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Design, Synthesis, and Biological Evaluation of 1,2,4-Triazole Derivatives as Potential Antimicrobial Agents

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Abstract

In this study, we aimed to develop new biologically active compounds with antibacterial properties. 4-amino-5-methyl-2*H*-1,2,4-triazol-3(4*H*)-one **(1)** was converted to the corresponding Schiff bases (**2**) with the reaction with a 4-anis aldehyde. Acetic acid ethyl esters containing [1,2,4] triazole ring **(3)** were synthesized by the condensation of compounds (**2**) with ethyl bromoacetate in basic media. The reaction of compounds (**3**) with hydrazine hydrate led to the formation of acid hydrazides (**4**). The reaction of hydrazide (**4**) with phenyl isothio - and phenyl isocyanate produced the corresponding carbothioamide (**5a**) and carboxamide (**5b**). The basic treatment of carbothioamide (**5a**) and. carboxamide (**5b**) produced 1,2,4-triazole (**6a, 6b**) compounds, respectively. The reactions of ((**6b**) with norfloxacin and ciprofloxacin in the presence of formaldehyde afforded the corresponding Mannich bases(**7a, 7b**). The structural assignments of the new compounds were based on elemental analysis and spectral (IR, ^{1}H-NMR, and ^{13}C-NMR) data. All newly synthesized compounds were screened for their antimicrobial activity. The in vitro antimicrobial activities of the compounds were evaluated against pathogenic microorganisms, and compounds 7a and 7b were found the most effective antimicrobial activity.

1. Introduction

Antimicrobial resistance (AMR), which has become a global problem, is a never-ending battle between humans and microbes. It is thought that the cause of 0.7 million deaths per year is microbial infection [1]. Antimicrobial resistance has become a major problem for health and development worldwide. Increasing antimicrobial resistance in the treatment of bacterial and fungal diseases has reached a dangerous level all over the world and affects people's health [2]. According to WHO studies on worldwide antimicrobial resistance, more than 10 million individuals are expected to be affected by multi-drug resistance diseases, with increased human mortality rates. Antimicrobial drug resistance is also likely to rise [3]. It is very important to increase research in this area to develop new and more effective antibacterial and antifungal drugs [4]. Standard antibiotic therapies such as -lactams, aminoglycosides, tetracyclines, sulfonamides, quinolines, and others failed to prevent the spread of antimicrobial resistance (AMR) in specific locations during the twentieth century by keeping bacteria inactive. As a result, there is a critical need in modern medicinal chemistry to develop and improve different ways of introducing a new active generation of antimicrobial drugs with alternative operating mechanisms in order to prevent antibiotic resistance in the future. When linked with other heterocyclic groups, 1,2,3-triazoles are remarkable scaffolds that have important biological effects [5-10]. N-containing heterocyclic chemicals are used in the pharmaceutical, agrochemical, and biological industries [11,12]. Among them, 1,2,3-triazoles and 1,2,4-triazoles exhibit a broad range of biological activities, such as antifungal [13-15], insomnia [16], anticancer [17], antineoplastic [18], antimicrobial [14,19–21,34], antibacterial [22,30], antioxidant [23,24,13], anti-inflammatory [25,26], antiviral [27,35], antimycobacterial [28,29], anticonvulsant [31], antidepressant [32], and anticoagulative [33] activities (Figure 1).

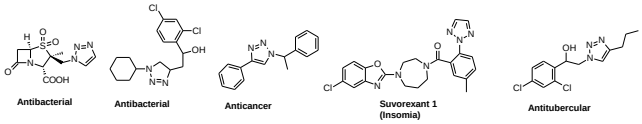

Figure 1. 1,3,4-Triazole-based biologically active agents.

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A nitrogen atom links to an alkyl or aryl group in a Schiff base, providing a nitrogen-carbon double bond [34]. The presence of an electron pair on nitrogen in the azomethine group and the presence of heterocyclic structures connected to carbon and nitrogen in the azomethine group are the primary reasons for the biological and chemical significance of Schiff bases [35]. Many scientists have synthesized 1,2,4-triazole-Schiff base derivatives, which are generated by condensation of the 1,2,4-triazoles and the Schiff base structure, and studied their diverse biological and physicochemical features because of the benefits of both of these structural classes [36, 37].

The Mannich reaction serves as a useful model for comprehending how various drug synthesis processes work. The nucleophilic substitution process occurred through a carbon Mannich base or a nitrogen Mannich base. Mannich bases derived from various heterocycles exhibit unique biological activities, such as antitubercular [38], antimalarial [39] anticancer [40] and analgesic [41] properties.

