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SYNTHESIS, AND BIOLOGICAL EVALUATION OF NEWLY SUNTHESIZED TRIAZOLOTRIAZINES AND TRIAZOLOTRAIZINES DERIVATIVES

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ABSTRACT

Novel 8-(4-fluorophenyl)-6,6-diphenyl-3-(pyrimidin-4-yl) -5,6,7,8-tetrahydro -6l5-[1,2,4] triazolo [4,3-a][1,3,5]diazaphosphinin-6-ol**(5) ,**8-(4-fluorophenyl)-6-phenyl-3-(pyrimidin-4-yl)-7,8-dihydro [1,2,4]triazolo [4,3-b] [1,2,4] triazine **(6),** 8-(4-fluorophenyl)-6-methyl-3-(pyrimidin-4-yl)-[1,2,4] triazolo [4,3-b][1,2,4]triazin-7(8H)-one **(7)** and 8-(4-fluorophenyl)-3-(pyrimidin-4-yl)-[1,2,4]triazolo[4,3-b][1,2,4]triazine-6,7(5H,8H)-dione **(8)** and 7-(pyrimidin-4-yl)-3,4-dihydro-[1,2,4]triazolo[4,e][1,2,4,5,3]tetrazaphosphinine **(10),** 3-(pyrimidin -4-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine **(11),** 6-(2-chloro -6- fluorophenyl)-3-(pyrimidin-4-yl)-1,5,6,7,8,8a -hexahydro-[1,2,4] triazolo [4,3-b] [1,2,4,5]tetrazine **(12),** 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine-6(5H)-thione **(13)** and 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazin-6(5H)-one **(14)** have been synthesized via heterocyclization of 3-(pyridin-4yl)-4-amino-5-substituted amino-1,2,4-triazole (**3**) and 3-(pyridin-4yl)-4-amino-5-hydrazino-1,2,4-triazole (**9**) with α,β-bifunctional reagents such as chloromethyldiphenyl-phosphanoxide, phenacyl bromide, pyruvic acid, diethyl oxalate, triethylphosphite, triethyl orthoformate, fluorinated benzaldehydes, ethyl chloroformate and carbon disulfide in different experimental conditions. The molecular structures of the synthesized target molecules were elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data, in addition to the fine elements analysis C.H.N.% data. The antimicrobial and anti-inflammatory effects of the obtained derivatives were evaluated and compared with that of the standard Indomethacin, Nalidixic acid and Nystatin.

The 1,2,4-triazole systems containing 1,3-pyrimidine moiety in particular the N3-(4-fluorophenyl)-5-(pyrimidin-4-yl)-4H-1,2,4-triazole-3,4-diamine **(3)** was initially used as precursor in order to synthesize of new heterocycles of potent pharmacological uses and various biological activities, such as photo chemical probe agents[14,15] anti-inflammatory[16], antimicrobial[17], anti-fungal and anti-viral agents. [18-22] The 1,2,4-triazole systems containing 1,3-pyrimidine moiety have considerable attention due to a large interesting observations[12]. The literature survey showed that a few work have been done on the triazole, tetrazoles and tetrazene derivatives[23]. The titled heterocyclic compounds have been synthesized via hetercyclization of 4-amino-5-substituted amino/hydrazino-1,2,4-triazoles and antimicrobial and anti-inflammatory properties were evaluated with

1. Introduction

Triazoles and nitrogen heterocycles containing triazole moiety are class of organic compounds of a a large range of applications in organic and bio-organic synthesis[1] such as synthons for plant growth regulators[2], drugs synthesis[3], surfactants[4], agrochemicals [5] and complexation agents.[6,7] They are possessing significant activities in biological systems such as: anti-inflammatory [8,9], antimicrobial [10,11], anticancer [12]. and molluscicidal activity.[13]

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diphenylphosphanoxide (0.01mol) in THF (100ml) with TEA (0.5ml) was refluxed for 4h, and then cooled down to give solid product. The obtained solid was washed for several times, filtered off and crystallized to give **5** yield 86%, crystals from dioxane; m.p.300-303°C, analysis for $C_{25}H_{21}N_7PFO$ (485), Cald. C,61.85; H,4.36; N,20.20; F, 3.91; P, 3.38; Found C,61.89; H, 4.40; N,20.17; F, 3.88, P, 3.40; FT-IR (ν cm^{-1}): 3084(aromatic CH), 2922.68(aliphatic CH), 2650 (P-OH), 1610(C=N), 1433(deformation of CH_2), 1388,1344(N-N-C-N), 1105.66,1039,945(P-N), 851, 8.04(aryl CH) and 667.84cm^{-1} (C-F); ^{1}HNMR(DMSO-d_6) δppm: 3.15(d,1H,C**H**-P), 3.98-3.99(1H, d, CH-P), 7.1-7.45(m, 14H, ArH's), 7.8,8.2(Two d, 2H pyrimidine H's), 8.79,8.88(d, 1H of pyrimidine) and 11.0(br., 1H, NHP), 14.4 (s, br., 1H, OH-P).

