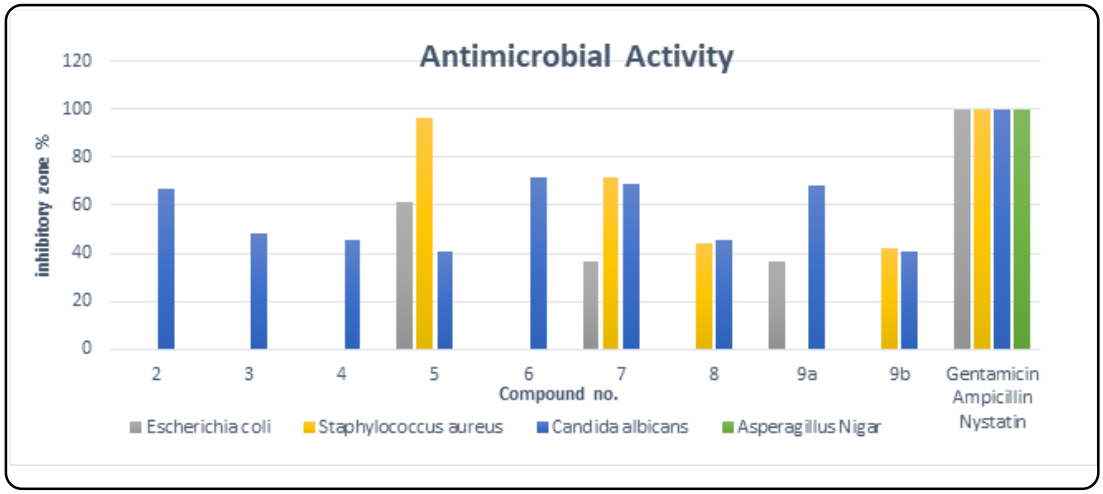


Fig. (2): the antibacterial activity index % of the investigated compounds

The antibacterial study provided that compounds **5, 7, 9a** have moderate activity against *Escherichia Coli*. Additionally, compound **5** showed high efficacy against *Staphylococcus Aureus*, while compounds **8**, **9b** revealed a moderate evaluation in comparison to ampicillin. On the other hand, all the investigated compounds were effective against *Candida Albicans* whereas, *Aspergillus Nigar* was resistant to all the tested compounds.

Thus, the strong antibacterial activity reported in compound **5** can be attributed to the oxadiazole moiety. Additionally, the antifungal efficacy against Candida Albicans increased in compound **6** may be a result of isatin derivative.

3.3. Cytotoxic Activity

The majority of the developed moieties were examined *in vitro* against MCF-7 (breast cancer cell lines) with the use of Doxorubicin as a drug reference with variable concentrations. The cytotoxicity evaluations were measured as growth inhibitory concentration (IC_{50}). The relation between IC_{50} of tested compounds in comparison to doxorubicin is concluded in **Table 2** and presented in **Fig. (3)**.

Table 2: Cytotoxicity values (IC50, µg/ml) of the tested derivatives against MCF-7 (breast cancer cell lines):

Compound No.	MCF-7 IC_{50} (µg/ml)
4	34
5	26
6	7
7	30.4
8	-
9a	10
Doxorubicin	5.6

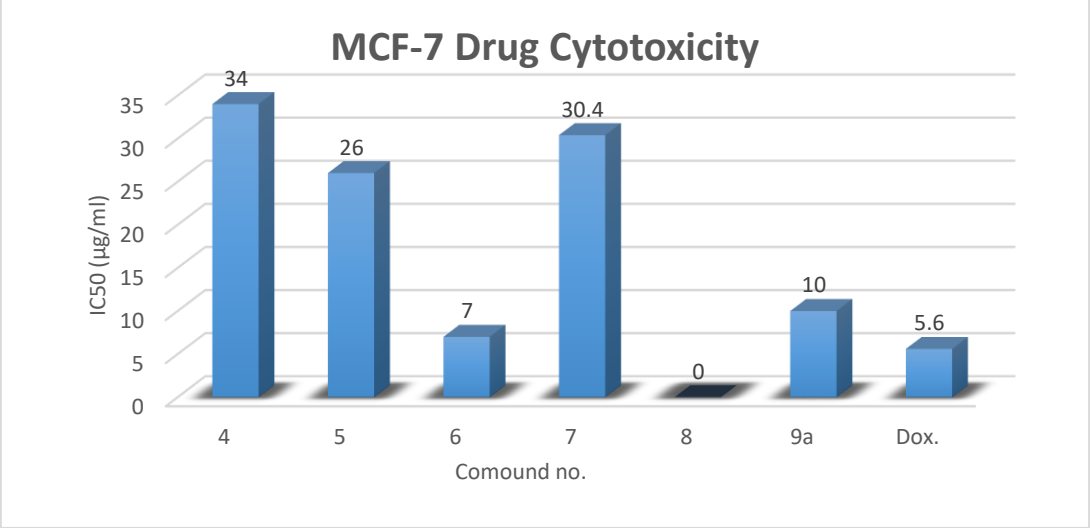


Fig. (3): Cytotoxicity (IC50, µg/ml) of the new derivatives in opposition to MCF-7

