A Convenient Synthesis of Some New Pyrazolo-Pyrimidine Derivatives with Potential Biological Activity

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Abstract: A series of pyrazolopyrimidines and their derivatives were synthesized using 2-[(N-methyl indolyl) methyl]-6-hydroxy-pyrazolo[3,4-*d*]pyrimidine as a starting material. Nineteen new heterocyclic containing a pyrimidine ring were thus prepared. The biological screening showed that many of these compounds have good antibacterial and antifungal activities. The structure assignments of the new compounds based on chemical and spectroscopic evidence. The detailed synthesis, spectroscopic data and biological properties are reported. [Nature and Science 2010;8(9):86-91]. (ISSN: 1545-0740).

Key words: Imidazole, pyrazole, pyrimidine, triazole, triazine, antibacterial activity.

1. Introduction

In the course of the program directed towards the synthesis of fused nitrogen heterocyclic compound and as an extension of efforts directed towards the development of convenient synthetic approaches for the synthesis of fused pyrazolo[3,4-*d*]pyrimidine derivatives with an expected broad spectrum of biological activity. Studies have shown that pyrazolopyrimidines and its derivatives exhibit various biological activities such as antipyretic and analgesic activities [6]. In this article, we have synthesized some triazolo[4',3':2,3]pyrazolo[5,4-*d*]pyridine and 6-[pyrazolo-1-yl]pyrazolo[3,4-*d*]pyrimidine derivatives for study their utility as pharmacological agents.

2 – Experimental

Melting points were obtained on a Graffin apparatus and are uncorrected. Microanalysis was carried out at the Microanalytical Center, Cairo University and Organic Microanalysis Section, National Research Center. IR spectra were recorded on a Shimadzue 435 spectrometer, using KBr discs. ^{1}H-NMR spectro were performed on a Jeol NMR FXQ-200 MHZ spectrometer and EM 39090 MH2 NMR spectrometer, using TMS as internal standard. EIMS were recorded on GCMS-QP 1000EX, Mass spectrometer. Progress of the reactions was monitored by TLC using preconated aluminium sheets silica gel MERCK 60 F_{254} and was visualized by UV lamp.

2-[(N-Methylindolyl)methyl]-4H-6-chloro-7-methyl-pyrazolo[3,4-d]pyrimidine (C16H14N5Cl) (2).

A mixture of 2.29g 1 (1mmol), $POCl_3$ (20 cm^3) was refluxed 4h. The solid product was collected and crystallized from ethanol to give 0.21g 2 (66%); MP 241°C; IR (film):ő= 3252 (NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 2.18 (s, CH_3), 2.50 (s,N-CH_3), 3.18 (s,CH_2), 7.21-7.46 (m, Ar-H) and 10.63 (s, NH, exchangeable with D_2O)ppm; MS(EL, 70ev): m/z (%) = 311 (M^+, 21) and 235 (100, base peak).

Synthesis of triazolo-pyrazolo-pyrimidine derivatives 3 and 4

A mixture of 0.31g 2 (1mmol), hydrazine reagents, namely, acyl cyanohydrazine and benzoyl hydrazine (1mmol) in 30 cm3 butanol was refluxed 5h. The solid product was collected and crystallized to give 0.23g 3 (65%) and 0.24g 4 (62%).

2-[(N-Methylindodyl)methyl]-4H-5-cyanomethyl-8-methyl-1,2,4-triazolo[4',3':2,3] pyr-azolo[5,4-d]pyrimidine (C19H16N8) (3).

MP 265°C (BuOH); IR (film): ő = 3245 (NH) and 2218 (CN) cm-1; 1H-NMR (DMSO-d6): δ = 2.13 (s, CH3), 2.54 (s, N-CH3), 3.22 (s, CH2), 3.71 (s, CH2), 7.12-7.75 (m, Ar-H) and 10.62 (s, NH, exchangeable with D2O)ppm; MS (EL, 70ev): m/z (%) = 356 (M+, 100, base peak).

2-[(N-Methylindolyl)methyl]-4H-8-methyl-5-phenyl-1,2,4-triazolo[4',3':2,3]pyrazolo [5,4-d]pyrimidine (C23H19N7) (4).