Synthesis of 8-(4-fluorophenyl)-6-phenyl-3-(pyrimidin-4-yl)-7,8-dihydro-[1,2,4]triazolo[4,3-b][1,2,4]triazine (6) A mixture of **3** (0.01mol) and phenacyl bromide (0.01mol) in ethanolic KOH (5%, 50ml) was reflux for 2h, and cooled down .The reaction mixture was poured into ice-HCl mixture. The yield solid was filtered off, washed several times and recrystallized from dioxin- methanol mixture to give **6,** yield 88%, m.p.188-190°C analysis for $C_{20}H_{14}N_7F$ (373); Cald. C,64.68; H,3.80; N,26.40; F, 5.12 Found C,64.71; H,3.83; N,26.44; F, 5.15; FT-IR (ν cm^{-1}): 3099.20 and 2980 cm^{-1} (aromatic and aliphatic CH), 1605(C=N), 1429(deformation of CH_2), 930,832 (aryl CH) and 688 cm^{-1} (C-F).

Synthesis 8-(4-fluorophenyl)-6-methyl-3-(pyrimidin-4-yl)-[1,2,4]triazolo[4,3-b][1,2,4]triazin-7(8H)-one (7): A mixture of **3** (0.01mol) and sodium pyruvate (0.01mol, in H_2O 10ml)with aqueous NaOH (5%, 50ml) was refluxed for 2h, cooled down and then poured into ice-HCl mixture .The produced solid was filtered off, washed several times and recrystallized from EtOH to give **5,** yield 75%, m.p.308-310°C, analysis for $C_{15}H_{10}N_7FO$ of mol. wt. 323; cal.C,55.37; H,3.12; N,30.33; F, 5.88; found C,55.40; H,3.14; N,30.35; F, 5.90; FT- IR (ν cm^{-1}): 3090,2978 cm^{-1} (aromatic and aliphatic CH)1768(C=O), 1592(C=N), 1480.95 (deformation of CH_3), 1322(N-N-C-N), 1213(C-F), 836,816,762,729 (aromatic & pyrimidine CH), and 675 cm^{-1} (C-F)؛ MS (Int.%): 323(M^+,2.50), 295(44.5), 269(12.2),180(100), 155(1.10), 148(13.01), 105(20.1), 109(1.1), 79(58.9), 45 (27.74), 41(6.73).

Synthesis of 8-(4-fluorophenyl)-3-(pyrimidin-4-yl)-[1,2,4]triazolo[4,3-b][1,2,4]triazine-6,7(5H,8H)-

respect to that of Indomethacin, Nalidixic acid and Nystatin [2].

2. Experimental Section

Melting points were determined in an electrothermal Bibby Stuart Scientific melting point SMP (US). The IR spectra recorded (KBr discs) on a Perkin Elmer Spectrum RXI FT-IR systems No. 55529. ^{1}H/^{13}C-NMRwere determined for solution in detreated DMSO with a Bruker NMR Advance DPX 400 MH using TMS as an internal standard. Mass spectra were measured on a GCMS-Q 1000 Ex spectrometer. Electronic absorption spectra were recorded in DMF on Shimadzu UV and visible 3101 PC spectrophotometry. Microanalyses (CHNS elemental) and the anti-inflammatory and antimicrobial evaluation were carried out in Department of Pharmaceutical Microbiology, National Center for Radiation Research and Technology, Nasr City, Egypt. Compound **1** was prepared [17] according to the reported method by direct hydrazinolysis of 4-pyridyl-$CONHNHCS_2K$.