Based on these observations, we believed that new bioactive compounds could be developed and synthesized in a series of 1,2,4-triazol-3-one derivatives in this research.

2. Experimental

2.1. Material and Methods

All the chemicals were purchased from Fluka Chemie AG Buchs (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Bu¨chi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets (silica gel 60 GF254) and “0.25 mm thick silica gel plate. The mobile phase was ethyl acetate: diethyl ether, 1:1 (v:v) and detection was made using UV light. FT-IR spectra were recorded as potassium bromide pellets using a Perkin Elmer 1600 series FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d6 on a BRUKER AVANCE II 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me4Si as an internal reference, *J* values are given in Hz. The mass spectra were obtained on a Quattro LC–MS (70 eV) instrument.

2.1.1. 4-[(Z)-(4-methoxybenzylidene) amino]-5-methyl -2,4-dihydro-3H-1,2,4-triazol-3-one (2)

A solution of the corresponding compound **1** (10 mmol) in absolute ethanol was refluxed with anisaldehyde (10 mmol) for 3 h. After cooling the mixture to room temperature, a White solid appeared. This crude product was recrystallized from dimethyl sulfoxide/water (1:2) to afford the desired product.

Yield: 88 %, m.p: 190-192 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3167 (NH), 3041 (aromatic CH), 1687 (C=O), 1512 (C=N). ^{1}H NMR (DMSO-*d6*, δ ppm): 2.26 (3H, s, CH_3), 3.82 (3H, s, O-CH_3), 7.05 (2H, d, *J*= 8.0 Hz, arH), 7.78 (2H, d, *J*= 8.0 Hz, arH), 9.61 (1H, s, CH), 11.76 (1H, s, NH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.59 (CH_3), 55.87 (O-CH_3), arC: [114.93 (2CH), 126.46 (C), 129.93 (2CH), 144.68 (C)], 154.40 (CH), 151.80 (triazole C-3), 162.34 (triazole C-5). EI MS m/z (%): 233.40 ([M+1]$^{+}$, 100), 103.33 (37), 114.21 (36), 215.22 (28), 182.20 (25), 152.26 (21).

2.1.2. Ethyl {4-[(*Z*)-(4-methoxybenzylidene) amino]-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}acetate (3)

Compound **2** (10 mmol) was refluxed with 1 equivalent of sodium in absolute ethanol for 2 h. Then, ethyl bromoacetate

(10 mmol) was added and refluxed for an additional 8 h. After evaporating the solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol/water (1:2) to afford compound **4**.

Yield: 80 %, mp. 180-182 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3058 (aromatic CH), 1685 (C=O), 1720 (C=O), 1510 (C=N). ^{1}H NMR (DMSO-*d6*, δ ppm): 1.22 (2H, s, CH_2), 2.31 (2H, s, CH_2), 3.84 (3H, s, CH_3), 4.17 (3H, t, *J*= 4.0 Hz, CH_3), 4.57 (3H, s, O-CH_3), 7.07 (2H, d, *J*= 8.0 Hz, arH), 7.80 (2H, d, *J*= 8.0 Hz, arH), 9.56 (1H, s, CH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.40 (CH_3), 38.25 (CH_2), 49.20 (CH_2), 50.12 (CH_3), 55.87 (O-CH_3), arC: [111. 20 (2CH), 122.52 (C), 128.12 (2CH), 141.85 (C)], 155.41 (CH), 152.79 (triazole C-3), 164.70 (triazole C-5), 170.64 (C=O). EI MS m/z (%): 319.20 ([M+1]$^{+}$, 100), 247.30 (85), 149.45 (53).

2.1.3. 2-{4-[(*Z*)-(4-methoxybenzylidene)amino]-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl} acetohydrazide (4)

A solution of compound **3** (10 mmol) in ethanol was refluxed with hydrazine hydrate (30 mmol) for 5 h (controlled with TLC). After cooling it to room temperature, acetone was added to the mixture and was kept overnight in cold. The resulting solid separation was collected by

filtration and recrystallized from ethanol to afford the desired product.

