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Synthesis and Biological Evaluation of Some New 1,2,3-Triazole Derivatives As Anti-microbial Agents

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ABSTRACT

A series of 1,2,3-triazole derivatives bearing different chemical entities were prepared starting from 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide, compound **2**. The purity of all new compounds was checked by TLC and elucidation of their structures was confirmed by **IR**, **^{1}H** and **^{13}C NMR** along with High Resolution Mass Spectrometry (**HRMS**). All the target compounds were evaluated for their possible antimicrobial activity. Most of the tested compounds showed moderate to good antibacterial activity against most of the bacterial strains used in comparison with ciprofloxacin as a reference drug. The most active compounds were **4a**, **9a**, **9b**, and **9f**. Results of antifungal activity revealed that most of the tested compounds showed a good antifungal activity in comparison to fluconazole as a reference drug. Compounds **4a, 9c, 9d** and **9f** were the most active ones.

Indexing terms/ Keywords

1,2,3-Triazole; Synthesis; Antibacterial activity; Antifungal activity.

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1- INTRODUCTION

1,2,3-Triazoles are an important class of heterocycles due to their wide range of applications as synthetic intermediates and pharmaceuticals [1-2]. Several therapeutically interesting 1,2,3-triazoles have been reported, including anti-HIV agents [3-6], antimicrobial compounds [7], ß$_3$-selective adrenergic receptor agonists [8], kinase inhibitors [9-10] and other enzyme inhibitors [11-12]. The 1,2,3-triazole moiety is also present in a number of drugs, for example, the ß-lactam antibiotic tazobactam [13] and the cephalosporin cefatrizine [14].

Morbidity and mortality due to enteric bacterial infections have caused important health problems worldwide, mainly in the developing countries [15-16]. Toxicity and resistance to the drugs also have played an important role in treatment failure [17]. Consequently, there is an urgent need to screen new compounds for the development of new antibacterial agents.

We report simple and efficient methods for preparation of 1,2,3-triazole derivatives bearing different chemical entities that promise superior antimicrobial activity.

2- RESULTS AND DISCUSSION

2.1. Chemistry

The key intermediate ethyl 2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetate, compound 1 was prepared according to a reported procedure and its structure was confirmed by matching its physical and spectral data with the reported one[18].

The novel 2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetohydrazide, compound **2** was obtained through condensation of compound **1** with hydrazine hydrate by heating the reactants in ethanol at reflux for 6h, **Scheme 1**. **IR** spectrum of compound **2** showed two bands at 3290 and 3160 cm^{-1} due to NH and NH_2 functions, in addition to a band at 1658 corresponding to carbonyl group. **^{1}H NMR** spectrum of compound **2** showed signals derived from hydrazide structure appeared at 4.42 ppm (-$NHNH_2$) and 9.57 (-$NHNH_2$) ppm integrating for two and one protons, respectively (exchangeable with D_2O). In addition, the spectrum also showed singlet signal at 5.10 ppm for (NCH_2). **HRMS** of compound **2**: Found 218.1033, Calculated for (**$C_{10}H_{12}N_5O^+$**) 218.1036.

Hydrazide **2** was allowed to react with phthalic anhydride in glacial acetic acid by heating the reactants at reflux for 2 h to afford the new phthalimide derivative **3** in a 78% yield, **Scheme 1**. **IR** spectrum of compound **3** showed one band at 3260 cm^{-1} due to (–NH) stretching and two bands at 1699 and 1648 for carbonyl groups. **^{1}H NMR** spectrum of compound **3** displayed no signals belonging to –NH_2 group. **HRMS** of compound **3**: Found 348.1087, Calculated for (**$C_{18}H_{14}N_5O_3^+$**) 348.1091.

The novel thiosemicarbazide derivatives **4a-c** were obtained in 76-84% yields through the reaction of acid hydrazide **2** with the appropriate isothiocyanate by heating the reactants at reflux in ethanol for 2 h. Furthermore, the thiosemicarbazides **4a-c** were cyclized in hot NaOH solution as a base catalyzed reaction to obtain the novel 5-mercapto-4-substituted-4*H*-1,2,4-triazole derivatives **5a-c**.

The **IR** spectra of compounds **4a-c** showed strong absorption bands at 3265-3234 cm^{-1} and 1666-1652 cm^{-1} for the NH and C=O groups, respectively. In addition to a strong absorption band at 1190 cm-1 due to (C=S) group. Meanwhile **IR** spectra of compounds **5a-c** displayed no bands belonging to NH and C=O groups; instead a new bands at 2988-2962 cm^{-1} for SH group.

