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SYNTHESIS OF SOME NOVEL 1, 3, 5-TRISUBSTITUTED [1,2,4]TRIAZOLE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AGENTS

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تم فى هذا العمل تحضير بعض مشتقات١،٣،٥ - الجديده لحلقة [١،٢،٤] التريازول وبعض قواعد شيف المحضره منهم. تم التأكيد من التراكيب البنائيه للمركبات المستهدفة باستخدام الرنين النووى المغناطيسي، التحليل الطيفى للكتلة والتحاليل الدقيقة للعناصر. هذا وقد تم اختبار المركبات المستهدفة كمضادات للبكتريا (موجبة وسالبة الجرام) وقد ثبت أن هناك فاعلية لبعض المركبات المختبرة وأعطت نتائج مساوية أو متفوقة على المضادات الحيوية المرجعية المستعملة مثل الأمبسيللين وحمض الناليديكسيك

In the present work, some new 1,3,5-trisubstituted[1,2,4]-triazole derivatives and their Schiff's bases were synthesized. The chemical structure of the target compounds was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, FAB-MS, EI-HRMS spectra and elemental analyses. The title compounds were tested for their in vitro antibacterial activity against Gram-positive and Gram-negative ones using ampicillin and nalidixic acid as reference drugs. Some of them showed antibacterial activity more significant than the reference drugs.

INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem due to the emergence of resistant strain of bacteria to major classes of antibacterial agents. The emergence of multidrug resistance of Gram-positive pathogens is a problem of ever increasing significance[1]. Organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus*

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epidermids (MRSE), vancomycin-resitant enterococci (VRE), penicillin and cephalosporin-resistant streptococci are continuously challenging chemists, physicians and patients[S]. Consequently, the search of new chemotherapeutic agents constitutes real challenge facing microbiologists, Pharmacologists as well as medicinal chemists.

Currently, 1,2,4-triazole nucleus has been incorporated into a wide variety of the pharmacological activities. The following [1,2,4]-triazole derivatives are applied in medicine: alprazolam (tranquilizer), estazolam (hypnotic, sedative, tranquilizer), rilmazafon (hypnotic, anxiolytic, used in the case of neurotic insomnia), benatradin (diuretic), trapidil (hypotensive), trazodon (antidepressant,anxiolytic), etoperidone (antidepressant), nefazo-done (antidepressant, 5-HT2 A-antagonist), anastrozole, letrozole, vorozole (antineoplastics, non-steroidal competitive aromatase inhibitors), ribavirin (the potent antiviral N-nucleoside), fluconazole, itraconazole, terconazole (the powerful azole antifungal agents)[2].

The literature survey revealed that depending on the type of substituent, the derivatives of [1,2,4]triazole have a high potential for biological activity, possessing antibacterial[3-7] and antitumor[8-11] properties. The other ones show also anti-inflammatory[12], antihypertensive[13], anticonvulsant[14], and antiviral[15].

Moreover, through the various molecules designed and synthesized as new antibacterial agents, it was demonstrated that 1,2,4-triazoles and their derivatives could be considered as possible antimicrobial agents[16-22].

The above mentioned facts motivate our interest to synthesize new 1,3,5-trisubstituteded [1,2,4]-triazoles and their Schiff bases as potential antibacterial agents.

EXPERIMENTAL

Chemistry

Materials and equipment

Melting points were determined on an electrical melting point apparatus (stuart Scientific, UK), and are uncorrected. Silica gel 60 F_{254} (Merck) aluminum plates were used for thin layer chromatography. Dichloromethane/methanol (9:1 v/v) and Cyclohexane/EtOAc (3:2 v/v) were used as developing systems and the spots were visualized by ultraviolet light. IR spectra were recorded on a Shimadzu spectrophotometer (IR-470) as potassium bromide discs, at the Faculty of Pharmacy, Assiut University, Egypt. ^{1}H-NMR spectra were performed on Varian EM-360, 60 MHz spectrometer at the Faculty of Pharmacy, Assiut University, Egypt. DMSO-d_6 was used as a solvent and the chemical shifts are given in δ (ppm) values downfield from TMS as internal standard. ^{1}H-NMR and ^{13}C-NMR spectra of some compounds were taken using Bruker DPX 300 MHz instrument at Organic Chemistry institute, Bonn, Germany. DMSO-d_6 and $CDCl_3$ were used as solvent and the chemical shifts are

given in δ (ppm) values. The chemical shifts of the remaining protons of the deuterated solvents served as internal standard: δ ^{1}H: 2.49 ppm, ^{13}C: 39.7 ppm and δ ^{1}H: 7.26 ppm, ^{13}C: 77.36 ppm for DMSO-d_6 and $CDCl_3$ respectively. The EI-HRMS was obtained using El-Finnigan MAT 95XL (Thermo Finnigan, Bremen) at Organic Chemistry institute, Bonn, Germany. FAB-MS were carried out using JOEL JMS600 mass spectrometer at analyses center, Assiut University, Egypt. Elemental microanalyses were performed on a Perkin-Elmer 240 elemental analyzer at the Department of Chemistry, Faculty of Science, Assiut University, Egypt.

Compounds **9, 11, 12, 13, 15, 16, 17, 19** and **20** were prepared as reported[23].