Yield: 73 %, m.p: 189-191 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3302 (NH_2), 3114 (NH), 3063 (aromatic CH), 1678 (C=O), 1703 (C=O), 1510 (C=N). ^{1}H NMR (DMSO-*d6*, δ ppm): 2.08 (2H, d, *J*= 8.0 Hz, CH_2), 3.43 (2H, brs, NH_2+H_2O), 4.19 (3H, s, CH_3), 5.24 (3H, d, *J*= 4.0 Hz, CH_3), 6.88 (1H, s, arH), 7.40 (1H, d, *J*= 12.0 Hz, arH), 7.66 (1H, s, arH), 8.55 (1H, s, arH), 9.19 (1H, s, CH), 10.20 (1H, s, NH). ^{13}C NMR (DMSO-*d6*, δ ppm): 12.98 (CH_3), 39.75 (CH_2), 41.10 (CH_2), 54.17 (O-CH_3), arC: [110.23 (CH), 111.74 (CH), 112.36 (CH), 120.47 (CH), 139.52 (C), 141.85 (C)], 155.37 (CH), 151.94 (triazole C-3), 166.64 (triazole C-5), 171.04 (C=O). EI MS m/z (%): 305.40 ([M+1]$^+$, 100), 273.18 (85), 190.12 (61).

General Procedure for the Synthesis of Compounds 5a-b

To a solution of corresponding compound 4 (10 mmol) in dichloremethane, phenylisocyanate (**for 5b**) (20 mmol) or phenyl isothiocyanate **(for 5a)** (20 mmol), was added and the mixture was stirred at room temperature for 24 hours. After evaporating the solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol to yield the target compounds.

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2-[(4-{[(1Z)-(4-methoxyphenyl)methylene]amino}-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-N-phenylhydrazinecarbothioamide (5a)

Yield, 91 %; mp. 157-159 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3112 and 3213 (3NH), 3067 (aromatic CH), 1687 (C=O), 1713 (C=O), 1578 (C=N), 1120 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.09 (2H, d, *J*= 12.0 Hz, CH_2), 3.37 (3H, s, CH_3), 4.45 (3H, s, CH_3), 7.14-7.18 (3H, m, arH),7.32-7.37 (3H, m, arH), 7.43-7.55 (3H, m, arH), 9.69 (1H, s, NH), 9.74 (1H, s, NH), 9.89 (1H, s, NH), 10.29 (1H, s, CH).^{13}C NMR (DMSO-*d6*, δ ppm): 12.10 (CH_2), 48.70 (CH_3), 56.45 (CH_3), arC: [111.20 (CH), 112.43 (CH), 117.64 (CH), 118.49 (CH), 120.13 (CH), 123.07 (CH), 126.73 (CH), 130.01 (CH), 138.10 (CH), 140.06 (C), 141.46 (C), 150.32 (C)], 154.37 (CH), 155.69 (triazole C-3), 158.30 (triazole C-5), 170.33 (C=O), 186.14 (C=S). EI MS m/z (%): 440.09 ([M+1]$^+$, 100), 319.41 (85), 200.76 (61), 174.20 (33).

2.1.4. 2-[(4-{[(1Z)-(4-methoxyphenyl)methylene] amino}-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetyl]-N-phenylhydrazine carboxamide (5b)

Yield, 90 %; mp. 160-162 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3117 and 3215 (3NH), 3054 (aromatic CH), 1679 (C=O), 1748 (C=O), 1571 (C=N), 1129 (C=S). ^{1}H NMR (DMSO-d_6, δ ppm): 2.12 (2H, s, CH_2), 4.38 (3H, s, CH_3), 5.28 (3H, s, O-CH_3), 6.95-6.97 (2H, m, arH), 7.23-7.27 (3H, m, arH), 7.46-7.50 (3H, m, arH), 7.98 (1H, s, arH), 8.21 (1H, s, NH), 8.70 (1H, s, NH), 8.79 (1H, s, NH), 9.97 (1H, s, CH).^{13}C NMR (DMSO-*d6*, δ ppm): 19.33 (CH_2), 49.41 (CH_3), 53.79 (CH_3), arC: [110.74 (CH), 118.14 (CH), 120.31 (2CH), 124.73 (2CH), 129.07 (2CH), 131.70 (CH), 136.49 (C), 138.36 (C), 140.19 (C)], 148.02 (CH), 153.55 (triazole C-3), 159.64 (triazole C-5), 170.76 (C=O), 173.42 (C=O). EI MS m/z (%): 424.87 ([M+1]$^+$, 100), 301.95 (81), 213.20 (73), 198.10 (49).