Synthesis of *N3-(4-fluorophenyl)-5-(pyrimidin-4-yl)-4H-1,2,4-triazole-3,4-diamine* (3): An equimolar mixture of **1** and 4-fluoroaniline in DMF-ethanol (1:1, 100ml) was refluxed for 4h, cooled for a while and poured into ice. was washed for several times, filtered off and crystallized to give **3**: yield 74% crystal from dioxane; m.p. 225-227°C, analysis for $C_{12}H_{10}N_7F$ (271); Cald. C,53.13; H,3.72; F, 7.00; N,31.11; Found C,53.16; H,3.77; F, 6.96; N,31.15). The UV spectrum gave [λ_{max} (Log ε)]: 374(0.85)nm; FT-IR (ν cm^{-1}): 3350, 3230 (NH_2, NH**),** 1601,1585(C=N), 830,780 (aromatic CH, pyrimidine CH) and 705 cm^{-1} (C-F); ^{1}HNMR(DMSO-d_6) δppm: 5.87(τ,2H,NH_2), 7.2,7.4(each τ,4H,aromatic protons), 7.82,8.041(d, 2H of pyrimidine), 8.77,8.81(d, 1H of pyrimi-dine) and at 14.22 ppm (NH of substituted amino);^{13}C NMR (DMSO-d_6) δppm: 158(C_1 of C-F), 115.13,114.99(C_2 & C_3 of aryl), 119.58,119.99 (C_4&C_5 of aryl), 132.99(C_6 of aryl), 147.36(C_3& C_5 of triazole), 129.75(C_2 of pyrimidine), 121.63(C_5 & C_6 of pyrimidine), 150.82,150.04 ppm (C_4 of pyrimidine); MS (Int.%) : 271(5.5), 254(5.21), 227(4.21), 208(37.71), 166(5.8), 104(4.85), 79(2.11), 45(100), 41(12.18).

Synthesis of 8-(4-fluorophenyl)-6,6-diphenyl-3-(pyrimidin-4-yl)-5,6,7,8-tetrahydro-6l5-[1,2,4] triazolo [4,3-a][1,3,5]diazaphosphinin-6-ol (5): A mixture of **3** (0.01mol) and chloromethyl-

C,41.61; H,3.01; N,55.44; IR (ν cm^{-1}): 3150, 3105(NH, NH of tetrazine & triazole), 1610, 1588(C=N), 1348(NCN), 850,820,730,710 and 690 cm^{-1} (pyrimidine CH)؛ MS (Int.%): 202(0.01%),194(100), 164(15.0),148(2.10), 106(22.8), 123(12.1), 79(28.1), 45(40.15), 41(12.12).

Synthesis of 6-(2-chloro-6-fluorophenyl)-3-(pyrimidin-4-yl)-1,5,6,7,8,8a-hexahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (12): A mixture of **9** (0.01mol) and 2-chloro-6-fluorobenzeldehyde (0.01mol) in ethanol (100ml) piperidine (0.5ml) was refluxed for 12h and then cooled down. The solid product was filtered off, washed several times and recrystallized from dioxan to give **12,** yield 72%; m.p.257-259ºC, analysis for $C_{13}H_{10}N_8FCl$ of mol.wt.332; cal.C,46.93; H,3.03; N,33.68; F, 5.71; Cl, 10.66; found C,46.98; H,3.05; N,33.70; F, 5.74; 10.70; UV [DMF, λ_{max} nm (Log ε)]: 267(1.11) and 261(1.01) nm;FT- IR (ν cm^{-1}): 3296,3158 and 3100 cm^{-1}(3NH), 3020(aromatic CH), 1602,1568 cm^{-1} (C=N), 13.9(N-N-C-N), 1216(C-F), 821,734 cm^{-1} (aromatic and pyrimidine CH), 685 cm^{-1} (C-Cl); ^{13}C nmr(DMSO-d_6) δ ppm: 147(C_3,C_5 of triazole), 133,122,211,151 and 150 (four carbons of pyridine), 136,134(C-F&CCl of aryl), 45(C_6 of –CH-tetrazine) and 129,128 ppm (four carbons of aryl).

Synthetic 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine-6(5H)-thione (13): A mixture of **9** (0.01mol), CS_2 (0.02mol) in DMF (50ml) was refluxed for 2h, cooled down and then poure onto ice. The resulted solid was filtered off washed several times, and and recrystallized from THF to give **13,** yield 84%; m.p.238-240ºC, analysis for $C_7H_6N_8S$(234); Cald.C,35.89; H,2.58; N,47.84; S,13.69; Found C,35.91; H,2.61; N,47.86; S,13.71. UV [λ_{max} (Log ε)]:316(1.5) nm; FT-IR (ν cm^{-1}): 3296,3158(NHNH), 2402,1812 ($\overset{-}{S}\text{----}\overset{+}{N}H_3$),1602(C=N), 1308(NCSN), 820 and 733 cm^{-1} (pyrimidine CH); ^{1}HNMR (DMSO-d_6) δ ppm: δ2.5(s, br., 1H, -NH- tetrazine), 5.8(s, br., 1H,SH), 8.031- 8.76(m, 2H of pyrimidine), 8.88-8.92(s, 1H of pyrimidine), 14.21 ppm(s, br., 1H, NH of tetrazine); ^{13}C NMR(DMSO-d_6) δppm: 169(C_1 of C=S),148(C_2, C_3 of 1,2,4-triazole), 134(C_2 of pyrimidin-4-yl), 122.121(C_4,C_5 of pyrimidine), 151(C_6 of pyrimidine); MS (Int.%): 234(1.00%), 194(100), 179(1.51),163(3.30), 123(4.20), 107(15.15),79(18.9), 45(58.48), 41(15.40).

