World Journal of Chemistry 4 (1): 66-72, 2009
ISSN 1817-3128

Synthesized and Antimicrobial Evaluations of Some New Pyrazolo[4,3-*c*]Pyridazines Using 4-(6-Methyl-3-oxo-2,3-dihydro-pyrazolo[4,3-c]pyridazin-5-yl)Benzonitriles As Synthon

[1]*Ahmed A. Fayed,* [1]*Eman M. Flefel,* [2]*Nermien, M. Sabry and* [3]*Hanaa M. Hosni*

[1]Department of Photochemistry, National Research Center, Dokki, Cairo, Egypt
[2]Applied Organic Chemistry, National Research Center, Dokki, Cairo, Egypt
[3]Department of Pesticide Chemistry, National Research Centre, Dokki, Cairo, Egypt

Abstract: A series of new heterocyclic compounds 2-15 were synthesized from 3-oxo-1-(4-cyanophenyl)-4*H*-7-methyl-pyrazolo[4,3-*c*]-pyridazine (1) as starting. The structure assignment of the new compounds is based on chemical and spectroscopic evidence. The biological screening showed that many of these obtained compounds have good antimicrobial activities comparable to Streptomycin®, Erythromycin® and Fusidic acid as reference drugs.

Key words: Pyrazolopyridazine, Triazolopyrazolopyridazine, Triazinopyrazolopyridazine, Antimicrobial activities

INTRODUCTION

In previous work we reported that certain substituted pyridines, pyrimidines and their chiral macrocyclic derivatives have antidepressant, antimicrobial, anticancer, analgesic and anticonvulsant activities [1-6]. The chemistry of pyrazole and fused heterocyclic pyrazole derivatives have been considered to be an interesting class of heterocycles owing to their synthetic and effective biological activity [7-12]. Pyrazolopyridazine derivatives has received considerable attention owing to their variety of biological activities, especially as inhibitors of PDE5 extracted from human platelets [13], HIV-1 reverse transcriptase [14], human ERK2 [15], cyclin-dependent Kinases [16]. In view of these observations and as continuation of our previous work on heterocyclic chemistry, we have synthesized some new heterocyclic compounds for evaluation as antimicrobial activities in comparison to Streptomycin®, Erythromycin® and Fusidic acid as active drugs.

MATERIALS AND METHODS

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data were obtained from the micro analytical unit, Cairo University, Cairo, Egypt. The results were in favorable agreements with the calculated values. The IR spectra (Kbr,ν, cm^{-1}) were recorded on a Perkin-Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in δ (ppm) downfield from tetramethylsilane as an internal standard. The mass spectra were performed using VG 2AB-3F spectrometer (70 ev). All reactions were followed by TLC (Silica gel, aluminum sheets 60 F_{254}, Merck). The physical and spectral data are given in Tables 1 and 2.

3-Chloro-1-(4-cyanophenyl)-7-methyl-pyrazolo[4,3-c]pyridazine (2): A mixture of 3-oxo-1-(4-cyanophenyl)-4*H*-7-methyl-pyrazolo[4,3-*c*]pyridazine (1) (0.25 g, 0.001 mol) and $POCl_3$ (30 ml) was refluxed for 3 h. After cooling, the reaction mixture was poured into ice-water. The obtained solid was collected by filtration and crystallized from ethanol to give 2 as brown powder.

1-(4-Cyanophenyl) -4H-5-oxo-7- methyl-imidazo [2`,3`:5,1]pyrazolo[4,3-c]-pyridazine (3) and 1-(4-Cyanophenyl)-8-oxo-11-methyl-benzo[2`,3`:3,2]-pyrimidino[2`,3`:5,1]-pyrazolo[4,3- c]pyridazine (4): A solution of 2 (0.27 g, 0.001 mol) and glycine or anthranilic acid (0.001 mol) in n-butanol (30 ml) was refluxed for 3 h. The reaction mixture was evaporated under reduced pressure, the residue was refluxed in acetic anhydride (20 ml) for 2 h. After cooling, the obtained solid was filtered off, dried and crystallized from the proper solvent to give compound 3.