Synthesis of methyl (4-methoxy-benzoyl)thiocarbamate (10)

To a solution of 4-methoxy-benzoyl chloride **2** (6.43 g, 37,84 mmol) in acetone 60 mL, potassium thiocyanate (3.68 g, 37.84 mmol, 1.0 eq.) was added under efficient stirring. The reaction mixture was refluxed at 60°C for 90 min and monitored by TLC until the start disappeared and the intermediate 4-methoxybenzoyl isothiocyanate **6** was formed. Methanol (3.8 ml, 94.60 mmol, 2.5 eq.) was added dropwise to the reaction mixture. The mixture was refluxed for 8 h then cooled, filtered, washed with acetone (15 ml), and the filtrate was evaporated. The residue was purified by silica gel chromatography to afford the product as yellowish white oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.13 (br, 1H, NH), 7.74 (d, *J* = 8.5 Hz, 2H), 7.88 (d, *J* = 8.5 Hz, 2H), 4.19 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): 190.65, 163.67, 161.88, 129.96, 124.79, 114.29, 59.52, 55.60.

EI-HRMS (*m/z*): calcd. for $C_{10}H_{11}NO_3S$ 225.0460, found 225.0453 $[M]^+$.

Synthesis of 3-methoxy-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-[1,2,4] triazole (14)

Methyl (4-methoxybenzoyl)thio-carbamate **10** (5.41 g, 24 mmol, 1 eq.) and *p*-nitro-phenylhydrazine (3.66 g; 24 mmol, 1 eq.) were suspended in ethanol (45.0 ml). The reaction mixture was refluxed for 15 h. During the course of the reaction H_2S evolution was observed. The reaction mixture was cooled and the precipitated product was filtered washed with cold ethanol. The residue was dried in vacuum to afford the triazole derivative **14** as yellow solid.

IR (cm^{-1}): 3080 (Ar. C-H), 2980 (aliph. C-H), 1601 (C=N), 1528 (NO_2, asym.), 1340 (NO_2, sym.), 1180, 1070 (C-O), 827 (*p*-disubstituted benzene).

^{1}H-NMR (300 MHz, DMSO): δ 8.30 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 3.97 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3).

^{13}C-NMR (75 MHz, DMSO): δ 168.55, 161.32, 154.18, 146.81, 143.11, 130.90, 126.19, 125.30, 119.71, 114.72, 57.12, 55.76.

EI-HRMS (*m/z*): calcd. for $C_{16}H_{14}N_4O_4$ 326.1015, found 326.1014 $[M]^+$.

Synthesis of 1-(4-aminophenyl)-3-methoxy-5-(4-methoxyphenyl)-1-*H*-[1,2,4] triazole (18)

3-Methoxy-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1H-[1,2,4] triazole **14** (4.50 g, 13.8 mmol) was dissolved in ethanol (100 mL) and the solution was washed with argon 3 times. Pd/C10% (5.1 g) was added portionwise and the reaction mixture was stirred in autoclave under H_2 atmosphere under 20 bar pressure for 20 h. The reaction mixture was filtered through celite pad and washed with ethanol (80 ml) and hot methanol (5x100 ml). The filtrate was evaporated to afford the desired compound **18**. The compound was purified by recystallization from ethanol.

IR (cm^{-1}): 3340 (NH_2), 3060 (Ar. C-H), 2930 (aliph. C-H), 1634, 1599 (C=C, C=N), 1178, 1040 (C-O), 828 (*p*-disubstituted benzene).

^{1}H-NMR (300 MHz, DMSO): 7.37 (d, *J* = 8.6 Hz, 2H), 6.99 (d, *J* = 8.6 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.58 (d, *J* = 8.5 Hz, 2H), 5.49 (br s, 2H, NH_2), 3.90 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3).

^{13}C-NMR (75 MHz, DMSO): δ 167.68, 160.68, 152.71, 149.91, 130.18, 127.48, 126.75, 120.44, 114.31, 114.09, 56.68, 55.67.

EI-HRMS (*m/z*): calcd. for $C_{16}H_{16}N_4O_2$ 296.1273, found 296.1275 $[M]^+$.

General procedure for synthesis of Schiff bases (21-44)

To a suspension of triazole derivative **17, 18, 19** or **20** (1.18 mmol) in ethanol (15 mL), an equimolar amount of the appropriate aldehyde (1.18 mmol) was added. The suspension was gently heated until a clear solution was obtained. Then few drops of glacial acetic acid were added and the solution was refluxed for 7-8 h on a water-bath. The reaction mixture was cooled to the ambient temperature and the formed precipitate was filtered and recrystallized from a mixture of ethanol and water (9:1) to yield the compounds **21-44**.

1-[4-(Benzylidene-amino)phenyl]-3-methoxy-5-(phenyl)-1-*H*-[1,2,4]triazole (21)

^{1}H-NMR (60 MHz, DMSO): 9.1 (s, 1H, N=CH), 7.9-8.6 (m, 14H, Ar H), 4.4 (s, 3H, OCH_3).

1-{4-[(4-Bromobenzylidene)amino]-phenyl}-3-methoxy-5-(phenyl)-1-*H*-[1,2,4] triazole (22)

IR (cm^{-1}): 3010 (Ar. C-H), 2940 (aliph. C-H), 1612, 1591 (C=C, C=N), 1173 (C-O), 832 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.2 (s, 1H, N=CH), 7.9-8.6 (m, 13H, Ar H), 4.45 (s, 3H, OCH_3).