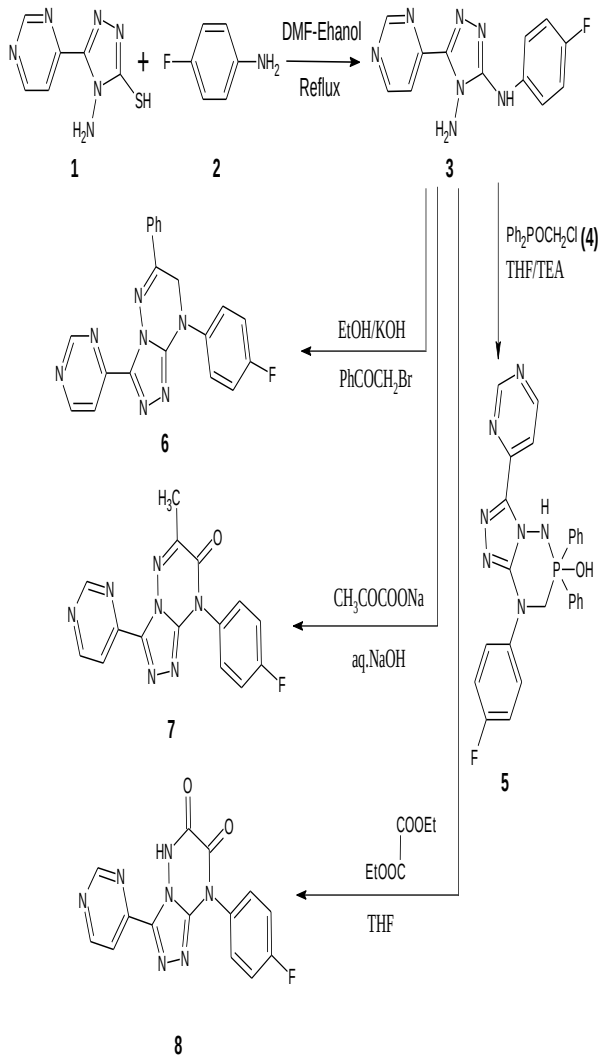
dione (8) : Equimolar mixture of **3** and diethyl oxalate in THF (100ml) was refluxed for 4h,and cooled down. The obtained solid was filtered off, washed several times and recrystallized from EtOH to give **8,** yield 79%; m.p.180-182ºC, analysis for $C_{14}H_8N_7FO_2$ (325); Cald. C,51.70; H,2.48; N,30.14; F, 5.84; Found C,51.79; H,2.51; N,30.14, F, 5.88; IR (ν cm^{-1}): 3250(NH), 3085(aromatic CH), 2699.50(C-OH), 1769, 1631.49(2C=O), 1592, 1547(C=N), 1212 (C-F), 836,818,762,729(aromatic and pyrimidine CH).

Synthesis of 3-Hydrazinyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-4-amine (9): A mixture of **1** (0.01 mol) and hydrazine hydrate (0.04 mol) in ethanol (100ml) was refluxed for 4h, cooled down and a 3 drops acetic acid. The produced solid was filtered off, washed several times and from EtOH to give **9,** yield 81%; m.p.160-162ºC, analysis for $C_6H_8N_8$ (192); Cald. C,37.50; H,4.20; N,58.31; Found C,37.52; H,4.21; N,58.33. UV [DMF, λ_{max} nm (Log ε)]: 311(2.2) nm; FT-IR (ν cm^{-1}): 3350, (NH_2), 3159(NH), 3080(aromatic CH), 1605(deformation NH_2), 1570(C=N), 1313(N-N-CN), and 822,735,707,687 cm^{-1} (3CH of pyrimidine); ^{1}HNMR (DMSO-d_6) δppm: 4.3(s, br., 2H,NH_2 of hydrazino), 5.85(s, br., 2H, NH_2 of 1,2,4-triazole), 7.82,7.83(d, 2H of pyrimidine), 8.85,8.81(d, 1H of pyrimidine), 14.2 ppm (s, br., 1H,NH_2 of hydrazino).