MP 215°C (EtOH); IR (film): ő = 3253 (NH) cm-1; 1H-NMR (DMSO-d6): δ = 1.93 (s, CH3), 2.25 (s, N-CH3), 3.23 (3, CH2), 7.21-7.85 (m, Ar-H) and 11.12 (s, NH, exchangeable with D2O)ppm; MS (El,

70eV): m/z(%) = 393 (M+, 21) and at 290 (100, base peak).

2-[(N-Methylindolyl)methyl]-5-amino-4H-8-methyl-1,2,4-triazolo[4',3':2,3]pyrazolo [5,4-d]pyrimidine (C17H16N8) (5).

A mixture of 0.31g 2 (1m mol), 0.09g thiosemicarbazide (1m mol), in 20 cm3 acetic acid was refluxed 5hr. The reaction mixture was cooled, poured into water, the solid product was filtered off, and crystallized from ethanol to give 0.18g 5 (56%), MP>300°C; IR (film): ŭ = 3315 – 3261 (NH, NH2) cm-1; 1H-NHR (DMSO-d6): δ = 1.97 (s, CH3), 2.24 (s, N-CH3), 3.37 (s, CH2), 6.18 (s, NH, exchangeable with D2O), 7.21-7.54 (m, Ar-H) and 11.31 (s, NH2, exchangeable with D2O) ppm; MS (El, 70eV): m/z(%) = 332 (M+, 40) and at 235 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-1,2,3,4-tetrazolo[4',3':2,3]pyrazolo[5,4-d]pyri-midine (C16H14N8) (6).

A mixture of 0.31g 2 (1m mol), 0.19g sod. azide (5m mol) in 30 cm3 acetic acid was refluxed 3h. The solid product was collected and crystallized from acetic acid to give 0.22g 6 (69%); MP 180°C; IR (film): ŭ = 3240 (NH) cm-1; 1H-NH R (DMSO-d6): δ = 2.11 (s, CH3), 2.63 (s, N-CH3), 3.51 (s, CH2), 7.14-7.68 (m, Ar-H) and at 10.89 (s, NH, exchangeable with D2O) ppm; MS (El, 70eV): m/z (%) = 318 (M+, 14) and at 262 (100, base peak).

Synthesis of amino derivatives of pyrazolo pyrimidine 7 and 8.

A mixture of 0.31g 2 (1m mol) and amino reagents, namely, anilineand hydrazine hydrate (1m mol) in 30 cm3 acetic acid was refluxed 4h. The reaction mixture was cooled, poured into water, the solid product was filtered off, and crystallized to give 0.26g 7 (71%) and 0.21g 8 (68%).

2-[(N-Methylindolyl)methyl]-6-anilino-4H-7-methylpyrazolo[3,4-d]pyrimidine (C22H20N6) (7).

MP 241°C (MeOH); IR(film): ŭ = 3320-3265 (2NH) cm-1; 1H-NMR (DMSO-d6): δ = 2.11 (s, CH3), 2.42 (s, NCH3), 3.63 (s, CH2), 6.23 (s, NH, exchangeable with D2O), 6.92-7.46 (m, Ar-H) and 10.28 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 368 (M+, 14) and at 275 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-6-hydrazino-7-methylpyrazolo[3,4-d]pyrimidine (C16H17N7) (8).

MP 266°C (BuOH); IR(film): ŭ = 3332-3246 (2NH, NH2); cm-1; 1H-NMR (DMSO-d6): δ = 1.89 (s, CH3), 2.14 (s, NCH3), 3.51 (s, CH2), 6.93-7.42 (m, Ar-H) and 9.43 (s, NH, exchangeable with D2O), 10.25 (s, NH, exchangeable with D2O) and 11.31 (s, NH2 exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 307 (M+, 100, base peak).

Synthesis of fused rings 9 and 10 by reaction with amino acids.

A mixture of 0.31g 2 (1m mole) and amino acids, namely, glycine and anthranilic acid (1m mol) in 30 cm3 ethanol was heated under reflux 4h. The solid separated was refluxed with AC2O for 3h. The solid product obtained after cooling was collected and crystallized to give 0.18g 9 (54%) and 0.20 g 10 (51%).