1-{4-[(4-Chlorobenzylidene)amino]-phenyl}-3-methoxy-5-(phenyl)-1-*H*-[1,2,4] triazole (23)

IR (cm^{-1}): 3090 (Ar. C-H), 2940 (aliph. C-H), 1612, 1583 (C=C, C=N), 1078 (C-O), 832 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.2 (s, 1H, N=CH), 8.5 (d, *J* = 8.6 Hz, 2H, Ar H), 7.8-8.3 (m, 11H, Ar H), 4.4 (s, 3H, OCH_3).

3-Methoxy-1-{4-[(4-methoxybenzyl-idene)amino]phenyl}-5-(phenyl)-1-*H*-[1,2,4] triazole (24)

IR (cm^{-1}): 3090 (Ar. C-H), 2935 (aliph. C-H), 1621, 1593 (C=C, C=N), 1156, 1101 (C-O), 825 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.0 (s, 1H, N=CH), 8.5 (d, *J* = 8.6 Hz, 2H, Ar H), 7.8-8.2 (m, 9H, Ar H), 7.5 (d, *J* = 8.5 Hz, 2H, Ar H), 4.4 (s, 3H, OCH_3), 4.2 (s, 3H, OCH_3).

3-Methoxy-1-{4-[(4-N,N-dimethyl-aminobenzylidene)amino]phenyl}-5-(phenyl)-1-*H*-[1,2,4] triazole (25)

IR (cm^{-1}): 3065 (Ar. C-H), 2905 (aliph. C-H), 1598, 1570 (C=C, C=N), 1178 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.1 (s, 1H, N=CH), 8.6 (d, *J* = 8.5 Hz, 2H, Ar H), 7.8-8.3 (m, 9H, Ar H), 7.3 (d, *J* = 8.6 Hz, 2H, Ar H), 4.4 (s, 3H, OCH_3), 3.3 (s, 6H, $N(CH_3)_2$.

FAB-MS m/z, (%): 398 $[M+1]^+$ (5%), 420 $[M+ Na]^+$ (4%), 93.08 (100%).

3-Methoxy-1-{4-[(4-nitrobenzyl-idene)amino]phenyl}-5-(phenyl)-1-*H*-[1,2,4] triazole (26)

IR (cm^{-1}): 3030 (Ar. C-H), 2985 (aliph. C-H), 1616, 1586 (C=C, C=N), 1527 (NO_2, asym.), 1333 (NO_2, sym.), 1179, 1099 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.6 (s, 1H, N=CH), 9.2 (d, *J* = 8.6 Hz, 2H, Ar H), 8.4 (d, *J* = 8.5 Hz, 2H, Ar H), 7.9-8.2 (m, 9H, Ar H), 4.4 (s, 3H, OCH_3).

FAB-MS m/z, (%): 400.17 $[M+1]^+$ (13%), 57.05 (100%).

1-{4-[(4-Hydroxybenzylidene)-amino]phenyl}-3-methoxy-5-(phenyl)-1-*H*-[1,2,4] triazole (27)

IR (cm^{-1}): 3420 (OH), 3080 (Ar. C-H), 2970 (aliph. C-H), 1611, 1580 (C=C, C=N), 1170 (C-O), 823 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 11.2 (br s, 1H, OH), 9.3 (s, 1H, N=CH), 8.5 (d, *J* = 8.5 Hz, 2H, Ar H), 7.8-8.2 (m, 9H, Ar H), 7.5 (d, *J* = 8.5 Hz, 2H, Ar H), 4.3 (s, 3H, OCH_3).

FAB-MS m/z, (%): 371.20 $[M+1]^+$ (6%), 50.99 (100%).

1-{4-[(4-Carboxybenzylidene)-amino]phenyl}-3-methoxy-5-(phenyl)-1-*H*-[1,2,4] triazole (28)

IR (cm^{-1}): 3470 (OH), 3030 (Ar. C-H), 2930 (aliph. C-H), 1679 (C=O), 1610 (C=N), 1162 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 11.1 (br s, 1H, COOH), 9.4 (s, 1H, N=CH), 8.5-8.7 (m, 5H, Ar H), 7.8-8.1 (m, 8H, Ar H), 4.3 (s, 3H, OCH_3).

1-{4-[(4-Carboxymethyloxybenzyl-idene)amino]phenyl}-3-methoxy-5-(phenyl)-1-*H*-[1,2,4] triazole (29)

IR (cm^{-1}): 3440 (OH), 3035 (Ar. C-H), 2920 (aliph. C-H), 1730 (C=O), 1614, 1502 (C=C, C=N), 1185, 1085 (C-O), 837 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 10.8 (br s, 1H, COOH), 9.3 (s, 1H, N=CH), 8.5 (d, *J* = 8.6 Hz, 2H, Ar H), 7.5-8.1 (m, 11H, Ar H), 5.2 (s, 2H, O-CH_2-), 4.3 (s, 3H, OCH_3).

FAB-MS m/z, (%): 429.02 $[M+1]^+$ (3%), 50.99 (100%).