Synthesis of 7-(pyrimidin-4-yl)-3,4-dihydro-[1,2,4]triazolo[4,3-e][1,2,4,5,3]tetrazaphosphinine (10): Equimolar of **9** and triethylphosphite in THF (100ml) and TEA (0.5ml) was refluxed for 4h, and cooled down. The obtained solid was filtered off, washed several times and recrystallized from THF to give **10** yield 74%; m.p.246-247ºC, analysis for $C_6H_5N_8P$ (220); Cald. C,32.74; H,2.29;N,50.90; P, 14.07; Found C,32.79; H,2.31; N,50.87; P, 14.05; FT-IR (ν cm^{-1}): 3100-2950 (s, br., NH-NH), 1651(P-NH), 1588(C=N), 1358(NCN), 100,1050,950 (P-N), 832,785,710 and 689 cm^{-1} (pyridine CH)؛ MS (Int.%): 219(221,M^+,1.50), 193(100), 162(41), 147(1.10), 78(18.10), 44(100), 40(30.08).

Synthesis of 3-(pyrimidin-4-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (11): A mixture of **9** (0.01mol) and triethylorthoformate (0.012mol) in THF (100ml) was refluxed for 4h, cooled. The produced solid was filtered, washed several times to give **11** yield 70% crystal from dioxan; m.p.230-231ºC, analysis for $C_7H_6N_8$ of mol.wt.202; cal.C,41.58; H,2.99; N,55.42; found

Structures of compounds **6-8** were confirmed by both the elemental analysis and spectral measurements. FT-IR spectrum of **6** showed a characteristic stretching vibration absorption bands at ν (cm^{-1}) 2980 and bending vibration bands1429 (cm^{-1}) of CH_2, while, that of **7** showed ν (cm^{-1}) at 2978 and bending vibration bands at 1480 (cm^{-1}) of CH_3, and additional bands at 1768 (cm^{-1}) of (C=O) stretching vibration, and compound **8** recorded ν (cm^{-1}) at 3250 (NH), 1769, 1631 (2C=O), 850-730 (C-F) and 1620 (C=N). The reported results of high potency for the synthesied1,2,4-triazolo systems as antimicrobial and anti-inflammatory agents were good motivation to conduct new creative of more derivatives of the same systems. Thus, it has been found that refluxing compound 1 with hydrazine hydrate in ethanol gave 3-(pyridin-4yl)-4-amino-5-hydrazino-1,2,4-triazole (**9**) the start for all the next syntheses.



Scheme 1: Synthsis of 1,2,4-triazolo derivatives **5-8**:

Synthetic of 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazin-6(5H)-one (14): A mixture of **9** (0.01mol) and ethyl chloroformate (0.012mol) with THF (100ml) and TEA(0.5ml, added drowse) was refluxed for 2h and then cooled down. Then obtained solid was filtered, washed several times and recrystallized from EtOH to give **14,** yield 71% crystal; m.p.170-171ºC, analysis for $C_7H_6N_8O$ of mol.wt.218; cal.C,38.54; H,2.77; N,51.36; found C,38.56; H,2.79; N,51.39. UV [λ_{max} (Log ε)]: 326(1.31)nm;FT- IR (ν cm^{-1}): 3149(b, NH-NH), 1624(-CONH), 1582(C=N), 1358(N-N-C-N), and 787,739 cm^{-1} (pyrimidine CH); MS (Int.%): 218(5.55), 194(100), 163(2.30), 105(8.55), 79(14.95), 45(100), 41(28.28)