According to the findings from the cytotoxic investigation, the majority of the examined substances demonstrated adequate response against MCF-7 in comparison to doxorubicin. On the basis of these values, the activity of newly generated moieties can be arranged in the declining order presented below:

6 > 9a > 5 > 7 > 4

Breast cancer cell line was resistant to compound **8**. The IC_{50} values indicated that the newly synthesized derivatives **5, 6, 7, 9a** are more effective against MCF-7 as compared with the starting material **4**. This confirms that the new derivatives enhanced the behavior of triazoles as cytotoxic drugs.

3.4. Molecular Docking

Molecular docking study was processed to inspect the potential binding profile of the newly synthesized derivatives **5** and **6** against four proposed microbial protein receptors namely enoyl-[acylcarrier-protein] reductase (FabI) of *Escherichia Coli*, enoyl-[acyl carrier protein] reductase (FabI) of *Staphylococcus Aureus*, cytochrome P-450-dependent enzyme lanosterol 14-α-demethylase (CYP51) from *Candida Albicans*, and the catalytic domain of glucoamylase from *Aspergillus Niger*. These targets were chosen to establish a connection between the theoretical antimicrobial effectiveness and experimental results, as these proteins are known to be vital for bacterial growth [52–55].

The stability of biological macromolecular structures relies mainly on the interaction of ligands and proteins, encompassing both polar and hydrophobic interactions. Notably, the principal factor dictating the binding of a specific drug within the target's binding pocket is the occurrence of hydrogen bonding [41, 56]. Consequently, these interactions assume a vital role in dictating the ligand's affinity for the protein's active site, its molecular recognition, and its placement within the binding site [40, 57]. Another pivotal factor that impact the stability of drug-protein complexes is the binding energies, wherein hydrophobic interactions between the ligand's lipophilic surface and the hydrophobic regions in the binding pocket wield a considerable impact [38, 39, 58]. The influence of polar bonding on binding energies primarily depends on the number of formed polar bonds and desolvation

energy [57]. Hence, for a thermodynamically favorable interaction between a drug and its receptor, it is essential to attain a proper geometric alignment of the ligand within the protein's active site. The agreement between the results of molecular docking studies and biochemical assays offers an opportunity for a systematic assessment of the efficacy of these drugs as potent treatments for microbial infections.

The findings of our molecular docking investigation revealed a broad-spectrum antimicrobial activity of compound **5** against *Escherichia Coli*, *Staphylococcus Aureus*, and *Candida Albicans*. This inference primarily stems from the observed low binding affinities of this compound towards the examined target receptors. The obtained binding free energies for these receptors are -7.6, -9.3, and -9.8 kcal/mol, respectively. Interestingly, a relatively higher binding affinity was obtained for the target protein of *Aspergillus Niger* (-5.4 kcal/mol). This information is especially noteworthy since it clarified the correlation between the docking results and the *in-vitro* antimicrobial assay, where compound **5** showed no *in-vitro* antimicrobial activity against *Aspergillus Niger*. **Fig. (4)** represents the active site interactions of compound **5** with the target proteins of *Escherichia Coli*, *Staphylococcus Aureus*, and *Candida Albicans*. Compound **5** was shown to dock deeply submerged into the main central channel binding site in both *Staphylococcus Aureus* and *Candida Albicans* receptors, meanwhile, it was detected in an active binding pocket on the surface of the protein in the case of *Escherichia Coli* **Fig. (4)**. In addition, a dense network of polar and hydrophobic interacting amino acid residues was detected in the binding profile of this drug with all tested protein receptors. Furthermore, essential key residues were pinpointed within the active site of the complexes formed by the synthesized compound and the target receptors, as illustrated in **Fig. (4C)**. For instance, the residues Phe92, Phe94, Phe204, Phe126, and Phe233 were detected in the binding sites of studied drug-target complexes. These residues are capable of contributing significantly to complex stabilization and drug orientation by exhibiting a thermodynamically favorable π-π interaction, enabling the development of a stable drug-protein complex. Thus, the findings of our molecular docking analysis of compound **5** against the microbial proteins of *Escherichia Coli*, *Staphylococcus Aureus*, and *Candida Albicans* indicate a strong likelihood for this compound to exhibit antimicrobial activity against these microbial strains.