1-{4-[(4-Bromobenzylidene)amino]-phenyl}-3-methoxy-5-(4-methoxy-phenyl)-1-*H*-[1,2,4] triazole (30)

IR (cm^{-1}): 3060 (Ar. C-H), 2945 (aliph. C-H), 1601, 1529 (C=C, C=N), 1179 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.4 (s, 1H, N=CH), 8.3-8.7 (m, 4H, Ar H), 8.0-8.2 (m, 4H, Ar H), 7.5-7.8 (m, 4H, Ar H), 4.4 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3).

1-{4-[(4-Carboxybenzylidene)-amino]phenyl}-3-methoxy-5-(4-methoxyphenyl)-1-*H*-[1,2,4] triazole (31)

IR (cm^{-1}): 3435(OH), 3035 (Ar. C-H), 2920 (aliph. C-H), 1675 (C=O), 1605, 1564 (C=C, C=N), 1180, 1105 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.4 (s, 1H, N=CH), 8.6-8.8 (m, 4H, Ar H), 7.8-8.0 (m, 4H, Ar H), 7.1-7.7 (m, 4H, Ar H), 4.3 (s, 3H, OCH_3), 1.5 (s, 3H, OCH_3).

FAB-MS m/z, (%): 428.18$[M]^+$ (12%), 93.10 (100%).

1-[4-(Benzylidene)amino)phenyl]-5-(4-*tert.*Butylphenyl)-3-methoxy-1-*H*-[1,2,4] triazole (32)

^{1}H-NMR (60 MHz, DMSO): 9.3 (s, 1H, N=CH), 8.3-8.6 (m, 4H, Ar H), 7.8-8.2 (m, 9H, Ar H), 4.4 (s, 3H, OCH_3), 1.5 (s, 9H, $C(CH_3)_3$).

1-{4-[(4-Bromobenzylidene)amino]-phenyl}-5-(4-*tert.*butylphenyl)-3-methoxy-1-*H*-[1,2,4] triazole (33)

IR (cm^{-1}): 3055 (Ar. C-H), 2930 (aliph. C-H), 1612, 1571 (C=C, C=N), 1160, 1047 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.2 (s, 1H, N=CH), 8.3-8.6 (m, 4H, Ar H), 7.8-8.2 (m, 8H, Ar H), 4.4 (s, 3H, OCH_3), 1.5 (s, 9H, $C(CH_3)_3$).

5-(4-*tert.*Butylphenyl)-1-{4-[(4-chlorobenzylidene)amino]phenyl}-3-methoxy-1-*H*-[1,2,4] triazole (34)

IR (cm^{-1}): 3040 (Ar. C-H), 2930 (aliph. C-H), 1615, 1582 (C=C, C=N), 1184, 1081 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.2 (s, 1H, N=CH), 8.5 (d, *J* = 8.5 Hz, 2H, Ar H), 7.7-8.2 (m, 10H, Ar H), 4.4 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

FAB-MS m/z, (%): 445.44 $[M+1]^+$ (5%), 93.04 (100%).

5-(4-*tert.*Butylphenyl)-3-methoxy-1-{4-[(4-methoxybenzylidene)amino]-phenyl}-1-*H*-[1,2,4] triazole (35)

IR (cm^{-1}): 3060 (Ar. C-H), 2925 (aliph. C-H), 1615, 1581 (C=C,

C=N), 1160 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.1 (s, 1H, N=CH), 8.4-8.6 (m, 2H, Ar H), 7.6-8.0 (m, 6H, Ar H), 7.1-7.5 (m, 4H, Ar H), 4.4 (s, 3H, OCH_3), 4.2 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

5-(4-*tert.*Butylphenyl)-3-methoxy-1-{4-[(4-N,N-dimethylaminobenzyl-idene)-amino]phenyl}-1-*H*-[1,2,4] triazole (36)

IR (cm^{-1}): 3080 (Ar. C-H), 2925 (aliph. C-H), 1583 (C=N), 1161 (C-O), 815 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.1 (s, 1H, N=CH), 8.5 (d, *J* = 8.5 Hz, 2H, Ar H), 7.9-8.2 (m, 8H, Ar H), 7.5 (d, *J* = 8.5 Hz, 2H, Ar H), 4.4 (s, 3H, OCH_3), 3.3 (s, 6H, $N(CH_3)_2$), 1.4 (s, 9H, $C(CH_3)_3$).

FAB-MS m/z, (%): 453.38 $[M]^+$ (13%), 454.43 $[M+1]^+$ (80%), 455.21 $[M+2]^+$ (18%), 323.24 (100%).

5-(4-*tert.*Butylphenyl)-3-methoxy-1-{4-[(4-nitrobenzylidene)amino]-phenyl}-1-*H*-[1,2,4] triazole (37)

IR (cm^{-1}): 3075 (Ar. C-H), 2930 (aliph. C-H), 1616, 1589 (C=C, C=N), 1530 (NO_2, asym.), 1330 (NO_2, sym.), 1162, 1048 (C-O), 825 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.5 (s, 1H, N=CH), 8.7-9.1 (m, 4H, Ar H), 7.8-8.2 (m, 8H, Ar H), 4.3 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

5-(4-*tert.*Butylphenyl)-1-{4-[(4-hydroxybenzylidene)amino]phenyl}-3-methoxy-1-*H*-[1,2,4] triazole (38)

IR (cm^{-1}): 3395 (OH), 3070 (Ar. C-H), 2930 (aliph. C-H), 1601, 1562 (C=C, C=N), 1154 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 10.9 (s, 1H, OH), 9.2 (s, 1H, N=CH), 8.5 (d, *J* = 8.6 Hz, 2H, Ar H), 7.8-8.2 (m, 8H, Ar H), 7.5 (d, *J* = 8.6 Hz, 2H, Ar H),4.3 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

FAB-MS m/z, (%): 427.21 $[M+1]^+$ (18%), 323.09 (100%).