3. Results and Discussion

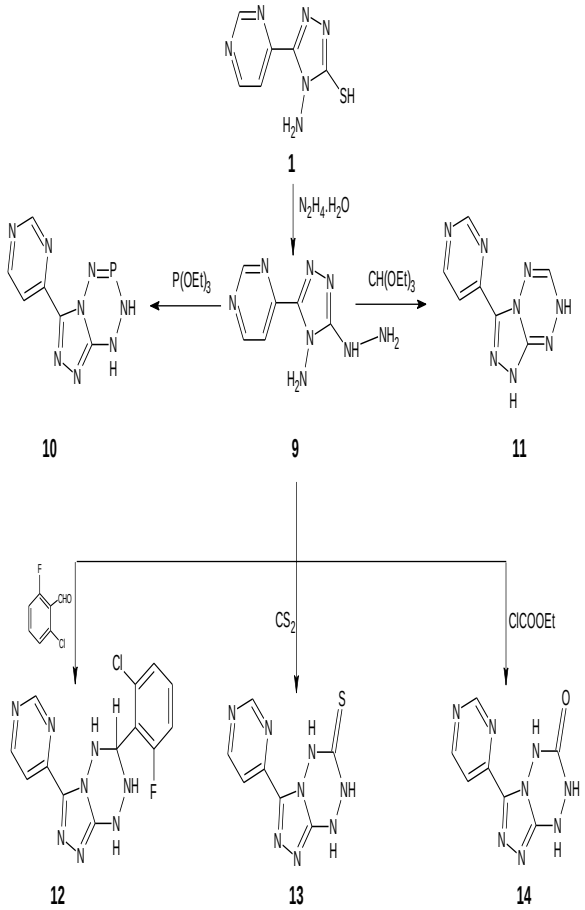
Nucleophilic displacement of mercapto group in 4-amino-5-mercapto-3-(pyrimidin-4-yl)-1,2,4-triazole (**1**) by primary aromatic amines such as p-fluoroaniline **(2)** in DMF-Ethanol mixture (1:1) under reflux yielded 4-amino-5-substituted amino-1,2,4-triazole derivative **3**. Structure of **3** was confirmed mainly from disappearance of the significant stretching frequency absorption of the mercapto functional group at 2600-2500cm^{-1} in the FT-IR spectrum. The ^{1}HNMR spectrum which showed the N-H proton of at δ 9.6 ppm with NH_2 proton of the amino triazole at 5.8 ppm is significant supporting evidence. The UV spectrum of compound 2 exhibit λ_{max} at 374 nm while that of compound **1** at 317 nm, which is another evidence for the formation of compound **3** formation, (Scheme 1).

Synthesis of 8-(4-fluorophenyl)-6,6-diphenyl-3-(pyrimidin-4-yl)-5,6,7,8-tetrahydro-6l5-[1,2,4] triazolo [4,3-a][1,3,5]diazaphosphinin-6-ol (**5**) (Scheme1) was accomplished by stirring of compound **3** with diphenyl(choloromethyl)phosphanoxide **(4)** in warm THF via ring closure reaction. Structure of **5** was deduced from appearance of characteristic stretching vibration bands of P-OH, P-N and NH functional groups at 2650, 1105, 3227, cm^{-1} in the FT- IR spectrum. ^{1}HNMR spectrum gives supported evidence to the structure which exhibited a resonated signal at δ 3.2 (br., 2H, C**H**$_2$-P), 11 ppm (**b**, 1H, NH-P), 14.4(s, br., 1H, P-OH). The target 1,2,4-triazolo derivatives (**5-8**) have been obtained from heterocyclizaion of compound **3** with phenacyl bromide in ethanolic KOH, pyruvic acid in NaOH and diethyl oxalate in THF / DMF[24] under reflux conditions (Scheme 1).

321, 734 cm^{-1} of substituted pyridine aromatic moieties. MS of **10** recorded M^+ at 219 (221, M+1) and 193 (100). UV spectral data of both the compounds **3** and **12** explain that the heterocyclization would inhibits the electronic transition and this cause the hypsochromic effect "shift to the shorter wavelength, thus, λ_{max} of **3** was 311 nm while that of **12** was 267nm. Finally, 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine-6(5H)-thione (**13**) and 3-(pyrimidin-4-yl)-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazin-6(5H)-one **(14)** were synthesized from refluxing 5-hydrazine-4-amino-1,2,4-triazole (**9**) with triethylortho-formate (TEA/THF) and /or with CS_2 (in DMF) (Scheme 2). Formation of compound 14 may be concluded to be firstly by synthesized via esterification of 9 and secondly by elimination of one mole of ethanol, while formation of **13** via addition to S=C=S followed by elimination of H_2S [25]. Structures of **13** and **14** were deduced from their elemental and spectral analysis thus the FT-IR spectrum of **14** showed the characteristic stretching vibration absorption bands ν (cm^{-1}) at 3249 (NH), 1624(CONH), while compound **13** showed at ν (cm^{-1}) 3269 (NH), 2402 (SH) and 1634 (CONH), in addition to ν (cm^{-1}) 1602, 1308, which is related to C=N, NCSN. ^{1}HNMR spectrum of **13** showed signals at δ 2.5(**s**, br., 1H, NH of tetrazine), 14.21 ppm (**s**, 1H, NH of tetrazine), and δ 5.8 of SH proton.

Additional evidence for the structure of compound **13** was obtained from ^{13}C NMR which showed signals at δ 169(C_1-C=S), 148(C_2, C_3-triazole), 134(C_4-pyrimidine) and 122,121, 151,150 ppm of pyrimidine carbons. Mass Fragmentation of compound of both compounds **13** and **14** exhibited M^+ at 233(1.00) and at 217(5.55) respectively**,** with a parent peak at 193 for both of them which confirm their structure [Schemes 3&4].