2-[(N-Methylindolyl)methyl]-4,6-dihydro-5-oxo-8-methyl-imidazo[3',2':2,3]pyrazolo[5, 4-d]pyrimidine (C18H16N6O) (9).

MP> 300°C (AcOH); IR(film): ŭ = 3310(NH) and 1705 (C=O) cm-1; 1H-NMR (DMSO-d6): δ = 2.14 (s, CH3), 2.38 (s, NCH3), 3.24 (s, CH2), 3.91, (s, CH2), 7.16-7.35 (m, Ar-H) and 11.26 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 332 (M+, 34) and at 237 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-5-oxo-11-methyl-benzo[d]pyrimidino[3',2':2,3]pyraz-olo[5,4-d]pyrimidine (C23H18N6O) (10).

MP 290°C (EtOH); IR(film): ŭ = 3245(NH) and 1690 (C=O) cm-1; 1H-NMR (DMSO-d6): δ = 1.83 (s, CH3), 2.27 (s, NCH3), 3.64 (s, CH2), 6.45, (s, NH exchangeable with D2O), and 7.24-7.61 (m, Ar-H)ppm; MS (El, 70eV): m/z(%) = 394 (M+, 18) and at 250 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-5,8-dimethyl-1,2,4-triazolo[4',3':2,3] pyrazolo [5,4-d]pyrimidine (C18H17N7) (11).

A solution of 0.31g 8 (1m mol) in acetic anhydride (20 ml) was refluxed for 2h. After cooling, the solid product was collected and crystallized from ethanol to give 0.20g 11 (32%), MP 254°C; IR (film): ŭ = 3229 (NH) cm-1; 1H-NMR (DMSO-d6): δ= 2.11 (s, CH3), 2.43 (s, NCH3), 2.98 (s, CH3), 2.57 (s, CH2), 6.93-7.38 (m, Ar-H) 11.41 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z (%) = 331, (M+, 23) and at 250 (100, base pea1k).

Synthesis of pyrazolo derivatives of pyrazolo pyrimidine 12-14

A mixture of 0.30g 8 (1m mol) and active methylene reagents, namely, acetyl acetone, ethyl acetoacetate and ethyl cyanoacetate in 30 cm3 ethanol was refluxed 5h. The solid product was collected and crystallized to give 0.25g 12 (68%), 0.23g 13 (63%) and 0.26g 14 (71%).

2-[(N-Methylindolyl)methyl]-4H-6-[3,5-dimethyl-pyrazolo-1-yl]pyra-zolo[3,4d]pyrimi-dine (C21H21N7) (12).

MP> 270°C (EtOH); IR(film): ὐ = 3247(NH) cm-1; 1H-NMR (DMSO-d6): δ = 2.15 (s, CH3), 2.36 (s, 2CH3), 2.55 (s, NCH2), 3.68, (s, CH2), 7.13-7.45 (m, Ar-H) and 10.84 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 371 (M+, 16) and at 312 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-6-[3-hydroxy-5-methyl-pyrazolo-1-yl] pyrazolo [3,4-d]pyrimidine (C20H19N7O) (13).

MP 300°C (MeOH); IR(film): ὐ = 3430-3265 (OH, NH) cm-1; 1H-NMR (DMSO-d6): δ = 1.95 (s, CH3), 2.24 (s, NCH3), 2.73 (s, CH3), 3.62, (s, CH2), 6.53 (s, NH, exchangeable with D2O),6.96-7.54 (m, Ar-H) and 13.14 (s, OH, exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 373 (M+, 24) and at 235 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-6-[3-amino-1H-5-oxo-pyrazolo-1-yl]pyrazolo[3,4-d] pyrimidine (C19H18N8O) (14).

MP > 300°C (EtOH); IR(film): ὐ = 3318-3225 (2NH, NH2) and 1693 (C=O) cm-1; 1H-NMR (DMSO-d6): δ = 2.26 (s, CH3), 2.45 (s, NCH3), 3.62 (s, CH2), 6.48 (s, NH, exchangeable with D2O), 7.15-7.68 (m, Ar-H) and 10.21 (s, CH, exchangeable with D2O) and 11.46 (s, NH2, exchangeable with D2O)ppm; MS (El, 70eV): m/z(%) = 374 (M+, 100, base peak).