5-(4-*tert.*Butylphenyl)-1-{4-[(2-hydroxybenzylidene)amino]phenyl}-3-methoxy-1-*H*-[1,2,4] triazole (39)

IR (cm^{-1}): 3390 (OH), 3035 (Ar. C-H), 2930 (aliph. C-H), 1609 (C=N), 1181, 1045 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 12.7 (s, 1H, OH), 9.6 (s, 1H, N=CH), 7.9-8.4 (m, 10H, Ar H), 7.4-7.7 (m, 2H, Ar H), 4.3 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

5-(4-*tert.*Butylphenyl)-1-{4-[(4-carboxybenzylidene)amino]phenyl}-3-methoxy-1-*H*-[1,2,4] triazole (40)

IR (cm^{-1}): 3445 (OH), 3090 (Ar. C-H), 2935 (aliph. C-H), 1679 (C=O), 1613, 1570 (C=C, C=N), 1185 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 10.9 (s, 1H, COOH), 9.5 (s, 1H, N=CH), 8.4-8.9 (m, 4H, Ar H), 7.8-8.3 (m, 8H, Ar H), 4.3 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

FAB-MS m/z, (%): 455.42 $[M+1]^+$ (21%), 456.47 $[M+2]^+$ (7%), 323.30 (100%).

5-(4-*tert.*Butylphenyl)-1-{4-[(4-carboxymethyloxybenzylidene)-amino]phenyl}-3-methoxy-1-*H*-[1,2,4] triazole (41)

^{1}H-NMR (60 MHz, DMSO): 11.0 (s, 1H, COOH), 9.3 (s, 1H, N=CH), 8.4-8.6 (m, 2H, Ar H), 7.8-8.3 (m, 6H, Ar H), 7.5-7.7 (m, 2H, Ar H), 7.1-7.3 (m, 2H, Ar H), 5.2 (s, 2H, O-CH_2-), 4.3 (s, 3H, OCH_3), 1.4 (s, 9H, $C(CH_3)_3$).

1-{4-[(4-Bromobenzylidene)amino]-phenyl}-3-ethoxy-5-(2,5-diflouro-phenyl)-1-*H*-[1,2,4] triazole (42)

IR (cm^{-1}): 3080 (Ar. C-H), 2925 (aliph. C-H), 1579, 1540 (C=C, C=N), 1149 (C-O), 825 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.3 (s, 1H, N=CH), 8.3-8.6 (m, 4H, Ar H), 7.7-8.2 (m, 7H, Ar H), 4.6 (t, *J* = 7.3 Hz, 2H, -OCH_2CH_3), 1.5 (t, *J* = 7.3 Hz, 3H, -OCH_2CH_3).

FAB-MS m/z, (%): 483.28 $[M+1]^+$ (7.6%), 484.32 $[M+2]^+$ (3%), 317.23 (100%).

1-{4-[(4-Carboxybenzylidene)-amino]phenyl}-3-ethoxy-5-(2,5-diflourophenyl)-1-*H*-[1,2,4] triazole (43)

IR (cm^{-1}): 3440 (OH), 3085 (Ar. C-H), 2960 (aliph. C-H), 1683 (C=O), 1615, 1571 (C=C, C=N), 1179 (C-O), 835 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.3 (s, 1H, N=CH), 8.4-8.7 (m, 5H, Ar H), 7.6-8.1 (m, 6H, Ar H), 4.6 (t, *J* = 7.3 Hz, 2H, -OCH_2CH_3), 1.5 (t, *J* = 7.3 Hz, 3H, -OCH_2CH_3).

3-Ethoxy-5-(2,5-diflourophenyl)-1-{4-[(4-nitrobenzylidene)amino]-phenyl}-1-*H*-[1,2,4] triazole (44)

IR (cm^{-1}): 3065 (Ar. C-H), 2960 (aliph. C-H), 1619, 1570 (C=C, C=N), 1530 (NO_2, asym.), 1341 (NO_2, sym.), 1185 (C-O), 830 (*p*-disubstituted benzene).

^{1}H-NMR (60 MHz, DMSO): 9.6 (s, 1H, N=CH), 9.1 (d, *J* = 8.5 Hz, 2H, Ar H), 8.0-8.6 (m, 9H, Ar H), 7.6-8.1 (m, 6H, Ar H), 4.6 (t, *J* = 7.4 Hz, 2H, -OCH_2CH_3), 1.5 (t, *J* = 7.4 Hz, 3H, -OCH_2CH_3).

Antibacetrial Activity

The antibacterial activity of all the target compounds **17-44** was investigated *in vitro* at the department of microbiology and immunology, faculty of medicine, Assiut University. The title compounds were tested against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae* (clinical isolates obtained from Infection Control Unit, Assiut Univeristy Hospital, Faculty of Medicine, Assiut University) using agar cup diffusion method[24&25] for susceptibility screening, and two-fold dilution method[25] for MIC determination. Ampicillin was used as a reference antibiotic, and DMSO was used as a solvent control.