A hydrazino group is more nucleophilic and more basic than the amino group in the compound 9 and this enhances the cyclization firstly from hydrazine center followed by amino center. The 7-(pyrimidin-4-yl)-3,4-dihydro-[1,2,4]triazolo[4,3-e][1,2,4,5,3] tetrazaphosphinine(**10**) was obtained from refluxing compound **9** with triethyl phosphate in THF, while treatment of **9** with triethylorthoformate under these conditions, produced 3-(pyrimidin-4-yl)-1,7-dihydro-[1,2,4]triazolo[4,3-b][1,2,4,5] tetrazine (**11**). Also, cycloaddition reaction of **9** with aromatic aldehyde in boiling ethanol with 3 drops of piperidine, furnished 6-(2-chloro-6-fluorophenyl)-3-(pyrimidin-4-yl)-1,5,6,7,8,8a-hexahydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine **12**. Structures **10-12** (Scheme 2) were elucidated by considering the data of FT- IR spectroscopy which showed the characteristic stretching vibration absorption bands at ν (cm^{-1}) 3100-2950 cm^{-1} of (**1**) (NH, SH) and ν (cm^{-1}) 3269, 3158 and 3100 cm^{-1} (HN) of **10**, **11** and **12** and additional bands at ν (cm^{-1}) 1568, 1602cm^{-1} (P=N, C=N), 613 (C-F).



Scheme 2: Synthesis of triazolotetrazine derivatives **10-14**:

In view of the observations, the aim of this work is the synthesis of some new fused 1,2,4-triazole with 1,2,4-triazine-1,2,4,5-tetrazine nucleus containing a various functional groups in an attempt to enhance that antimicrobial and anti-inflammatory effects, in hope to design a semi-drugs.

A. Antimicrobial activity: The synthesized compounds have been evaluated for their antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosae* as (-ve) bacteria *Staphylococcus aureus*as (+ve) bacteria, in addition, *Candida albicans*as fungi using applied standard method [12] DMF used as solvent and Nalidexic acid and Nystatin were used as reference drugs. The inhibition zone an MIC for the screening was reported in Table 1.

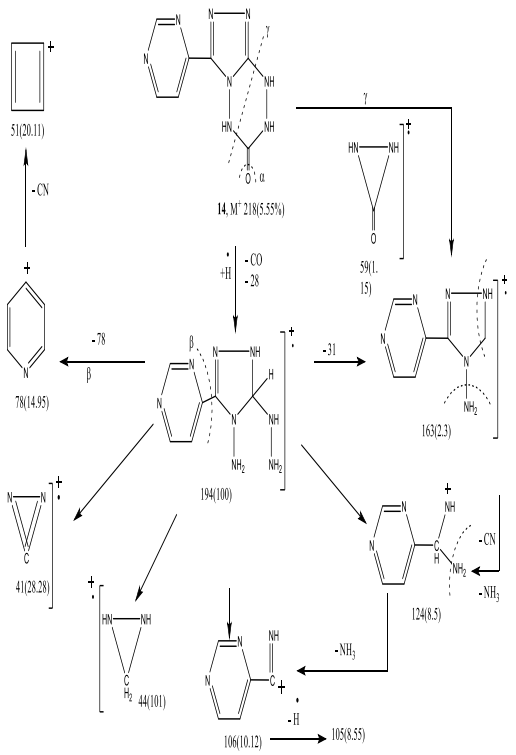
B. Anti-inflammatory activity: The anti-inflammatory activity of the selected compounds were evaluated by carrageenan- induced paw edema method [13,14] with reference drug indomethacin as a suspension in 24 tweens 80, using method of Winks et al [13,14]. The percentage of biological inhibition is calculated as following:

$$\%\ inhibtion = \frac{wt.of\ paw\ edema\ of\ control - wt\ of\ paw\ edema\ of\ related.}{wt.of\ paw\ edema\ of\ control.} * 100$$