General Method For The Synthesis of Compounds 6a, 6b

A solution of corresponding compound **5** (10 mmol) in 2 N NaOH was refluxed for 3 h. The resulting solution was cooled to room temperature and acidified to pH 3-4 with 37% HCl. The precipitate formed was filtered, washed with water and recrystallized from ethanol/water (1:1) to afford the desired compounds

2.1.5. 2-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-4-{[(1E)-(4 methoxyphenyl) methylene] amino}-5-methyl-2,4-dihydro-3H-1,2,4-triazol-3-one (6a)

Yield, 71 %; mp. 180-182 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3071 (aromatic CH), 1691 (C=O), 1573 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.02 (2H, s, CH_2), 4.79 (3H, s, CH_3), 5.06 (3H, s, O-CH_3), 6.91-7.57 (9H, m, arH), 8.56 (1H, s, CH), 13.37 (1H, s, SH). ^{13}C NMR (DMSO-*d6*, δ ppm): 49.09 (CH_2), 50.76 (CH_3), 57.12 (O-CH_3), arC: [110.98 (CH), 112.09 (CH), 113.79 (2CH), 119.03 (2CH), 121.93 (CH), 123.73 (CH), 125.04 (CH), 130.55 (C), 134.77 (C), 139.10 (C)], 148.45 (CH), 151.77 (triazole C-3), 153.20 (triazole C-3), 158.88 (triazole C-5), 160.30 (triazole C-5). EI MS m/z (%): 422.30 ([M+1]$^+$, 100), 198.22 (73), 219.33 (54).

2.1.6. 4-{[(1E)-(4-methoxyphenyl)methylene] amino}-5-methyl-2-[(5-oxo-4-phenyl-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (6b)

Yield, 73 %; mp. 188-190 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3077 (aromatic CH), 1698 (C=O), 1578 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.24 (2H, s, CH_2), 3.35 (3H, s, CH_3), 5.76 (3H, s, O-CH_3), 6.93-6.97 (3H, m, arH), 7.23-7.27 (3H, m, arH), 7.48-7.50 (3H, m, arH), 8.00 (1H, s, CH), 8.84 (1H, s, NH). ^{13}C NMR (DMSO-*d6*, δ ppm): 49.29 (CH_2), 51.37 (CH_3), 58.34 (O-CH_3), arC: [113.54 (CH), 114.09 (CH), 115.66 (2CH), 117.30 (2CH), 119.35 (CH), 120.31 (CH), 121.38 (C), 129.36 (C), 138.54 (C)], 143.53 (CH), 153.76 (triazole C-3), 155.20 (triazole C-3), 159.23 (triazole C-5),

161.57 (triazole C-5). EI MS m/z (%): 406.78 ($[M+1]^+$, 100), 119.20 (71), 298.25 (55).

2.2. General Method for the Synthesis of Compounds 7a-b

In the solution of corresponding compounds **6a and 6b** (10 mmol) in dimethyl formamide (5 mmol), norfloxacin or ciprofloxacin (10 mmol) was added and the mixture was stirred at room temperature in the presence of formaldehyde (50 mmol) for 24 h. The solid precipitated was filtered off and recrystallized from dimethylsulfoxide: water (1:1, v/v).

(E)-7-(4-((3-((4-(4-methoxybenzylideneamino)-3-methyl -5-oxo-4,5-dihydro-1,2,4-triazol-1-yl)methyl)-5-oxo-4-phenyl-4,5-dihydro-1,2,4-triazol-1-yl)methyl) piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (7a)

Yield, 80 %; mp. 193-195 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3324 (OH), 3110 (NH), 3075 (aromatic CH), 1703 (C=O), 1716 (C=O), 1578 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.18 (3H, s, CH_3), 1.31 (2H, s, CH_2), 2.73 (2H, s, CH_2), 2.83 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.35 (8H, s, $4CH_2+H_2O$), 3.81 (3H, s, -OCH_3), 6.96-8.65 (11H, m, arH), 8.94 (1H, s, CH), 8.99 (1H, s, CH), 9.19 (1H, s, CH), 15.22 (1H, s, OH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.78 (CH_3), 25.41 (CH_2), 28.78 (CH_2), 29.11 (CH_2), 30.03 (CH_2), 31.46 (CH_2), 33.89 (CH_2), 47.23 (CH_2), 51.76 (CH_2), 55.85 (O-CH_3), arC: [110.89 (CH), 111.75 (CH), 113.50 (CH), 119.30 (CH), 120.01 (CH), 121.28 (CH), 122.55 (CH), 123.52 (CH), 124.61 (CH), 126.74 (C), 127.39 (CH), 128.41 (CH), 129.47 (C), 130.53 (C), 132.34 (C), 133.79 (C), 135.06 (C), 136.80 (C), 139.80 (C)], 147.20 (CH), 148.85 (CH), 150.15 (CH), 155.20 (triazole C-3), 158.63 (triazole C-3), 160.61 (triazole C-5), 163.85 (triazole C-5), 170.11 (C=O), 171.06 (C=O). EI MS m/z (%): 301.85 (100), 749.52 ($[M+1]^+$, 77), 299.20 (63), 100.80 (41).