Contrary to compound **5**, compound **6** molecular docking results observed the lowest binding affinity for the receptor of *Staphylococcus Aureus* and *Candida Albicans*. The estimated binding energies of these receptors are -10.3 and -10.5 kcal/mol, respectively. However, compound **6** showed no *in-vitro* antimicrobial activity against *Staphylococcus Aureus*. This deviation is mainly because of the location of the drug in a binding site other than the main binding pocket in the main central channel of the enzyme and steric influence arises by compound **6** structural features. On the other hand, the findings of molecular docking are in line with the outcomes of the antimicrobial assay for *Escherichia Coli* and *Aspergillus Niger*, where these receptors revealed a higher binding affinity of -8.2 and -7.2 kcal/mol, respectively. **Fig. (5)** represents the active site interactions of compound **6** with the target enzyme of *Candida Albicans*. Notably, two polar bonds and a high extent of hydrophobic interactions were obtained in the active site of this complex **Fig. (5)**, Additionally, the studied drug was found to occupy the enzyme's primary binding site with nearly the whole ligand deeply submerged in the binding cavity **Fig. (5B)**. The location of compound **6** in the main active site was proved by the existence of the heme porphyrin residue in the binding site of this complex. Also, important key residues were included in the binding mechanism of this complex. For example, the residues Ph58, Ph230, Phe233, and Phe380 showed hydrophobic interaction with compound **6** **Fig. (5C)**, enabling the establishment of an energetically favored π-π interaction, assisting in this complex stabilization. These findings collectively indicate that compound **6** possesses inhibitory activity against the investigated protein receptors of *Candida Albicans.*

Based on the found binding affinities, hydrogen bonds, and potential hydrophobic interactions observed between compounds **5** and **6** and the receptor proteins of studied microbes, the present study proposes that these newly synthesized compounds have the potential to serve as effective antimicrobial agents by targeting these receptors. This assertion is corroborated by the findings of *in-vitro* antimicrobial activity assays. Consequently, further evaluation of these compounds for their antimicrobial efficacy is warranted through *in-vivo* studies aimed at assessing their antimicrobial properties.