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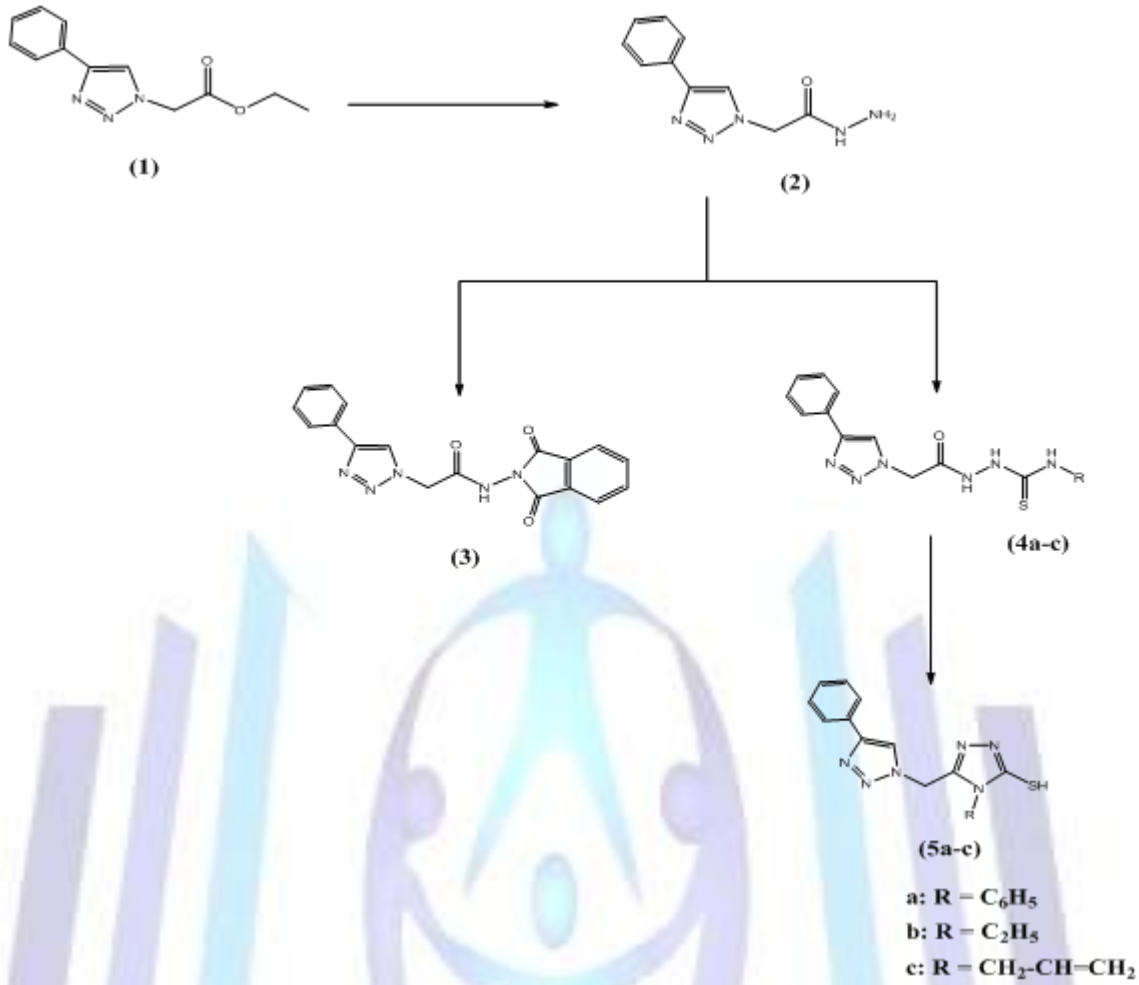

Scheme 1 Synthesis of compounds **2, 3, 4a-c,** and **5a-c**

Moreover, the novel oxadiazole **6** was synthesized in 87% yield through cyclization of the acid hydrazide **2** using potassium hydroxide and carbon disulfide in hot ethanolic solution. **IR** spectrum of compound **6** showed a band at 2961 cm^{-1} due to SH group. **HRMS** of compound **6**: Found 260.0594, Calculated for (**$C_{11}H_{10}N_5OS^+$**) 260.0601.

The intermediate potassium 2-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetyl)hydrazine carbodithioate **7** was prepared from hydrazide **2** and carbon disulfide in ethanolic potassium hydroxide solution with the aim to be cyclized to the novel triazole **8** under the effect of hydrazine hydrate. Structure of compound **8** was elucidated by spectral methods of analyses. **IR** spectrum of compound **8** showed two bands at 3270 and 3135 cm^{-1} due to (NH) stretching, a band at 2720 cm^{-1} for SH group and a strong band at 1299 cm^{-1} (C=S) stretching (thiol-thionetautomers). **IR** spectrum of compound **8** was devoid of absorption bands around 1660 cm^{-1} due to carbonyl group of hydrazide. ^{1}H NMR spectrum of compound **8** displayed no signalsbelonging to $–NH_2$ and –CONH groups; instead, a new singlet signal appeared at 5.68 ppm (exchangeable with D_2O) due to $–NH_2$ of triazole ring equivalent to two protons and a broad signal at 13.85 ppm due to exchanged –NH proton of triazole ring. **HRMS** of compound **8**: Found 274.0867, Calculated for (**$C_{11}H_{12}N_7S^+$**) 274.0869.

The target compounds; **9a-f** were synthesized by condensation of compound **2** with (un)substituted benzaldehydes or acetophenone **Scheme 2**. The structures of compounds **9a-f** were confirmed by spectral methods of analyses. The **IR** spectra of compounds **9a-f** showed strong absorption bands at 3255-3160 cm^{-1} and 1680-1668 cm^{-1} for the NH and C=O groups, respectively.