2.2.1. (E)-7-(4-((3-((4-(4-methoxybenzylideneamino)-3-methyl-5-oxo-4,5-dihydro-1,2,4-triazol-1-yl) methyl)-5-oxo-4-phenyl-4,5-dihydro-1,2,4-triazol-1-yl)methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (7b)

Yield, 78 %; mp. 190-192 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3320 (OH), 3108 (NH), 3061 (aromatic CH), 1710 (C=O), 1725 (C=O), 1581 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 1.17 (3H, s, CH_3), 1.42 (2H, s, CH_2), 2.73 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.39 (8H, s, $4CH_2+H_2O$), 4.14 (3H, d, *J*= 8.0 Hz, O-CH_3), 4.58 (3H, s, CH_3), 6.95-8.25 (11H, m, arH), 8.77 (1H, s, CH), 8.93 (1H, s, CH), 15.30 (1H, s, OH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.85 (CH_3), 26.10 (CH_2), 30.30 (CH_2), 39.52 (CH_2), 41.41 (CH_2), 42.85 (CH_2), 50.98 (CH_2), 51.36 (CH_2), 52.00 (CH_3), 56.18 (O-CH_3), arC: [110.03 (CH), 111.50 (CH), 115.55 (CH), 118.36 (CH), 120.18 (CH), 122.28 (CH), 123.61 (CH), 125.80 (CH), 126.78 (CH), 127.30 (C), 128.77 (CH), 129.60 (CH), 130.61 (C), 132.70 (C), 134.80 (C), 136.61 (C), 137.11 (C), 138.03 (C), 139.13 (C)], 148.12 (CH), 149.40 (CH), 154.79 (triazole C-3), 157.90 (triazole C-3), 160.66 (triazole C-5), 165.73 (triazole C-5), 169.80 (C=O), 171.28 (C=O). EI MS m/z (%): 257.85 (100), 759.80 ($[M+Na]^+$, 71), 360.12 (64), 414.30 (39).

2.3. General Method for the Synthesis of Compounds 8a-c.

A solution of the corresponding compound **4** (10 mmol) in

absolute ethanol was refluxed with an appropriate aldehyde

(10 mmol) for 3 hours. After cooling the mixture to room temperature, a white solid appeared. This crude product was recrystallized from dimethylsulfoxide/water (1:2) or ethanol to afford the desired product.

N'-[(1Z)-(4-methoxyphenyl)methylene]-2-(4-{[(1E)-(4-methoxyphenyl)methylene] amino}-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (8a)

Yield, 90 %; mp. 177-179 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3113 (NH), 3051 (aromatic CH), 1675 (C=O), 1570 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.13 (3H, s, CH_3), 2.31 (2H, s, CH_2), 4.79 (3H, s, CH_3), 5.30 (3H, s, CH_3), 6.96-7.83 (8H, m, arH), 8.64 (1H, s, CH), 9.61 (1H, s, CH), 11.51 (1H, s, NH).^{13}C NMR (DMSO-*d6*, δ ppm): 11.10 (CH_3), 11.46 (CH_3), 46.72 (CH_2), 55.85 (CH_3), arC: [114.76 (CH), 114.86 (CH), 114.99 (CH), 126.27 (C), 127.04 (C), 128.98 (CH), 129.05 (CH), 129.21 (CH), 130.09 (CH), 130.44 (CH), 145.27 (C), 154.01 (C)], 144.34 (CH), 144.62 (CH), 161.20 (triazole C-3), 162.15 (triazole C-3), 168.34 (C=O). EI MS m/z (%): 437.58 ($[M+1]^+$, 100), 217.52 (77), 120.63 (51), 109.41 (33).