2-[(N-Methlyindolyl)methyl]-4,7-dihydro-5,6-dioxo-9-ethyl[1,2,4]triazino[3',4':2,3] pyrazolo[5,4-d]pyrimidine (C18H15N7O2) (15).

A mixture of 0.30g 8 (1m mol), 0.15g diethyloxalate (1m mol) in 30 cm3 ethanol was refluxed 7h. The solid product was collected and crystallized from ethanol to give 0.21g 15 (58%); MP 240°C; IR (film): ὐ = 3284-3215 (2NH), 1685 (C=O) and 1704 (C=O) cm-1; 1H-NH R(DMSO-d6): δ = 1.87 (s, CH3), 2.25 (s, N-CH3), 3.52 (s, CH2), 7.23-7.51 (m, Ar-H), 9.68 (s, NH, exchangeable with D2O) and 10.37 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z (%) = 361 (M+, 41) and at 217 (100, base peak).

2-[(N-Methylindolyl)methyl]-4,6-dihydro-5-oxo-8-methyl[1,2,4]triazolo[3',4':2,3]pyraz-olo[5,4-d]pyrimidine (C17H15N7O) (16).

A mixture of 0.30g 8 (1m mol), 0.10g ethylochloroformate (1m mol) in 30 cm3 pyridine was refluxed 10h. Then, poured on dilute HCl. The solid product obtained was crystallized from ethanol to give 0.21g 16 (66%); MP 210°C; IR (film): ὐ = 3326-3252 (2NH), and 1695 (C=O) cm-1; 1H-NH R(DMSO-d6): δ = 2.14 (s, CH3), 2.63 (s, N-CH3), 3.52 (s, CH2), 6.28 (s, NH, exchangeable with D2O),7.13-7.52 (m, Ar-H), and 10.81 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z (%) = 333 (M+, 32) and at 235 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-6-cinnamoylhydrazino-7-methyl-pyrazolo[3,4-d]pyri-midine (C25H23N7O) (17).

A mixture of 0.30g 8 (1m mol), and 5 cm3 cinnamoyl chloride was heated on water bath for 5h. The solid product obtained was filtered, washed with benzene and crystallized from ethanol to give 0.32g 17 (73%); MP 195°C; IR (film): ὐ = 3290-3175 (2NH), and 1691 (C=O) cm-1; MS (El, 70eV): m/z (%) = 437 (M+, 15) and at 276 (100, base peak).

2-[(N-Methylindolyl)methyl]-4H-5-cinnamoylhydrazino-8-methyl[1,2,4]triazolo[4',3':2 , 3]pyrazolo[5,4-d]pyrimidine (C25H21N7) (18).

A mixture of 0.43g 17 (1m mol), dry xylene 5 cm3 and POCl3 5 cm3 was refluxed for 8h. The cooled reaction mixture was diluted with pet. ether and the residue was dissolved in water, neutralized with ammonia solution and the precipitated solid was filtered off and crystallized from ethanol to give 0.24g 18 (59%); MP 241°C; IR (film): ὐ = 3214 (NH) cm-1; 1H-NMR (DMSO-d6): δ = 2.23 (s, CH3), 2.45 (s, NCH3), 3.92 (s, CH2), 5.82 (d, CH=), 6.04 (d, CH=), 7.12-7.49 (m, Ar-H) and 10.23 (s, NH, exchangeable with D2O)ppm; MS (El, 70eV): m/z (%) = 419 (M+, 100, base peak).

2-[(N-Methylindolyl)methyl]-4H-7-methyl-6-cinnamoyl-thiosemic-arbazidopyrazolo [3, 4-d]pyrimidine (C26H24N8OS) (19).

A mixture of 0.30g 8 (1m mol), and 0.18 cinnamoyl isothiocyanate (1m mol) in 20 cm3 ethanol was refluxed for 2h. The solid product was collected and crystallized from ethanol to give 0.31g 19 (64%); MP 286°C; IR (film): ὐ = 3310-3230 (4NH), 1708 (C=O) and 1340 (C=O) cm-1; MS (El, 70eV): m/z (%) = 496 (M+, 34) and at 276 (100, base peak).