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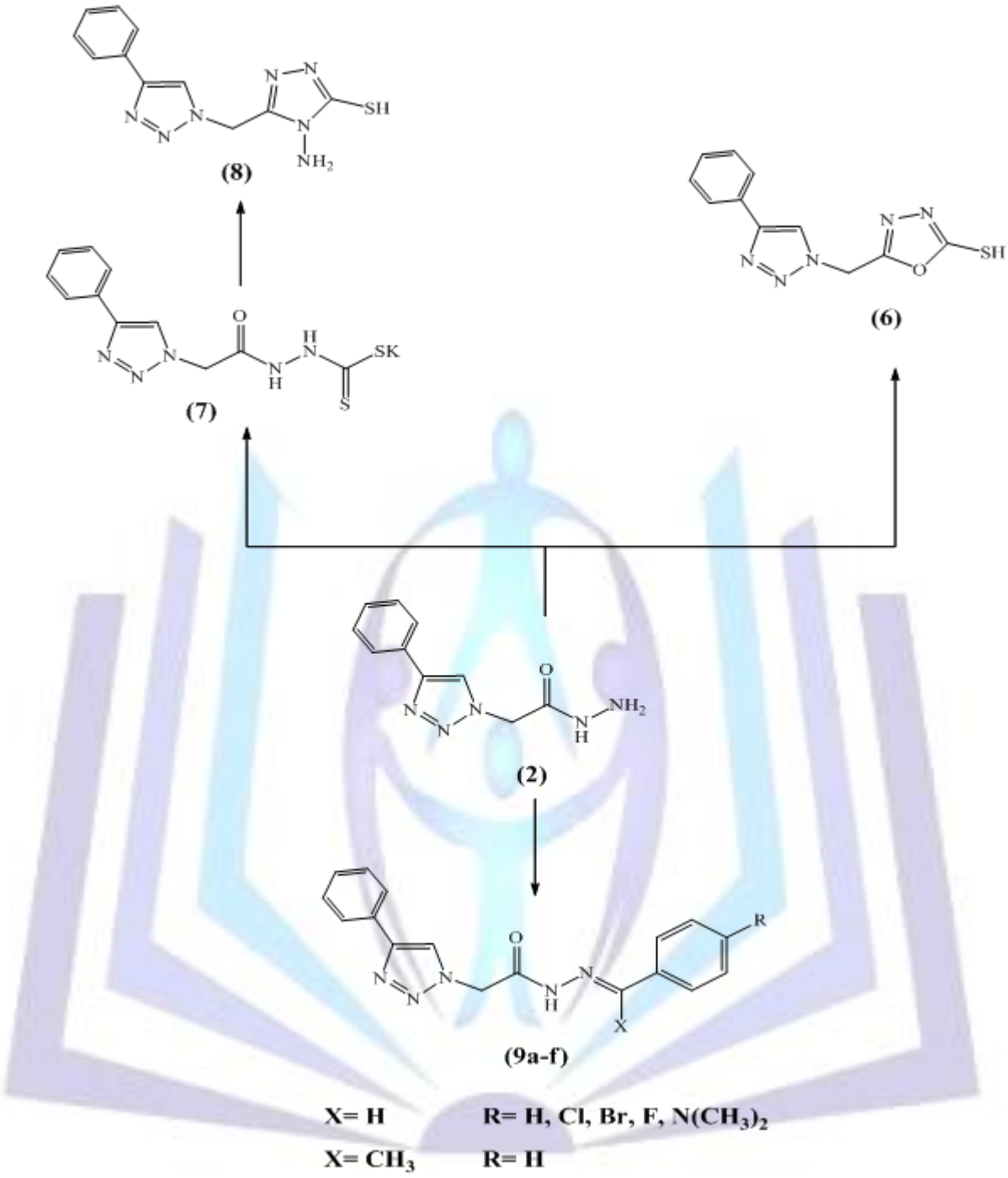

Scheme 2 Synthesis of compounds **6-8** and **9a-f**

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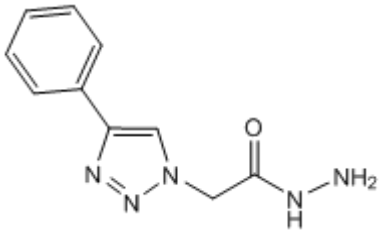
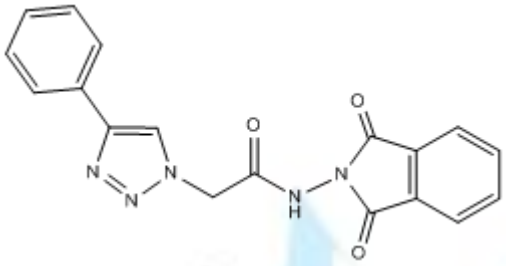
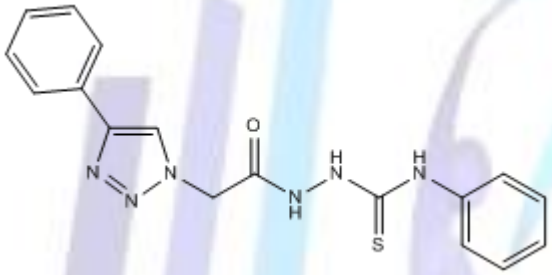
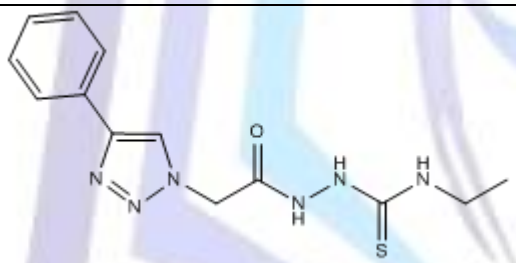
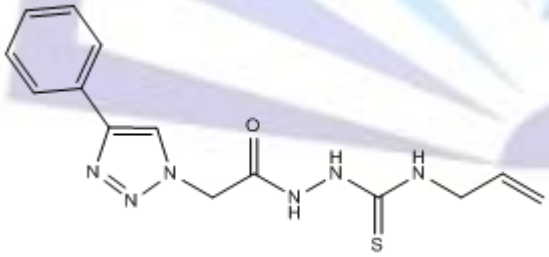
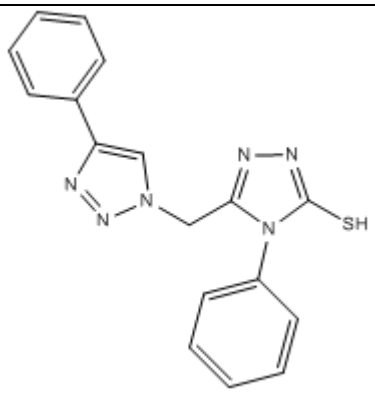
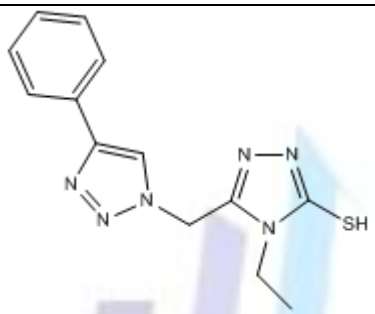
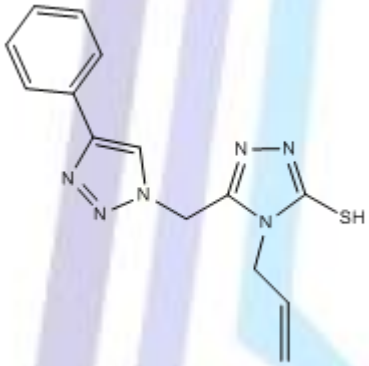
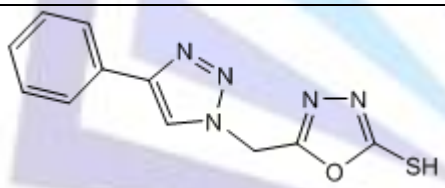
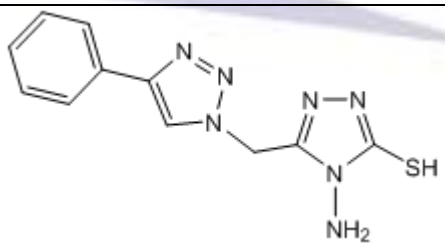
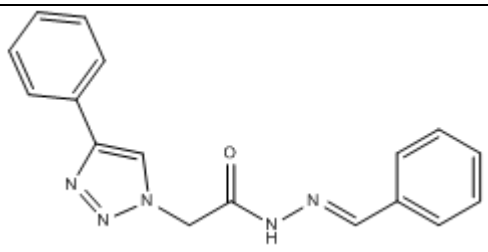
Table 1. Structures and yields of compounds **2, 3, 4a-c, 5a-c, 6, 8,** and **9a-f**