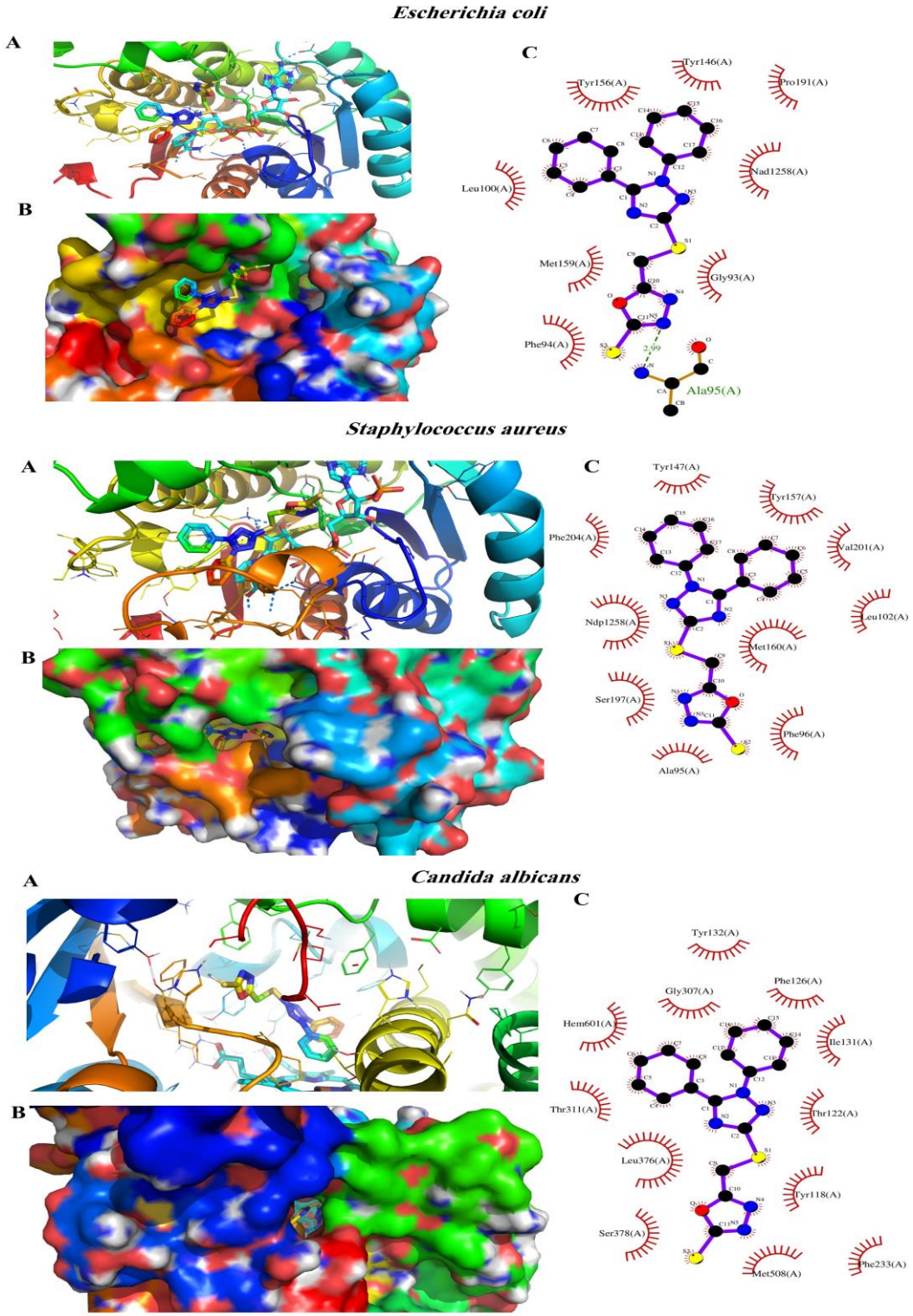


Fig. (4): Active site interactions of compound **5** against the microbial proteins of *Escherichia Coli*, *Staphylococcus Aureus*, *and Candida Albicans*, drug and the binding cavity of the protein are shown as a tube model, while residues are shown as a stick model (A), surface representation of the binding site occupied by substrates (B), residues exhibiting polar and hydrophobic interactions with the proposed drug (C).

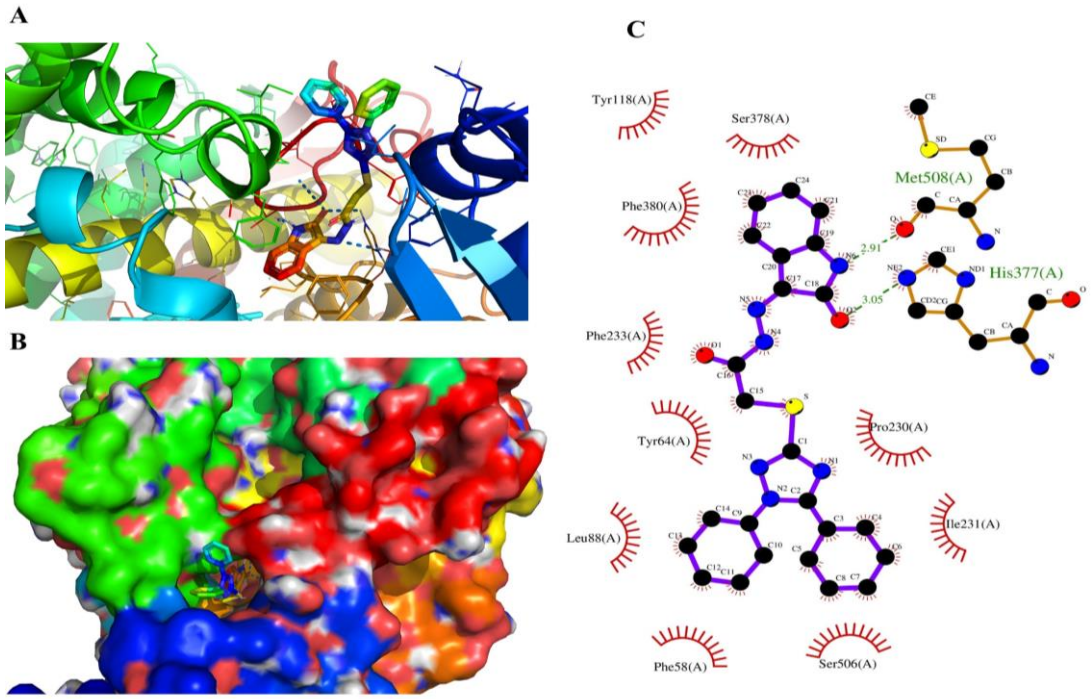


Fig. (5): Active site interactions of derivative **6** against the target enzyme of *Candida Albicans*, drug and the binding cavity of the protein are shown as tube model, while residues are shown as stick model (A), surface representation of the binding site occupied by substrates (B), residues exhibiting polar and hydrophobic interactions with the proposed drug (C).