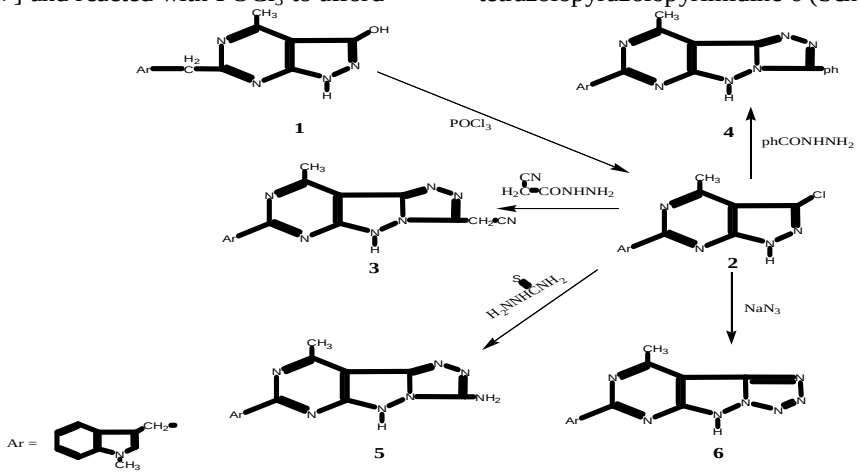
2-[(N-Methylindolyl)methyl]-4H-8-methyl-cinnamoylamino-[1,2,4]triazolo[4',3':2,3] pyrazolo[5,4-d]pyrimidine (C26H22N8O) (20).

A mixture of 0.50g 19 (1m mol), 0.22g dicyclohexylcarbazidiimide (DCC) (1m mol) in toluene 10 cm3 was refluxed for 5h. After cooling, the solid product was collected and crystallized from toluene to give 0.25g 20 (54%); MP 237°C; IR (film): ὐ = 3310-3256 (2NH) and 1678 (C=O) cm-1; 1H-NMR (DMSO-d6): δ = 2.11 (s, CH3), 2.26 (s,

NCH3), 3.67 (s, CH2), 5.41 (d, CH=), 5.83 (d, CH=), 6.41 (s, NH, exchangeable with D2O), 7.12-7.58 (m, Ar-H) and 11.23 (s, NH, exchangeable D2O)ppm; MS (El, 70eV): m/z (%) = 462 (M+, 19) and at 144 (100, base peak).