Product	No.	Yield (%)
	**2**	**82**
	**3**	**78**
	**4a**	**84**
	**4b**	**76**
	**4c**	**80**

	**5a**	**81**
	**5b**	**73**
	**5c**	**76**
	**6**	**87**
	**8**	**81**
	**9a**	**82**

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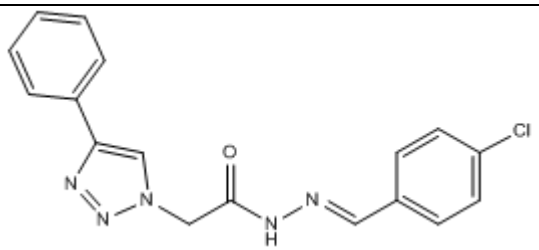
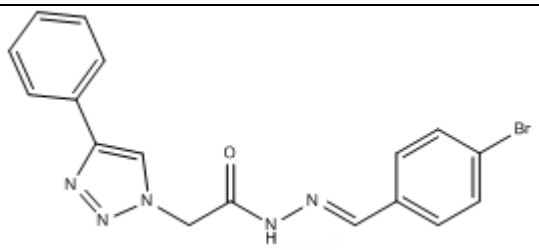
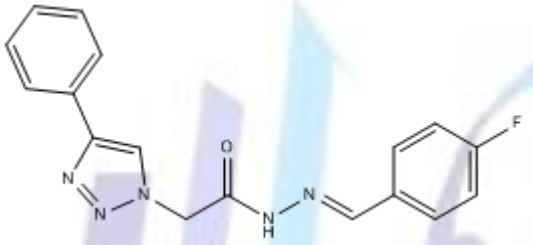
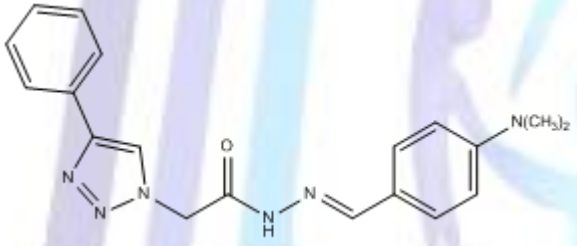
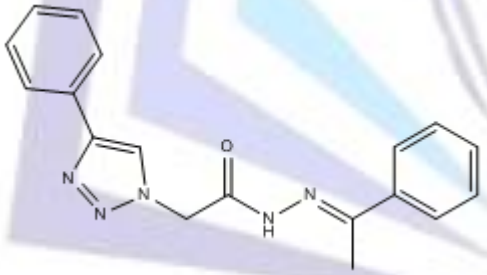
	**9b**	**85**
	**9c**	**87**
	**9d**	**84**
	**9e**	**78**
	**9f**	**84**

2.2. Biology

The results of the preliminary testing of the antibacterial activity of the final compounds are given in Table II. The synthesized compounds **2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f** were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus*as a representative of Gram positive strains and *Escherichia coli* as a Gram negative strain using ciprofloxacin as a reference drug. The result revealed that most of the newly synthesized compounds exhibited promising antibacterial activity comparable to ciprofloxacin against the test organisms (Table II).

The hydrazide derivative compound **2**, was found to exhibit antibacterial activity nearly 48% that of ciprofloxacin against *Staphylococcus aureus*, however it showed only 40% activity of ciprofloxacin against *E.Coli*.

According to the results in Table II, it can be noticed that conversion of hydrazide derivative, compound **2** into compound **3** decreased the activity against *Staphylococcus aureus* as it showed only 38% activity of ciprofloxacin.

Compounds **4a-c** were found to exhibit pronounced antibacterial activity which ranged from 60-73% that of standard drug against *Staphylococcus aureus* and 45-60% that of ciprofloxacin against *E.Coli*. It is worthy-mentioning that compound **4a** showed the highest activity (73%) against *Staphylococcus aureus* and 60% activity of ciprofloxacin against *E.Coli*.

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Furthermore, compounds **5a-c** exhibited moderate activity against *Staphylococcus aureus*and their activity was 53-60% that of ciprofloxacin, but they showed weak activity (45-47% that of ciprofloxacin) against *E.Coli* and that compound **5a** was the most active compound against *Staphylococcus aureus*as it showed 60% that of ciprofloxacin while it showed only 47% activity against *E.Coli*. It was noticed that cyclization of thiosemicarbazides **4a-c** to their corresponding triazole derivatives **5a-c** led to decrease of the antibacterial activity.

The oxadiazole derivative, compound **6** showed moderate activity against *Staphylococcus aureus*and its activity was 68% that of ciprofloxacin while it showed only 55% activity of ciprofloxacin against *E.Coli.* Meanwhile, the conversion of hydrazide **2** into the amino triazole derivative, compound **8** did not show a significant improvement in the antibacterial characters of compound **2**.