2.3.1. 2-(4-{[(1E)-(4-methoxyphenyl) methylene] amino}-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)-N'-[(1Z)-phenyl methylene] acetohydrazide (8b)

Yield, 89 %; mp. 173-175 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3116 (NH), 3071 (aromatic CH), 1703 (C=O), 1583 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.34 (2H, s, CH_2), 4.91 (6H, s, $2CH_3$), 7.43-8.26 (9H, m, arH), 9.73 (1H, s, NH), 11.61 (1H, s, CH), 11.70 (1H, s, CH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.44 (CH_3), 46.78 (CH_2), 55.91 (CH_3), arC: [127.45 (CH), 127.62 (CH), 128.27 (CH), 128.82 (CH), 129.27 (CH),

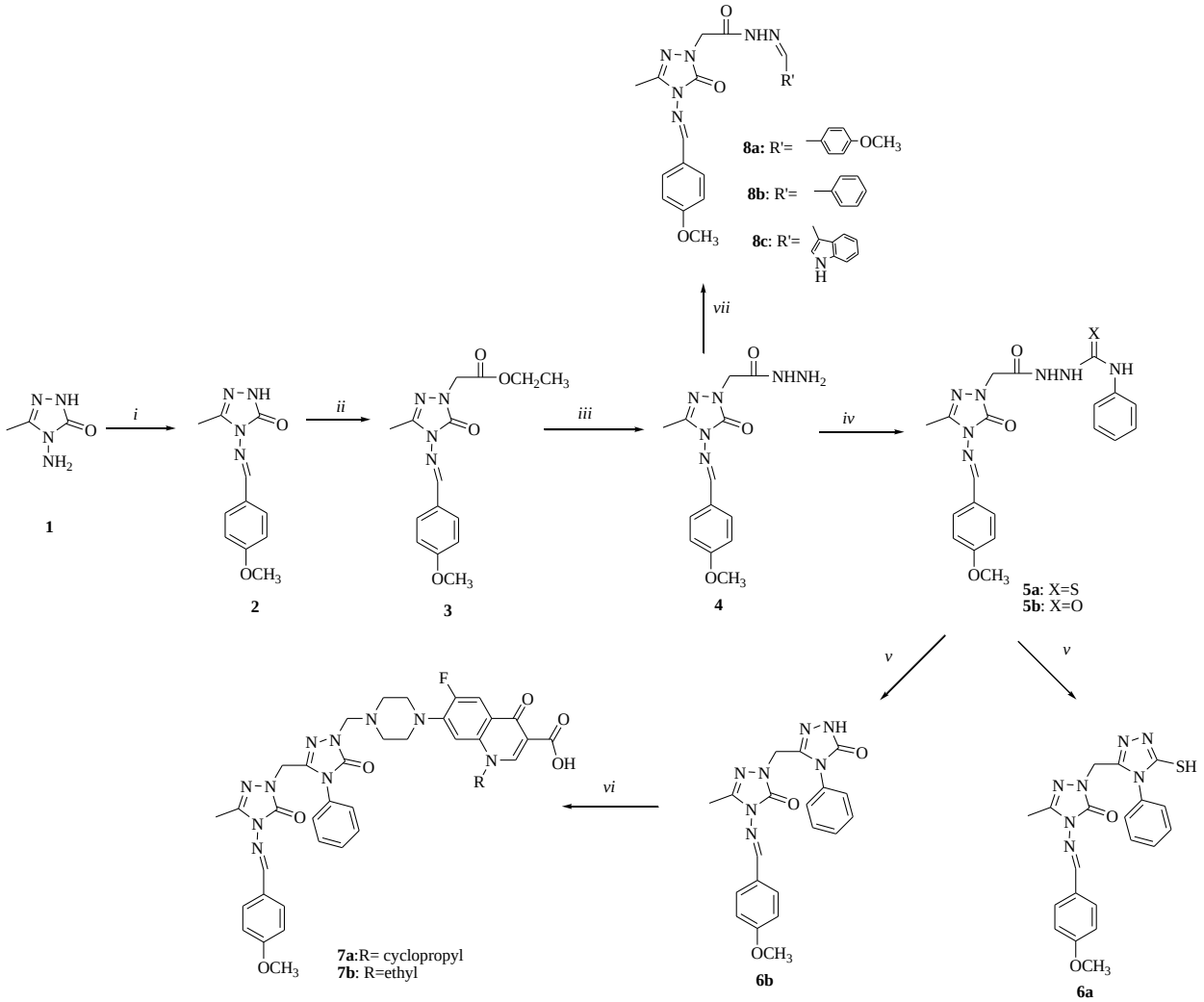
129.50 (CH), 130.51 (CH), 132.07 (CH), 133.79 (C), 134.35 (C), 143.83 (CH), 144.80 (C)], 148.17 (CH), 154.58 (CH), 150.69 (triazole C-3), 163.87 (triazole C-3), 168.21 (C=O). EI MS m/z (%): 407.12 ([M+1]$^+$, 100), 309.41 (73), 198.74 (49).