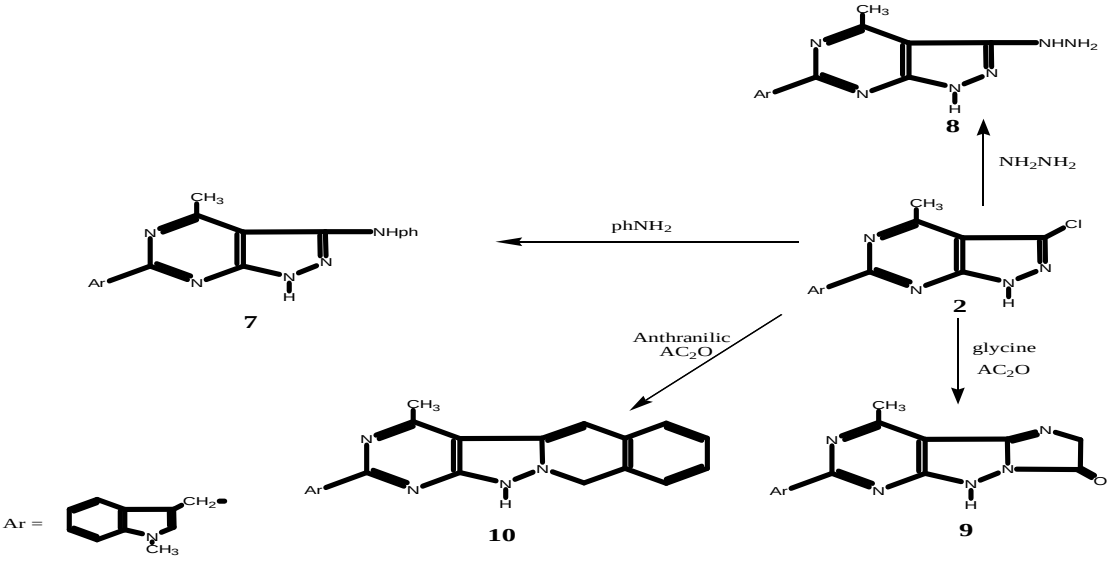
3. Result and Discussion

The starting material 1 was prepared according to reported method [7] and reacted with $POCl_3$ to afford compound 2. The reaction of compound 2 with hydrazine derivatives, namely, acylcyanohydrazine and/or benzoyl hydrazine in refluxing butanol afforded the corresponding triazolopyrazolopyrimidine derivatives 3 and 4 respectively. Compound 2 was reacted with thiosemicarbazide to afford 5. Also, the reaction of compound 2 with sodium azide afforded tetrazolopyrazolopyrimidine 6 (Scheme 1).



(Scheme 1)

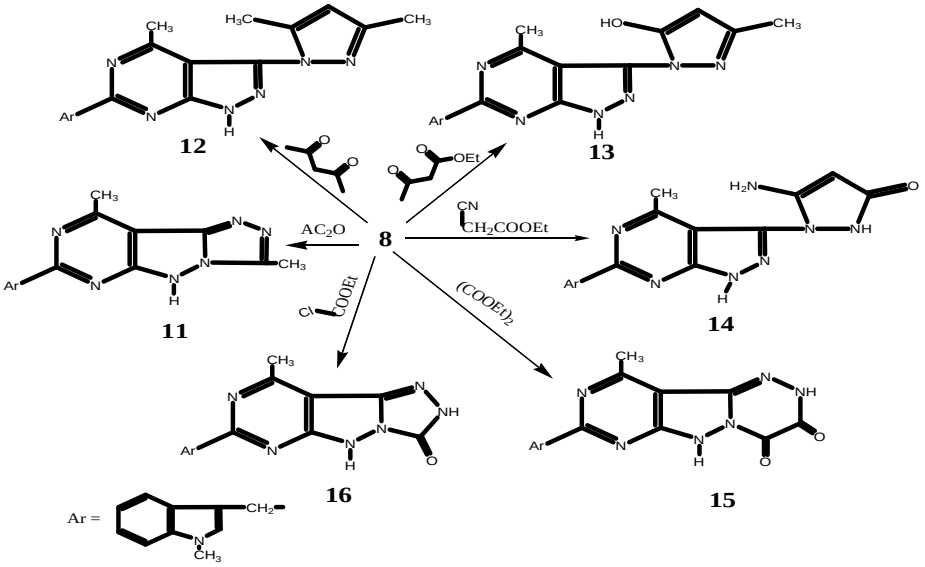
The reaction of compound 2 with amino derivatives, namely, aniline or hydrazine respectively afforded aniline and hydrazine derivatives 7 and 8 respectively. Reaction of 2 with amino acid, glycine or anthranilic acid, then refluxing with AC_2O to yield imidazolo pyrazolopyrimidine 9 and 10 respectively.



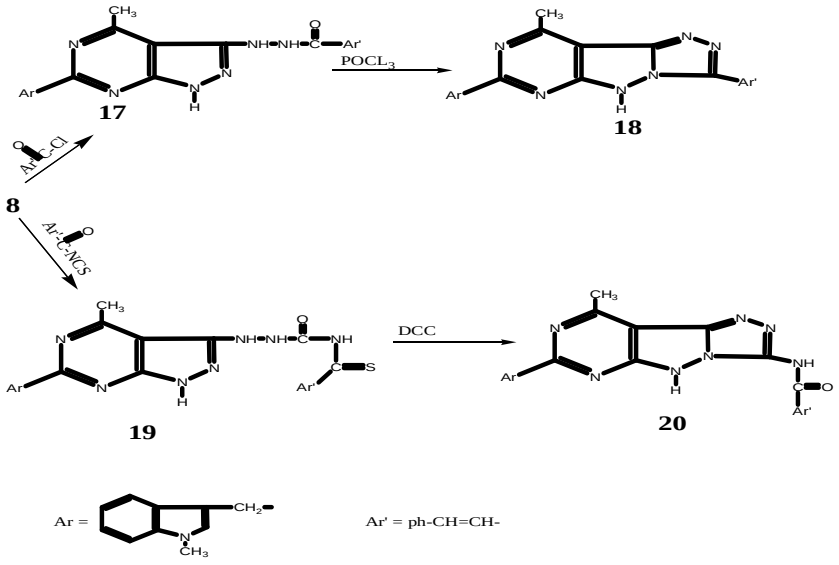
(Scheme 2)