Compounds **9a-f** were found to exhibit pronounced antibacterial activity which ranged from 65-85% that of standard drug against *Staphylococcus aureus* and 50-80% that of ciprofloxacin against *E.Coli*. It is worthy-mentioning that compound **9f** showed the highestactivity (85%) against *Staphylococcus aureus* while compound **9d** was the most active derivative against *E.Coli*(80% avtivity).

Table II. Inhibitory zone diameter (mm) of compounds **2, 3, 4a-c, 5a-c, 6, 8,** and **9a-f**

Compd. No.	Gram-positive bacteria *Staph. aureus*	Gram-negative bacteria *E. coli*	Fungi C. Albicans
2	**19**	**16**	**16**
3	**15**	**16**	**16**
4a	**29**	**24**	**28**
4b	**25**	**21**	**25**
4c	**24**	**18**	**26**
5a	**24**	**19**	**21**
5b	**20**	**18**	**22**
5c	**21**	**18**	**20**
6	**27**	**22**	**24**
8	**20**	**21**	**18**
9a	**28**	**20**	**26**
9b	**28**	**24**	**24**
9c	**26**	**20**	**30**
9d	**26**	**32**	**30**
9e	**26**	**24**	**32**
9f	**34**	**26**	**30**
Ciprofloxacin	**40**	**40**	**--**
Fluconazole	**--**	**--**	**40**

All the synthesized compounds **2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f** were tested as potential antifungal agents against *Candida albicans* using Fluconazole as a reference drug (Table II).

The results revealed that the tested compounds showed a varying degree of antifungal activity against the test organism. Compound **2** showed activity 40% that of fluconazole. Moreover, further derivatization of compound **2** with different (un)substituted benzaldehydes and acetophenone, compounds **9a-f** affords compounds with improved antifungal activity against *Candida albicans,* showing activity 50-80% that of fluconazole. Compound **9e** displayed the higher antifungal activity among the other derivatives as it showed 80% activity of fluconazole.

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3 CONCLUSION

In conclusion, several 1,2,3-triazole derivatives bearing different chemical entities were synthesized. A microbiological study was undertaken to evaluate the effect of the synthesized compounds on different bacterial and fungal strains. The results of the preliminary testing of the antibacterial activity of the final compounds revealed that the majority of the synthesized compounds show varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-positive bacteria was higher than against the Gram-negative bacteria. The triazole derivatives **4a**, **9a**, **9b**, and **9f**, displayed the highest activity. Results of antifungal activity revealed that all compounds showed a weak to a good antifungal activity and that compounds **9c**, **9d**, **9e** and **9f** were the most active ones.

4 EXPERIMENTAL

4.1. Chemistry

Reagents used for synthesis were purchased from Sigma-Aldrich (Gillingham – Dorest, UK) and MERCK (Schuchardt, Germany). All solvents were obtained from commercial suppliers and used without further purification. The starting material ethyl 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetate, compound **1**[18] was prepared according to reportedprocedure.

Melting points were determined on an electro thermal melting point apparatus [Stuart Scientific, model SMP3, England, UK], and were uncorrected. Pre-coated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for TLC monitoring of reactions. The developing solvent systems of $CHCl_3/CH_3OH$ (9.5:0.5 *V/V*) were used and the spots were detected at 254 nm wavelength using ultraviolet lamp (Spectroline, model CM-10, USA). The target compounds were crystallized from ethanol unless otherwise specified. IR spectra (KBr discs) were recorded on a shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University, Assiut. NMR Spectra were taken using a Varian Unity INOVA 400 MHz and Bruker AC250 MHz spectrometers for proton and carbon. All numbers referring to NMR data obtainedare in parts per million (ppm) relative to TMS as an internal standard, using DMSO-d_6, unless otherwise specified, as a solvent, and deuterium oxide was used for the detection of exchangeable protons.. High resolution mass spectrometric data were obtained using the EPSRC mass spectrometry centre in Swansea and Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Pump) at university of Aberdeen.

Synthesis of 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide (2)

To a solution of ethyl 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetate, compound **1**(3.17 g, 0.013 mol) in absolute ethanol (40 mL), hydrazine hydrate 99% (1.00 g, 0.02 mole) was added. The reaction mixture was refluxed for 6 h, and then cooled. The precipitated product was filtered, washed with cold ethanol, dried, and crystallized from ethanol as white crystals.

Synthesis of N-(1,3-dioxoisoindolin-2-yl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetamide (3)

A mixture of the hydrazide **1** (0.001 mol) and phthalic anhydride (0.001 mol) in glacial acetic acid (10 mL) was heated at reflux for 2 h, after cooling, the separated product was filtered and crystallized from DMF/H_2O.

General procedure for preparation of N-alkyl/aryl-2-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetyl)hydrazine carbothioamide (4a-c)

A mixture of the hydrazide **2** (0.002 mol) and the appropriate isothiocyanate (0.002 mol) in ethanol (10 mL) was heated at reflux for 2 h, after cooling, the separated product was filtered and crystallized from ethanol.

General procedure for preparation of 4-alkyl/aryl-5-((2-(4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol (5a-c)

Compounds **4a-c** (0.002 mol) were dissolved in NaOH (2N, 10 mL), then heated under reflux for 2 h. The solution was cooled, filtered and then acidified with HCl (2N). The separated solid was filtered and crystallized from DMF/H_2O.

Synthesis of 5-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (6)

The acid hydrazide **2** (0.002 mol) was stirred in ethanol (20 mL) containing potassium hydroxide (0.002 mol) for 1 h until a clear solution was obtained. Carbon disulfide (0.005 mol) was added dropwise to the stirred reaction mixture, and then it was heated under reflux for 6 h. The reaction mixture was concentrated, cooled, and acidified with diluted HCl. The separated product was filtered, washed with water, and crystallized from ethanol.