2.3.2. N'-[(1Z)-1H-indol-3-ylmethylene]-2-(4-{[(1E)-(4-methoxyphenyl)methylene] amino}-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (8c)

Yield, 91 %; mp. 180-182 ^{0}C. FT-IR (υ_{max}, cm^{-1}): 3120 (NH), 3080 (aromatic CH), 1710 (C=O), 1570 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm): 2.14 (2H, s, CH_2), 4.37 (3H, s, CH_3), 4.84 (3H, s, CH_3), 6.98-8.39 (8H, m, arH), 9.74 (1H, s, NH), 10.15 (1H, s, NH), 11.29 (1H, s, CH), 11.54 (1H, s, CH), 11.83 (1H, s, CH). ^{13}C NMR (DMSO-*d6*, δ ppm): 11.51 (CH_3), 47.20 (CH_2), 55.85 (CH_3), arC: [111.41 (CH), 112.50 (CH), 113.61 (CH), 114.39 (CH), 115.34 (CH), 118.52 (CH), 121.75 (CH), 124.60 (CH), 130.41 (C), 134.85 (C), 135.90 (C), 137.50 (C), 141.97 (C)], 148.85 (CH), 150.10 (CH), 158.52 (triazole C-3), 160.28 (triazole C-3), 170.01 (C=O). EI MS m/z (%): 446.10 ([M+1]$^+$, 100), 250.47 (73), 100.19 (49).

3. Results And Discussion

The goal of the study is to create new antibacterial hybrids that include a variety of pharmacophore groups. The previous observation of Scheme 1 was completed and intermediate molecular processes were accomplished. The components were synthesized using traditional methods, and the synthesis was finalized using the thin-layer chromatography (TLC) method. The basic spectral and physicochemical data formed the basis for all compound structures.



Scheme 1. Reactions and conditions**.** *i*: anisaldehyde, 115-120 °C, oil bath, 2 h*ii*: absolute ethanol, Na, ethyl bromoacetate, reflux for 8 h; *iii*: absolute ethanol, hydrazine hydrate, reflux for 12 h; i*v*: phenyl isothiocyanate (for **6a**), or phenyl isocyanate(for **6b**) in dichloro-methane, room temperature for 24 h; *v*: 2 N NaOH in ethanol/water (1:1), reflux for 6h *vi*: HCl, formaldehyde, ciprofloxacin (**for 7a**),norfloxacin (**for 7b**),

By investigating on our previous study on antimicrobial drugs, we set out to develop new compounds with significant activity in our lab. **6a-b** are 1,2,4-triazole derivatives that were synthesized in this article. Compounds were characterized using ^{1}H and ^{13}C NMR, EI-MS, FT-IR, and mass spectrometry for all synthesized compounds. The known compound **1** was reacted with anisaldehyde and afforded the desired product of compound **2** known as a Schiff base. The characterization of this compound shows that the reaction was successfully achieved from the FT IR spectrum that the peak of NH_2 was not observed and the additional peak was added such as aromatic CH in 3041 cm^{-1}. Also in NMR spectra the aromatic protons resonated between 7.05-7.78 ppm and the imine –CH bond resonated downfield at 9.61 ppm in ^{1}H NMR spectra. In ^{13}C NMR the additional aromatic carbon atoms and imine carbon were detected. Another evidence of compound **2** is the molecular ion peak as M+1. Ethyl (4-{[(2,4-dichlorophenyl)methylene]amino}-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl)acetate **(3)** was synthesized from compound 2 with ethyl bromoacetate in the presence of a protic solution and resulted with an additional sharp C=O peak in 1720 cm^{-1}, the additional ester group (-OCH_2CH_3) protons were resonated respectively between 2.31 ppm and 4.57 ppm. The M+1 value shows the reliability of the corresponding compound **3**. FT IR data of the acetohydrazide derivative of compound **4** give details of the $–NHNH_2$ group in 3302 and 3114 cm^{-1} that take place instead of the ester group. The NH_2 and NH protons were resonated respectively in 3.43 and 9.20 ppm and controlled exchanging with D_2O solution. 2-{4-[(Z)-(4-methoxybenzylidene)amino]-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl}aceto hydrazide (**4**) was reacted with different iso(thio)cyanates to convert to carbothiohydrazide **5a** and carbohydrazide **5b** derivatives. Compared the carbo(thio)hydrazide derivatives (**5a-b**) with its starting material of compound **4**, an additional NH and C=S peak for compounds **5a**, or C=O peak for compound **5b** was seen in their FT IR spectra.