3.5. Structure Activity Relationships

On the basis of the cytotoxic experiments performed on particular newly developed compounds in opposition to breast cancer cell lines, structure activity relationships (SAR) were illustrated as follows: The majority of the compounds under investigation afforded sufficient cytotoxic effect as compared to the starting material **(4)**, this can be related to the ability of the interacted substituents to enhance the cytotoxic activity of the basic nucleus **(4)**.

Compound **(6)** attained the highest cytotoxic effect which can be referred to the active hydrazone moiety, in addition to the isatin derivative which is a potential cytotoxic agent whereas compound **(9a)** showed a considerable activity since it comprises an aryl linked electron-withdrawing group (Cl) with a Schiff base moiety. Furthermore, the oxadiazole ring developed in compound **(5)** has attributed in the observed activity. The moderate cytotoxic effect arisen in compound **(7)** may be related to the Schiff base moiety beside the electron donating (OH) group [7, 43, 59-62] **Fig. (6)**.

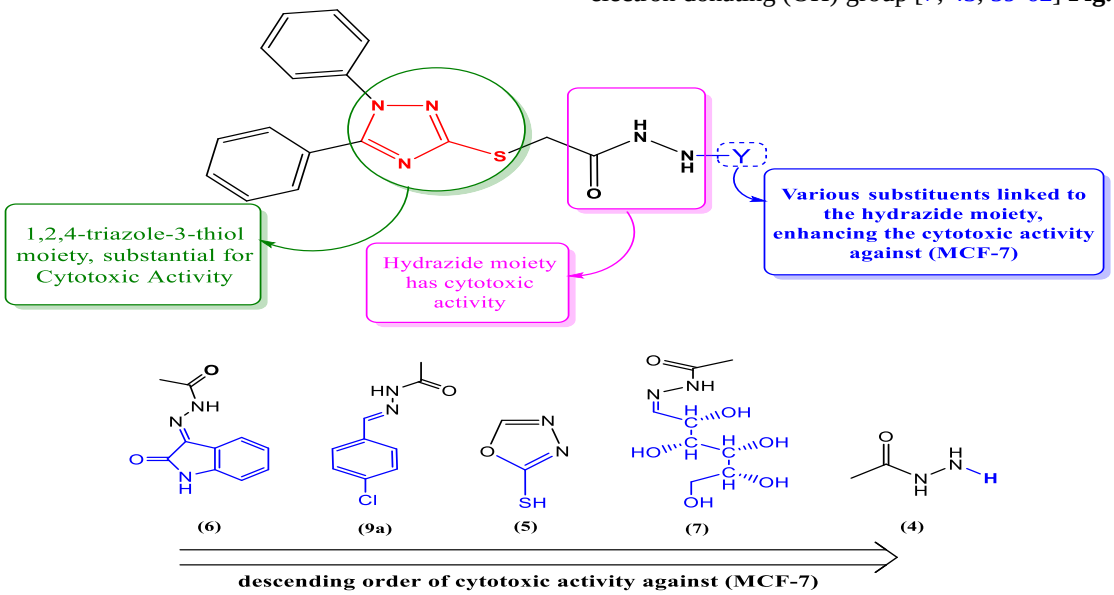


Fig. (6): structure-activity relationships SAR of the investisgated moieties against (MCF-7)

4. Conclusion

1,5-diphenyl-1H-1,2,4-triazole-3-thiol **(2)** was considered as the basis for the production of a novel design of heterocyclic compounds **(3-9)**. The derived structures were confirmed by spectroscopic data involving IR, Mass spectrum, ^{1}H-NMR, and ^{13}C-NMR. A number of the introduced moieties exhibited approximately moderate to high activity when examined against bacteria (Gram-positive and Gram-negative), and fungi strains. Also, the cytotoxic activity was tested for most of the created derivatives toward MCF-7 (breast cancer cell lines), compounds **6, 9a** revealed high evaluation as compared to Doxorubicin (ref. drug). Moreover, the design of targeted drug delivery systems has benefited greatly from the application of molecular docking in which the most effective compounds are expected to have higher binding affinity as compared to the reference drugs. Consequently, this work offers preliminary recommendations for further characterization and investigation of these newly synthesized compounds to reinforce their potential as biological agents.

5. Conflict of Interest

There are no conflicts to declare.

6. Funding

Authors declare that no funding has been received.

7. Acknowledgements

Not applicable.

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