Synthesis of potassium 2-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetyl)hydrazinecarbodithioate (7)

Carbon disulfide (0.015 mol) was added dropwise to an ice-cooled solution of ethanol (20 mL) containing KOH (0.01 mol) and hydrazide **2** (0.01 mol). The mixture was stirred for 14 h, and then dry diethyl ether (10 mL) was added. The separated solid was filtered and then washed twice with diethyl ether (20 mL). The obtained product was used in the next reaction without further purification.

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Synthesis of 4-amino-5-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol (8)

A mixture of intermediate 7 (0.005 mol) and hydrazine hydrate (99%; 0.01 mol) in ethanol (20 mL) was refluxed for 4 h. the reaction mixture was diluted with cold water, then neutralized by portion wise addition of concentrated HCl. The formed precipitate was filtered, washed with water, and crystallized from ethanol.

General procedure for preparation of N-arylidene-2-(4-phenyl-1H-1,2,3-triazol-1-yl)aceto-hydrazide (9a-f)

To a suspension of hydrazide2(0.002 mol) in ethanol (10 mL) and the appropriate aryl aldehyde (0.002 mol), 2 drops of glacial acetic acid were added, and then the reaction mixture was heated under reflux for 4-6 h. The reaction mixture was cooled and the precipitated product was filtered, washed with cold ether and crystallized from ethanol.

Spectral Data of New Compounds

2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide(2). Yield (82%), mp 186-188 ^{o}C; IR spectrum (v/cm^{-1}): 3290, 3160 (NH); 1658 (C=O); 1620, 1584, 1530, 1485, 1460 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 9.57 (s, 1H); 8.54 (s, 1H); 7.91-7.84 (m, 2H); 7.45 (t, J = 7.6 Hz, 2H); 738-7.28 (m, 1H); 5.10 (s, 2H); 4.42 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 164.79; 146.21; 130.72; 128.94; 127.88; 125.17; 122.82; 50.70; HRMS calcd for $C_{10}H_{11}N_5O$ $[M+H]^+$ 218.1036 Found 218.1033.

***N*-(1,3-dioxoisoindolin-2-yl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetamides (3)**. Yield (78%), mp 221-223^{o}C; IR spectrum (v/cm^{-1}): 3260 (NH); 1699; 1648 (C=O); 1610; 1594; 1520; 1487; 1460 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 8.62 (d, J = 1.0 Hz, 1H); 8.01-7.83 (m, 6H); 7.45 (t, J = 7.7 Hz, 2H); 7.34 (td, J = 7.2 Hz, 1.3 Hz, 1H); 5.56 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 165.33; 164.76; 146.32; 135.33; 130.55; 129.40; 128.94; 127.94; 125.18; 123.83; 123.11; 50.09; HRMS calcd for $C_{18}H_{13}N_5O_3$ $[M+H]^+$348.1091 Found 348.1087.

***N*-phenyl-2-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetyl)hydrazinecarbothioamide (4a)**. Yield (84%), mp 198-200 ^{o}C; IR spectrum (v/cm^{-1}): 3265 (NH); 1658 (C=O); 1610; 1590; 1527; 1482; 1460 (C=N/C=C); 1191 (C=S); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 10.56 (s, 1H); 9.79 (d, J = 9.6 Hz, 2H); 8.56 (s, 1H); 7.88 (d, J = 7.6 Hz, 2H); 7.50-7.30 (m, 7H); 7.20 (t, J = 7.3 Hz, 1H); 5.29 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 146.25; 138.97; 130.65; 128.95; 128.19; 127.91; 125.17; 122.97; 50.68; HRMS calcd for $C_{17}H_{16}N_6O_5$ $[M+H]^+$ 353.1179 Found 353.1178.

***N*-ethyl-2-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetyl)hydrazinecarbothioamide(4b)**. Yield (76%), mp 224-226 ^{o}C; IR spectrum (v/cm^{-1}): 3245 (NH); 1666 (C=O); 1610; 1590; 1527; 1482; 1460 (C=N/C=C); 1192 (C=S); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 10.31 (s, 1H); 9.34 (s, 1H); 8.54 (s, 1H); 8.16 (t, J = 5.6 Hz, 1H); 7.87 (d, J = 7.6 Hz, 2H); 7.45 (t, J = 7.6 Hz, 2H); 7.34 (t, J = 7.4 Hz, 1H); 5.23 (s, 2H); 3.49 (p, J = 6.9 Hz, 2H); 1.09 (t, J = 7.1 Hz, 3H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 169.40; 165.40; 146.27; 130.65; 128.95; 127.92; 125.17; 122.92; 50.64; 38.53; 14.50; HRMS calcd for $C_{13}H_{16}N_6O_5$ $[M+H]^+$ 305.1179 Found 305.1177.

***N*-allyl-2-(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetyl)hydrazinecarbothioamide(4c)**. Yield (80%), mp 217-219 ^{o}C; IR spectrum (v/cm^{-1}): 3234 (NH); 1652 (C=O); 1590; 1587; 1532; 1480; 1467 (C=N/C=C); 1191 (C=S); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 10.36 (s, 1H); 9.47 (s, 1H); 8.53 (s, 1H); 8.36 (t, J = 5.6 Hz, 1H); 7.91-7.84 (m, 2H); 7.45 (t, J = 7.6 Hz, 2H); 7.38-7.29 (m, 1H); 5.84 (ddt, J = 17.3, 10.2, 5.1 Hz, 1H); 5.25 (s, 2H); 5.19-5.03 (m, 2H); 4.14 (t, J = 5.4 Hz, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 165.47; 146.26; 134.80; 130.66; 128.95; 127.92; 125.17; 122.90; 115.32; 50.66; 45.90; HRMS calcd for $C_{14}H_{16}N_6O_5$ $[M+H]^+$ 317.1179 Found 317.1177.