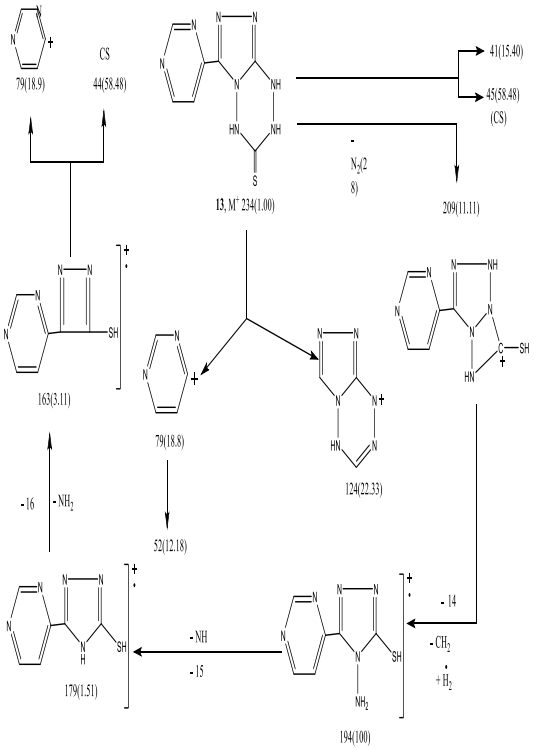
The observed results of antimicrobial activity and inflammatory are depicted in table 1 and table 2.

Table 1: The Antimicrobial Activity Screening of Some New Synthesized Compounds

MIC	inhibition Zone (mm)*									Fungi		
	E. coli			*P.aeruginosa*			*S. aureus*			*C. albicans*		
Conc.	100	50	25	100	50	25	100	50	25	100	50	25
Compd. No.												
5	26	19	-	31	20	-	17	-	-	14	-	-
8	24	13	-	18	-	-	19	-	-	13	-	-
10	22	11	-	16	-	-	20	-	-	13	-	-
11	24	11	-	11	-	-	19	-	-	12	-	-
9	22	11		22	11	-	20	-	-	12	-	-



Scheme 3: Mass Fragmentation of compound **13**



Scheme 4: Mass Fragmentation of compound **14**

Biocidal evaluation:

Recently, much attention has been focused on bridgehead nitrogen heterocycles especially containing 1,2,4-triazole, 1,2,4-triazine and 1,2,4,5-tetrazine derivatives because of their applications in medicine.

iii- Compounds **5, 8** and **13** showed a higher activities against bacteria *E.coli* at concentration 50 µg/disc.

iv- Quantitative structure activity relationships studies referred to the higher activity of the compounds 5, 8 and 11 is mainly due to these compounds containing fluorine, chlorine and phosphorus elements within the structure of 1,2,4-triazine and 1,2,4,5-tetrazine. On the other hand, the experimental results in Table 2 indicated that:-

i- Compounds **10** and **12** carrying phenyl and pyridine groups containing both the fluorine and chlorine are active against anti-inflammation, in compare the stander antibiotic used (Indomethacin).

ii-Both the compounds **10** and **12** containing mainly triazole and tetrazine rings with presence of both phosphorus and fluorine elements, incorporated with pyridyl moiety.

iii- A higher activity of the titled newly synthesized heterocyclic compounds is in agreement with the recent studies of phosphorus–fluorine bearing a heterocyclic nitrogen systems[15,16].

12	22	11	-	23	11	-	11	-	-	12	-	-
14	22	16	-	21	11	-	15	-	-	12	-	-
13	22	13	-	21	11	-	16	-	-	11	-	-
Nystatin	-	-	-	20	15	10	31	17	26	-	-	-
Nalidexic acid	-	-	-	-	-		-	-	-	-	40	-

*Concentration: 100,50 and 25 µg /disc .
Highly active : IZ=≥ 12
Moderately active: IZ= 9-12
Slightly active : IZ= 6-9; Not sensitive : IZ< 6 mm
MIC: minimum Inhibitory Concentration at 50 and or 25 µg /disc.

Table 2: The anti-inflammatory activity of some new synthesis compounds

Compd. No.	Dose Mg /Kg	Paw edema(g)* ± S.E.	% inhibition
9	25	0.55± 0.05	84.84
	5	0.58 ± 0.06	53.03
10	25	0.32 ± 0.05	68.16
	5	0.40 ± 0.06	51.51
12	25	0.36 ±0.03	51.51
	5	0.40 ±0.06	39.39
13	25	0.58 ±0.05	45.45
	5	0.66 ± 0.05	39.39
Control.	0	0.66 ± 0.05	0
Indomethacin	5	.032 ±0.02	51.51

S.E.: Standard Control.
*: significant difference from the control value at p< 0.05

Conclusions

The experimental results in **Table 1**, gave significant indications which can be explained as follows :

i- All tested compounds exhibited a higher activity against fungi used *C.abicans* in compare with Nystatin at 100 µg/disc concentration.

ii- All tested compounds recorded a higher to moderate activities against bacteria used *E.coli* ,*P. aeruginosa* and *S.aureus* in compare with Nistatin and Nalidexic acid at concentrations10 and 50 µg/discs.

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