The cyclization reaction of compound **5a-b** in basic media resulted in a 1,2,4-triazole ring in the molecule of compound **6** derivatives. The cyclization reactions of compounds **6a-b** were also supported by the NMR data, for **6a** –SH tautomeric form resonated approximately at 13.37 ppm whereas for the compound **6b** the -NH tautomeric form occurred and resonated at 8.84 ppm. The molecular ion peak of these compounds was detected in electron impact ionization techniques. In the mannich reaction with norfloxacin or ciprofloxacin in the presence of formaldehyde, compounds **6a-b** were allowed to bind the large molecule to the starting material to afford the target molecule **7a-b**. It is seen in the IR spectra that additional C=O peaks between 1703-1725 cm^{-1}, aromatic CH peak and in the ^{13}C or ^{1}H NMR spectra additional CH_2 carbon atoms and protons of piperazine ring, aromatic carbons and protons also were resonated differently from the starting material.

In excellent yields, arylmethyleneacetohydrazides **(8a-c)** were synthesized by reacting compound **4** with different aromatic aldehydes in ethanol solutions. ^{1}H-NMR, ^{13}C-NMR, IR, and mass spectroscopy methods can all be used to prove the synthesis of compounds **8a-c**. Additional signals coming from the arylidene moiety have been seen in the aromatic area of the ^{1}H-NMR spectra of compounds **8a-c**. Additionally, signals were observed at 9.61, 11.61, and 11.54 ppm, whereas the signal associated with the $–NHNH_2$ moiety, which had previously been observed at 3.43 ppm in the ^{1}H-NMR spectrum, disappeared.

Antimicrobial Activity

Antimicrobial activity or susceptibility tests were performed to determine the in-vitro activity of the antimicrobial agent against a particular bacterial species. Two techniques are used to measure susceptibility testing of microorganisms, including “diffusion” and “dilution”. The disc diffusion technique is a frequently used technique and the sensitivity of the antibiotic absorbed into paper discs (the solution to be measured by antibacterial activity) is based on the diffusion of the organism to the medium in which the organism is inoculated.

This study found that most of the compounds synthesized in this study were able to bind to the test chemicals (Table 1). There were seven compounds that showed outstanding activity on Gram-positive and Gram-negative bacteria of the test microorganisms with MIC values 0.24 g/mL, including 7a-b, which had fluoroquinolone nuclei in their structures. The carboxamides, **4a, 4b**, and triazoles, **5a, 5b,** which were obtained from intramolecular cyclisation of **4a, 4b,** displayed selective activity on a Gram-positive coccal bacterium, *Staphylococcus aureus* (Sa), and *Mycobacterium smegmatis* (Ms), atypical tuberculosis factor leading to morbidity and mortal

Table 1. Screening for the activity of newly synthesized compounds.

Comp No	Microorganisms and Minimal Inhibitory Concentrations (µg/mL)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
2	-	-	-	125	-	-	-	-	-
3	-	-	-	-	-	500	-	-	-
4a	31.3	31.3	31.3	125	-	-	62.5	-	-
4b	31.3	31.3	31.3	125	-	125	62.5	-	-
5a	-	-	-	62,5	-	-	31.3	-	-
5b	-	-	-	31.3	-	125	15.65	-	-
6a	-	-	-	125	-	-	31.3	-	-
6b	-	-	-	125	-	-	31.3	-	-
7a	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-	-
7b	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	-	-	-
8a	31.3	31.3	31.3	125	-	-	62.5	-	-
8b	62.5	31.3	31.3	62.5	125	125	62.5	125	125
8c	125	62.5	62.5	125	250	259	62.5	250	125
Amp.	0	8	128	0	5	5			
Strep.									
Flu								<8	<8

Ec*: Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 43288, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Bc: *Bacillus cereus* 702 Roma, Ms: *Mycobacterium smegmatis* ATCC607, Ca: *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251, Amp.: Ampicillin, Str.: Streptomycin, Flu.: Fluconazole, (—): no activity.

4. Conclusion

Norfloxacin and ciprofloxacin moieties have been converted into various pharmacophore heterocycles in various new hybrid drugs described in this work. Thus, we integrated all of these potentially useful chemotherapeutic units, namely the 1,2,4-triazole, norfloxacin, and ciprofloxacine moieties, in this study. Antimicrobial screening studies were also conducted as part of the study. The 1,2,4-triazole and ciprofloxacin or norfloxacin moiety (**7a, 7b**) synthesized compounds showed good-moderate activity against a variety of test microorganisms.

Declaration of Ethical Standards

The author of this article declares that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Conflict of Interests

The authors of this study have stated that they have no conflicts of interest that could have affected the outcomes.

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