4-phenyl-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol(5a). Yield (81%), mp 220-222 ^{o}C; IR spectrum (v/cm^{-1}): 2988 (SH); 1590; 1527; 1482; 1460 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 8.26 (s, 1H); 7.75 (d, *J* = 7.7 Hz, 2H); 7.51-7.38 (m, 5H); 7.37-7.28 (m, 3H); 5.26 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 168.71; 146.90; 146.34; 132.78; 130.32; 129.64; 129.39; 128.88; 127.98; 127.89; 125.19; 122.07; 44.53; HRMS calcd for $C_{17}H_{14}N_6S$ $[M+H]^+$ 335.1073 Found 335.1071.

4-ethyl-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol (5b). Yield (73%), mp 224-226 ^{o}C; IR spectrum (v/cm^{-1}): 3245 (NH); 2962 (SH); 1580; 1532; 1482; 1460 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 13.93 (s, 1H); 8.70 (s, 1H); 7.88 (d, J = 7.5 Hz, 2H); 7.44 (t, J = 7.6 Hz, 2H); 7.33 (t, J = 7.4 Hz, 1H); 5.94 (s, 2H); 4.04 (q, J = 7.1 Hz, 2H); 1.02 (t, J = 7.1 Hz, 3H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.23; 146.97; 146.88; 130.28; 128.94; 128.13; 125.30; 122.18; 44.15; 38.81; 12.89; HRMS calcd for $C_{13}H_{14}N_6S$ $[M+H]^+$287.1073 Found 287.1072.

4-allyl-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol (5c). Yield (76%), mp 237-239 ^{o}C; IR spectrum (v/cm^{-1}): 2971 (SH); 1591; 1530; 1482; 1457 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 14.00 (s, 1H); 8.65 (s, 1H); 7.90-7.82 (m, 2H); 7.49-7.40 (m, 2H); 7.38-7.28 (m, 1H); 5.85 (s, 2H); 5.73 (ddt, J = 17.3, 10.6, 5.2 Hz, 1H); 5.03 (dq, J = 10.3, 1.4 Hz, 1H); 4.93 (dt, J = 17.1, 1.4 Hz, 1H); 4.69 (dt, J = 5.2, 1.7 Hz, 1H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.76; 147.05; 146.81; 130.65; 130.37; 128.92; 128.06; 125.26; 125.25; 122.29; 117.44; 45.24; 44.15; HRMS calcd for $C_{14}H_{14}N_6S$ $[M+H]^+$ 299.1073 Found 299.1072.

5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-1,3,4-oxadiazole-2-thiol(6). Yield (87%), mp 262-264 ^{o}C; IR spectrum (v/cm^{-1}): 2961 (SH); 1601; 1537; 1482; 1457 (C=N/C=C); ^{1}H NMR spectrum (DMSO-d6): δ (ppm): 8.72 (d, J = 1.3 Hz, 1H); 7.92-7.84 (m, 2H); 7.48-7.39 (m, 2H); 7.33 (td, J = 7.4, 1.4 Hz, 1H); 5.98 (d, J = 1.6 Hz, 2H); ^{13}C NMR

spectrum (DMSO-d6): δ (ppm): 178.19; 158.23; 146.98; 130.23; 129.00; 128.23; 125.38; 122.46; 44.17; HRMS calcd for $C_{11}H_9N_5OS$ $[M+H]^+$ 260.0601 Found 260.0594.

4-amino-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)-4*H*-1,2,4-triazole-3-thiol(8). Yield (81%), mp 271-273 ^oC; IR spectrum (v/cm^{-1}): 3270; 3135 (NH_2 and NH); 2720 (SH); 1615; 1560; 1485; 1461 (C=N/C=C); 1299 (C=S); ^1H NMR spectrum (DMSO-d6): δ (ppm): 13.85 (s, 1H); 8.73 (d, *J* = 0.7 Hz, 1H); 8.62 (d, *J* = 0.7 Hz, 1H); 7.91-7.83 (m, 3H); 7.51-7.40 (m, 3H); 7.40-7.29 (m, 1H); 5.94 (s, 1H); 5.76 (s, 2H); 5.68 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 178.06; 167.21; 158.22; 147.25; 146.83; 146.58 130.47; 130.20; 128.89; 128.89; 128.18; 128.00; 125.28; 125.24; 122.44; 122.16; 44.04; 43.56; HRMS calcd for $C_{11}H_{11}N_7S$ $[M+H]^+$ 274.0869 Found 274.0867.

(*E*)-*N*-benzylidene-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide (9a). Yield (82%), mp 196-198 ^oC; IR spectrum (v/cm^{-1}): 3185 (NH); 1673 (C=O); 1608; 1583; 1483; 1458 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.90 (s, 1H); 8.56 (s, 1H); 8.08 (s, 1H); 7.92-7.83 (m, 2H); 7.80-7.73 (m, 2H); 7.51-7.41 (m, 5H); 7.39-7.30 (m, 1H); 5.76 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.38; 146.14; 144.57; 133.84; 130.80; 130.16; 128.95; 128.84; 127.83; 127.24; 127.07; 125.16; 125.12; 123.21; 50.71; HRMS calcd for $C_{17}H_{15}N_5O$ $[M+H]^+$ 306.1349 Found 306.1348.

(*E*)-*N*-(4-chlorobenzylidene)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl) acetohydrazide (9b). Yield (85%), mp 198-199 ^oC; IR spectrum (v/cm^{-1}): 3160 (NH); 1668 (C=O); 1610; 1578; 1488; 1461 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.96 (s, 1H); 8.56 (s, 1H); 8.06 (s, 1H); 7.87 (d, J = 7.5 Hz, 2H); 7.83-7.73 (m, 2H); 7.56-7.42 (m, 4H); 7.39-7.30 (m, 1H); 5.76 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.47; 162.21; 146.67; 146.27; 146.15; 143.27; 134.79; 134.59; 132.86; 130.79; 130.67; 128.94; 128.90; 128.88; 128.73; 127.89; 127.82; 125.16; 125.11; 123.18; 122.89; 51.15; 50.70; HRMS calcd for $C_{17}H_{14}ClN_5O$ $[M+H]^+$ 340.0960 Found 340.0962.