Agar cup diffusion method

38 Gm of Mueller-Hinton agar medium (MH) (Hi-Media, M 001) were added to 1 L of distilled water, heated to boiling to dissolve the

ingredients completely, and sterilized by autoclaving at 121°C for 30 minutes. High density inocula were made by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard unit with average turbidity 10^8 CFU mL[25]. The sterile petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50°C) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using sterile cork borer. 100 µL of the tested compounds (20 mg/mL in DMSO), the blank solvent, and ampicillin sodium (20 mg/mL in DMSO) were added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 h then the average diameters of the inhibition zones were measured in millimeters.

Minimum inhibitory concentration

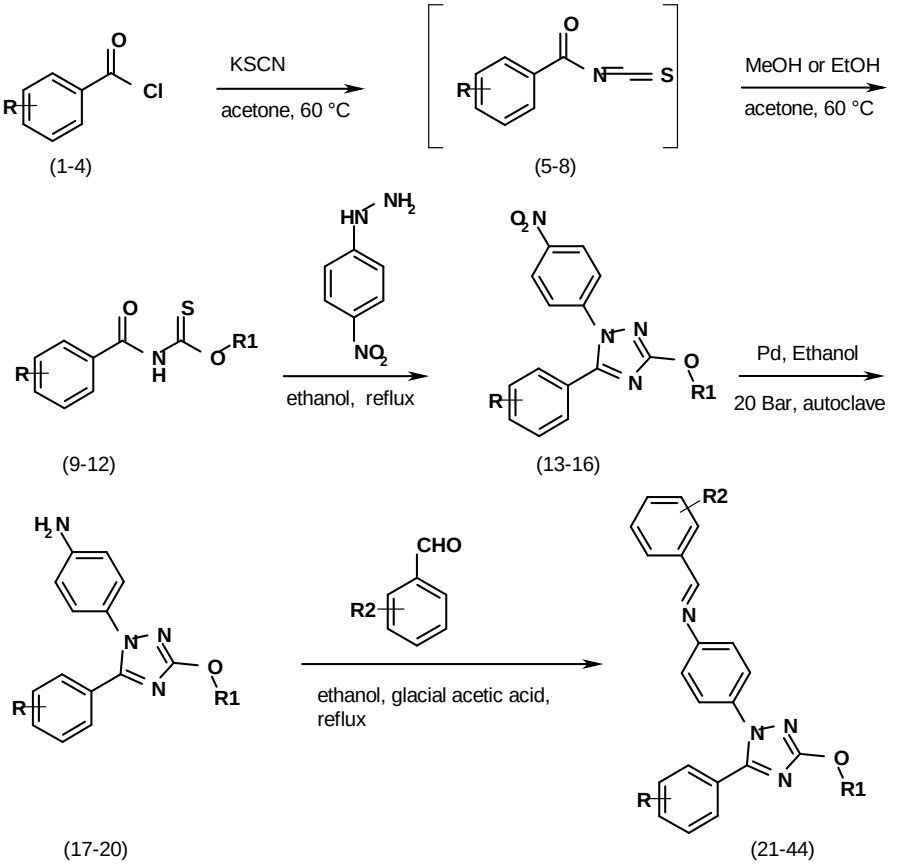
The MIC values were determined using two fold-dilution method[26] for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds, extrapolation of the resulting straight line to intersect with log concentration scale in the curve corresponded to log MIC, and MIC was obtained as antilog[27].

RESULTS AND DISCUSSION

Chemistry

Methyl (4-methoxybenzoyl)thiocarbamate **10** was prepared through the reaction of 4-methoxybenzoyl chloride **2** with potassium thiocyanate in acetone to give 4-methoxybenzoyl isothiocyanate intermediate **6,** followed by direct addition of methanol to the reaction mixture (Scheme 1). The structure of compound **10** was verified by ^{1}H-NMR, ^{13}C-NMR and EI-HRMS. The ^{1}H-NMR spectrum of this compound was characterized by the presence of amide NH at 9.13 ppm, two methoxy groups at 4.19 and 3.80 ppm and its ^{13}C-NMR showed C=O and C=S at 163.67 and 190.65 ppm respectively and the methoxy groups appeared at 59.52 and 55.60 ppm. EI-HRMS **10** showed a molecular ion at (*m/z*) 225.0460 (its calculated mass = 225.0453, see exp. part).

3-Methoxy-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-[1,2,4] triazole **14** was prepared in analogy to the reported procedure[28] by reacting methyl (4-methoxybenzoyl)thiocarbamate **10** with *p*-nitrophenylhydrazine in ethanol, the reaction was ended after H_2S evolution was ceased completely (Scheme 1). The ^{1}H-NMR and ^{13}C-NMR spectra revealed the disappearance of the amide NH and both C=O and C=S signals and appearance of new aromatic moiety which introduced to the triazole ring. Its EI-HRMS revealed (*m/z*) at 326.1014 $[M]^+$ and the calculated one



1,5 R = H, **2,6** R= 4-OCH_3, **3,7** R = 4-$C(CH_3)_3$, **4,8** R = 2,5-di(F), **9,13,17** R = H, R1 = CH_3, **10,14,18** R = 4-OCH_3, R1 = CH_3, **11,15,19** R = 4- $C(CH_3)_3$, R1 = CH_3, **12,16,20** R = 2,5-Di(F), R1 = CH_2CH_3.