On the other hand, reaction of compound 8 with acetic anhydride afforded triazolopyrazolopyrimidine 11 (Scheme 2). The reaction of compound 8 with active methylene reagents namely, acetyl acetone, ethyl acetoacetate or ethyl cyanoacetate in refluxing afforded the corresponding pyrazolo derivatives of pyrazolo pyrimidine 12-14 respectively. Reaction of compound 8 with diethyl oxalate afforded triazino derivative 15. Also, reaction of 8 with ethyl chloroformate afforded triazolo derivative 16 (Scheme 3).

Compound 17 was obtained by the nucleophilic attack of hydrazine derivative 8 to cinnanoyl chloride, the cyclization of 7 was achieved by refluxing in POCl3 and dry xylene affording triazolopyrazolopyrimidine 18. Also, compound 19 thiosenicarbazide derivative was obtained by refluxing of compound 8 and cinnamoyl isothiocyanate in ethanol. The cyclization of 19 was achieved by refluxing with DCC in ethanol affording triazolopyrazolopyrimidine 20 (Scheme 4).



(Scheme 3)



(Scheme 4)

Biological Activity

Most of the synthesized compounds **3,5,8,9,13,14,15** and **18** were screened in vitro for their antimicrobial activities against four species of bacteria (*Bacillus cereur, E. coli, Staphylococcus aureus, and Serratia marcescens*) and species of fungi (*Aspergillus flavus, Aspergillus niger, Candida albicans, Geotrichum candidum, Scopulariopsis breuicaulis, and Trichophyton rubrum*) using the disc diffusion method [8,9]. Chloramphenicol (5%) and terbinafine (5%) were used as a standard, respectively. Samples were dissolved in dimethyl formamide to a concentration of 5%, and filter paper discs (whatman no. 3.5 mm in diameter) were impregnated with the solutions. The discs were placed on the surface of solidified nutrient agar dishes seeded by the test bacteria or Czapek's Dox agar dishes seeded by the test fungi. The inhibition zones were measured in millimeters by the end of the incubation period (24h at 37°C for bacteria and 28°C for fungi).

Antibacterial Activity:

The results indicated that all of the screened compounds were active against all the tested bacterial species. Compound 5 was very active but compound 18 was weak active (Table 1).

Antifungal Activity

The results indicated that most of the screened compounds were active against all the tested fungal species. Compound 9 was more active but compound 13 was inactive (Table 2).

Table 1: Antibacterial activity of some synthesized compounds: inhibition zones in mm

Comp. No.	1	2	3	4
3	+	++	++	-
5	++	+++	++	+
8	+	+	+	-
9	-	-	+	+
13	+	-	+	-
14	++	+	-	-
15	+	-	+	+
18	-	+	+	-

1. *Bacillus cereus*
2. *E. coli*
3. *Staphylococcus aureus*
4. *Serratia marcescens*

+++ very active ++ moderate active
+ weak active - inactive

Table 2: Antifungal activity of some synthesized compounds

Comp. No.	1	2	3	4	5	6
3	+	+	++	+	+	+
5	-	+	-	-	+	+
8	-	-	+	-	+	-
9	+	++	+	++	+	+
13	-	-	-	-	-	-
14	-	+	+	-	-	+
15	+	+	+	+	-	-
18	+	-	-	++	+	+

1. Aspergillus flavus 2.Aspergillus niger
3. candida albicans 4.Geotrichum candidum
5. Scopulariopsis breuicaulis
6. 6.Trichophyton rubrum

+++ very active ++ moderate active +weak activ- inactive

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