(*E*)-*N*-(4-bromobenzylidene)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide (9c). Yield (87%), mp 201-203 ^oC; IR spectrum (v/cm^{-1}): 3165 (NH); 1680 (C=O); 1609; 1580; 1483; 1461 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.96 (s, 1H); 8.55 (s, 1H); 8.05 (s, 1H); 7.91-7.83 (m, 2H); 7.76-7.69 (m, 2H); 7.69-7.61 (m, 3H); 7.51-7.42 (m, 2H); 7.39-7.30 (m, 1H); 5.76 (s, 2H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.48; 146.15; 143.18; 133.14; 131.86; 131.82; 130.78; 129.10; 128.96; 128.94; 127.83; 125.16; 125.11; 123.40; 123.18; 50.70; HRMS calcd for $C_{17}H_{14}BrN_5O[M+H]^+$384.0454 Found 384.0455.

(*E*)-*N*-(4-fluorobenzylidene)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetohydrazide (9d). Yield (84%), mp 199-200 ^oC; IR spectrum (v/cm^{-1}): 3175 (NH); 1677 (C=O); 1606; 1581; 1486; 1462 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.90 (s, 1H); 8.56 (d, J = 2.5 Hz, 1H); 8.07 (d, J = 2.5 Hz, 1H); 7.92-7.75 (m, 5H); 7.46 (td, J = 7.7, 2.6 Hz, 3H); 7.39-7.25 (m, 1H); 5.75 (d, J = 2.2 Hz, 1H); 5.30 (d, J = 2.0 Hz, 1H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 167.39; 146.13; 143.40; 130.79; 130.50; 129.33; 129.25; 128.94; 127.82; 125.15; 125.10; 123.18; 116.00; 115.78; 50.70; HRMS calcd for $C_{17}H_{14}FN_5O$ $[M+H]^+$ 324.1255 Found 324.1252.

(*E*)-*N*-(4-(dimethylamino)benzylidene)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)aceto- hydrazide (9e). Yield (78%), mp 192-194 ^oC; IR spectrum (v/cm^{-1}): 3255 (NH); 1677 (C=O); 1613; 1576; 1487; 1461 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.58 (d, J = 2.9 Hz, 1H); 8.58-8.52 (m, 1H); 7.96-7.82 (m, 3H); 7.60-7.41 (m, 4H); 7.35 (ddd, J = 8.9, 5.3, 3.2 Hz, 1H); 6.74 (dt, J = 9.0, 2.0 Hz, 2H); 5.69 (d, J = 3.2 Hz, 2H); 3.07-2.84 (m, 6H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 166.66; 151.52; 146.09; 145.37; 130.83; 128.93; 128.60; 128.35; 127.79; 125.14; 125.10; 123.21; 121.13; 111.76; 50.65; 39.77; 30.70; HRMS calcd for $C_{19}H_{20}N_6O$ $[M+H]^+$ 349.1771 Found 349.1765.

(*E*)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-*N*-(1-phenylethylidene)acetohydrazide(9f). Yield (84%), mp 196-198^oC; IR spectrum (v/cm^{-1}): 3165 (NH); 1673 (C=O); 1610; 1576; 1487; 1461 (C=N/C=C); ^1H NMR spectrum (DMSO-d6): δ (ppm): 11.12 (s, 1H); 8.57 (s, 1H); 7.94-7.84 (m, 5H); 7.50-7.40 (m, 4H); 7.37-7.32 (m, 1H); 5.80 (s, 2H); 2.31 (s, 3H); ^{13}C NMR spectrum (DMSO-d6): δ (ppm): 168.31; 149.28; 146.16; 137.83; 130.85; 129.33; 128.94; 128.41; 127.81; 126.29; 125.13; 123.20; 51.23; 13.80; HRMS calcd for $C_{18}H_{17}N_5O$ $[M+H]^+$320.1506 Found 320.1503.

4.2. Biology

4.2.1. Antibacterial screening Organisms and culture conditions

The used bacterial cultures were obtained from Assiut University Mycological Center (AUMC), Assiut University. The synthesized compounds (**2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f**) were tested for their *in-vitro* antibacterial activity in comparison to ciprofloxacin as a reference drug using the standard agar cup diffusion method[19] against *Staphylococcus aureus*(AUMC B54) as a representative of Gram positive strains, while the Gram negative strains were represented by *Escherichia coli* (AUMC B69).

Materials and method

Bacterial strains were individually cultured for 48 h in 100 mL conical flasks containing 30 mL Nutrient Agar (NA). Assay was done in 10 cm sterile Petri dishes in which one mL bacterial suspension and 15 mL of NA were poured. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. The test compounds (**2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f**) and ciprofloxacin were dissolved in dimethyl sulfoxide (100 µmol/mL) and were pipeted in the cavities. In addition, other cavities were pipeted with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28 ± 2°C for 48 h.

The radii of inhibition zones (in mm) of triplicate sets were measured and the results are cited in table **II**.

4.2.2. Antifungal screening *Organisms and culture conditions*

The used Sabouraud Agar (SA) media were prepared in Assiut University Mycological Center (AUMC), Assiut University. The test compounds (**2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f**) were evaluated for their antifungal activity *in-vitro*, in comparison to fluconazole as a reference drug using the standard agar cup diffusion method[20] against a pathogenic fungal species *Candida albicans* (Robin) Berkhout (AUMC 418).

Materials and method

Spore suspension in sterile distilled water was prepared from 7 days old culture of the test fungi growing on Sabouraud's dextrose broth (30 mL) media in 100 mL conical flasks. The final spore concentration was nearly 5×104 spores/mL. About 15 mL of the growth medium was introduced on sterilized Petri dishes of 10 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer and was filled with the solutions of the test compounds (**2**, **3**, **4a-c, 5a-c, 6, 8,** and **9a-f**) and fluconazole (100 µmol/mL in DMSO). In addition, other cavities were impregnated with the solvent (DMSO) and served as a negative control. The seeded plates were incubated at 28±2°C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are displayed in table **II**.

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