Compd. No	R	R1	R2	Compd. No	R	R1	R2
21	H	CH_3	H	**33**	4-$C(CH_3)_3$	CH_3	4-Br
22	H	CH_3	4-Br	**34**	4-$C(CH_3)_3$	CH_3	4-Cl
23	H	CH_3	4-Cl	**35**	4-$C(CH_3)_3$	CH_3	4-OCH_3
24	H	CH_3	4-OCH_3	**36**	4-$C(CH_3)_3$	CH_3	4-$N(CH_3)_2$
25	H	CH_3	4-$N(CH_3)_2$	**37**	4-$C(CH_3)_3$	CH_3	NO_2
26	H	CH_3	NO_2	**38**	4-$C(CH_3)_3$	CH_3	4-OH
27	H	CH_3	4-OH	**39**	4-$C(CH_3)_3$	CH_3	2-OH
28	H	CH_3	4-COOH	**40**	4-$C(CH_3)_3$	CH_3	4-COOH
29	H	CH_3	4-O-CH_2COOH	**41**	4-$C(CH_3)_3$	CH_3	4-O-CH_2COOH
30	4-OCH_3	CH_3	4-Br	**42**	2,5-di(F)	CH_2CH_3	4-Br
31	4-OCH_3	CH_3	4-COOH	**43**	2,5-di(F)	CH_2CH_3	4-COOH
32	4-$C(CH_3)_3$	CH_3	H	**44**	2,5-di(F)	CH_2CH_3	4-NO_2

Scheme 1: Synthesis of 1,3,5-trisbstituted-*1H*-[*1,2,4*]-triazole derivatives (**21-44**).

is 326.1015 and this confirmed the structure of compound **14**.

1-(4-aminophenyl)-3-methoxy-5-(4-methoxyphenyl)-1-*H*-[1,2,4] triazole **18** was prepared by catalytic reduction of the nitro derivative **14** using Pd/C10% catalyst (Scheme 1). The ^{1}H-NMR of compound 18 showed broad signal at 5.49 ppm which is a strong evidence for the formed NH_2. Its structure was confirmed by ^{13}C-NMR and EI-HRMS which showed (*m/z*) at 296.1275 $[M]^+$ and the calculated one is 296.1273.

Schiff bases of 1,3,5-trisubstituted [1,2,4] triazole **21-44** were prepared by the reaction of triazole derivatives **17-20** with equimolar amounts of the appropriate (un)substituted benzaldehydes in ethanol in presence of few drops of glacial acetic acid (Scheme 1). Comparative study of the ^{1}H-NMR of aminotriazole derivatives **17, 18, 19** or **20** and their Schiff bases **21-44**, easily revealed the disappearance of the NH_2 group signal and appearance of N=CH signals at 9.1-9.6 ppm in addition to the introduced aromatic moiety. The structures of formed Schiff bases were also confirmed by elemental analyses (Table 1) and compounds **25-27, 29, 31, 34, 36,38, 40** and **42** were further confirmed using FAB-MS (exp. part).

Table 1: Yields, melting points and analytical data of the new compounds.

Compd. No.	Formula	MW	Elemental Analyses, Calc./Found			Yield [%]	Mp [°C]
			C	H	N		
10	$C_{10}H_{11}NO_3S$	225.26	HRMS*			91	Oil
14	$C_{16}H_{14}N_4O_4$	326.31	HRMS*			68	152-154
18	$C_{16}H_{16}N_4O_2$	296.32	HRMS*			71	204-206
21	$C_{22}H_{18}N_4O$	354.41	74.56 74.22	5.12 5.26	15.81 15.69	78	128-130
22	$C_{22}H_{17}BrN_4O$	433.31	60.98 60.62	3.95 3.53	12.93 12.85	88	139-141
23	$C_{22}H_{17}ClN_4O$	388.86	67.95 67.62	4.41 4.56	14.41 14.44	85	134-136
24	$C_{23}H_{20}N_4O_2$	384.44	71.86 71.90	5.24 5.39	14.57 14.39	86	118-120
25**	$C_{24}H_{23}N_5O.$ $0.5\ H_2O$	406.49	70.91 70.57	5.96 5.92	17.23 17.09	83	178-180
26**	$C_{22}H_{17}N_5O_3.$ $0.5\ H_2O$	408.42	64.69 64.74	4.45 4.62	17.15 17.15	80	210-212
27**	$C_{22}H_{18}N_4O_2.$ $0.5\ H_2O$	379.42	69.64 69.28	5.06 5.26	14.77 14.90	79	195-197

Table 1: Continued.

Compd. No.	Formula	MW	Elemental Analyses, Calc./Found			Yield [%]	Mp [°C]
28	$C_{23}H_{18}N_4O_3$	398.42	69.34 69.38	4.55 4.59	14.06 13.96	72	233-235
29**	$C_{24}H_{20}N_4O_4$. 0.5 H_2O	437.46	65.89 65.70	4.85 4.90	12.81 12.75	71	200-202
30	$C_{23}H_{19}BrN_4O_2$	463.34	59.62 59.65	4.13 4.36	12.09 12.18	68	154-156
31**	$C_{24}H_{20}N_4O_4$	428.45	67.28 66.81	4.71 4.92	13.08 12.89	67	220-222
32	$C_{26}H_{26}N_4O$	410.52	76.07 75.89	6.38 6.59	13.65 13.34	72	141-143
33	$C_{26}H_{25}BrN_4O$	489.42	63.81 63.52	5.15 5.35	11.45 11.27	81	179-181
34**	$C_{26}H_{25}ClN_4O$. 0.5 H_2O	453.97	68.78 69.12	5.78 5.75	12.34 12.20	70	180-182
35	$C_{27}H_{28}N_4O_2$	440.55	73.61 73.23	6.41 6.37	12.72 12.51	65	130-132
36**	$C_{28}H_{31}N_5O$. 0.5 H_2O	462.60	72.70 72.86	6.99 6.73	15.14 14.94	66	148-150
37	$C_{26}H_{25}N_5O_3$	455.52	68.56 67.90	5.53 5.48	15.37 15.26	72	208-210
38**	$C_{26}H_{26}N_4O_2$. 0.5 H_2O	435.53	71.70 72.03	6.26 6.36	12.86 12.89	74	220-222
39	$C_{26}H_{26}N_4O_2$	426.52	73.22 72.55	6.14 6.12	13.14 13.07	70	157-159
40**	$C_{27}H_{26}N_4O_3$. 0.5 H_2O	463.54	69.96 69.54	5.88 5.84	12.09 12.06	69	230-232
41	$C_{28}H_{28}N_4O_4$	484.56	69.41 69.11	5.82 5.98	11.56 11.13	62	210-212
42**	$C_{23}H_{17}BrF_2N_4O$	483.32	57.16 56.75	3.55 3.66	11.59 11.53	68	114-116
43	$C_{24}H_{18}F_2N_4O_3$. 0.5 H_2O	457.44	63.01 63.35	4.20 4.37	12.25 12.39	80	191-193
44	$C_{23}H_{17}F_2N_5O_3$	449.42	61.47 61.59	3.81 4.33	15.58 15.39	79	149-151

* Compounds **14, 18, 20** were confirmed by HRMS (see the experimental part).

** Compounds **25-27, 29, 31, 34, 36, 38, 40** and **42** were further confirmed by FAB-MS (see the experimental part).

Antibacterial activity

The newly synthesized compounds **17-43** were tested for their in vitro antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and Bacillus cereus as representatives of Gram-positive strains and *Escherichia coli* and *Klebsiella pneumoniae* as representatives of Gram-negative ones using ampicillin and nalidixic acid as reference drugs. The results revealed that most of the newly synthesized compounds exhibited promising antibacterial activity against all the test organisms (Table 2). Thus compounds **17-20** which were substituted by 4-aminophenyl at the 1-position of [1,2,4]triazoles showed antibacterial activity similar to nalidixic acid and higher than that of ampicillin against all tested strains. Among the synthesized Schiff bases, compound **40** was the most potent one against all strains. Its antibacterial potency was superior to that of ampicillin and to that of nalidixic acid against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus* and *Escherichia coli*.

Compounds **22, 25, 28, 31, 33, 36, 37**, and **43** exhibited pronounced activity against all strains.

On the other hand, screening results revealed that in some cases, conversion of the amino derivatives to Schiff bases led to loss of antibacterial activity for example compounds **23, 27, 34, 35, 38** and **39**.

Moreover, compounds **21, 24, 29, 32, 41, 42** and **44** did not exhibit enhanced activity compared to the parent amino derivatives.

Also, it has been observed that introduction of carboxyl group at 4-position to the 1-phenyl substituent regardless of the substituents of the 5-phenyl moiety produced higly active compounds **28, 31, 40** and **43**. Replacement of this carboxyl group by oxymethylcarboxyl ($O\text{-}CH_2\text{-}COOH$) decreased the activity.

In general anticipation of SAR can not be attained.

Table 2: Antibacterial activity of the tested compounds (expressed as the inhibition zone diameter and as MIC µM/mL).

Compd. No.	MRSA		*Bacillus cereus*		*Klebsiella pneumoniae*		*E.coli*	
	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml	Inhibition zone (mm)	MIC µM/ml
17	32	25	32	23	32	25	32	25
18	27	30	28	25	23	60	24	53
19	31	30	27	30	26	39.1	25	50
20	28	25	29	25	25	50	26	39.1
21	16		16		15		14	
22	31	20	23	65	16	130	16	135
23	-ve		-ve		-ve		-ve	
24	17		16		15		15	
25	30	20	20	70	17	125	17	125
26	17		20		-ve		-ve	
27	-ve		-ve		-ve		-ve	
28	28	30	25	50	27	31.6	18	79
29	19		18		20		20	
30	18		-ve		25		18	
31	32	20	21	66	16	132	16	135
32	15		17		16		15	
33]	29	25	21	60	18	79	18	79
34	-ve		-ve		19		-ve	
35	-ve		16		-ve		-ve	
36	27	30	22	60	20	70	20	70
37	27	30	28	30	26	40	17	125
38	17		-ve		-ve		-ve	
39	-ve		-ve		-ve		-ve	
40	33	20	30	25	31	30	33	20
41	19		22		21		26	
42	-ve		20		21		17	
43	29	30	28	31	25	42	17	127
44	-ve		-ve		22		17	
Ampicillin	20	69	22	60	23	50	20	70
Nalidixic acid	27	30	20	67	30	20	28	25

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