Corresponding Author: Dr. Hanaa M. Hosni, Pesticide Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

Table 1: Physical properties of the new synthesized compounds

Comp. No.	M.P. (°C) Solvent of cryst.	Yield (%)	Mol. formula (M. wt)	Analysis Calcd (Found) C	H	N	S
2	198-200 (EtOH)	72	$C_{13}H_8ClN_5$ (269.69)	57.90 57.98	2.99 2.95	25.97 25.95	
3	176-178 (AcOH);	58	$C_{15}H_{10}N_6O$ (290.28)	62.07 62.00	3.47 3.52	28.95 29.00	
4	232-234 (BuOH)	51	$C_{20}H_{12}N_6O$ (352.35)	68.18 68.11	3.43 3.47	23.85 23.88	
5	221-222 (EtOH)	63	$C_{16}H_{10}N_8$ (314.31)	61.14 61.20	3.21 3.17	35.65 35.61	
6	276-277 (Pet. ether)	68	$C_{19}H_{13}N_5S$ (343.41)	66.45 66.39	3.82 3.78	20.39 20.35	9.34 9.30
7	254-256 (BuOH)	74	$C_{13}H_{11}N_7$ (265.28)	58.86 58.95	4.18 4.10	36.96 37.01	
8	179-180 (EtOH)	71	$C_{13}H_8N_8$ (276.26)	56.52 56.44	2.92 2.99	40.56 40.52	
9	296-298 (DMF/EtOH)	68	$C_{18}H_{15}N_7$ (329.36)	65.64 65.72	4.59 4.55	29.77 29.72	
10	.> 300 (EtOH)	61	$C_{17}H_{13}N_7O$ (331.34)	61.63 61.58	3.95 4.01	29.59 29.55	
11	294-295 (MeOH)	62	$C_{16}H_{12}N_8O$ (332.32)	57.83 57.91	3.64 3.58	33.72 33.75	
12	267-268 (EtOH)	52	$C_{16}H_{13}N_9$ (331.34)	58.00 58.09	3.95 3.89	38.05 38.10	
13	210-212 (EtOH)	71	$C_{14}H_9N_7O$ (291.27)	57.73 57.82	3.11 3.02	33.66 33.72	
14	.> 300 (EtOH)	58	$C_{20}H_{14}N_8$ (366.38)	65.57 65.50	3.85 3.92	30.58 30.52	
15	286-288 (EtOH)	58	$C_{15}H_9N_7O_2$ (319.28)	56.43 56.50	2.84 2.79	30.71 30.66	

Table 2: Spectral data of newly synthesized compounds

Comp. No.	Spectral data IR (ν,cm^{-1})	MS (m/z, %)	^{1}H NMR (DMSO-d_6, δ)
2	2218 (CN)	269 (M^+, 21), 167 (100)	2.31 (s, 3H, CH_3), 7.32-7.86 (m, 5H, Ar-H + pyridazine H)
3	2220 (CN), 1685(C=O)	290 (M^+, 32), 120 (100)	2.42 (s, 3H, CH_3), 4.23 (s, 2H, CH_2), 6.92-7.32 (m, 4H, Ar-H + pyridazine H)
4	2232 (CN), 1696(C=O)	352 (M^+, 42), 208 (100)	2.19 (s, 3H, CH_3), 7.14-7.48 (m, 9H, Ar-H + pyridazine H)
5	2218, 2224 (2CN)	314 (M^+, 100)	1.82 (s, 3H, CH_3), 3.51 (s, 2H, CH_2), 7.31-7.68 (m, 5H, Ar-H + pyridazine H)
6	2195 (CN)	343 (M^+, 21), 234 (100)	2.12 (s, 3H, CH_3), 6.85-7.62 (m, 10H, Ar-H + pyridazine H)
7	3345-3251 (NH, NH_2), 2227 (CN)	265 (M^+, 41), 194 (100)	2.31 (s, 3H, CH_3), 6.34 (s, 2H, NH_2 exchangeable with D_2O), 6.92-7.30 (m, 5H, Ar-H + pyridazine H), 11.23 (s, H, NH exchangeable with D_2O)
8	2227 (CN), 1620(C=N)	276 (M^+, 43), 208 (100)	2.63 (s, 3H, CH_3), 7.41-7.82 (m, 5H, Ar-H + pyridazine H)
9	2214 (CN)	329 (M^+, 53), 234 (100)	2.24 (s, 3H, CH_3), 2.91 (s, 6H, $2CH_3$), 6.91-7.43 (m, 5H, Ar-H + pyridazine H + pyrazole H)
10	2210 (CN), 1690(C=O)	331 (M^+, 100)	2.15 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 3.42 (s, 2H, CH_2), 7.14-7.68 (m, 5H, Ar-H + pyridazine H)
11	3260 (NH, NH_2), 2218 (CN), 1680 (C=O)	332 (M^+, 13), 234 (100)	1.96 (s, 3H, CH_3), 5.98 (s, 2H, NH_2 exchangeable with D_2O), 6.84-7.32 (m, 6H, Ar-H + pyridazine H + pyrazole H), 11.21 (s, H, NH exchangeable with D_2O)
12	3310-3215 (NH_2), 2221 (CN)	331 (M^+, 24), 234 (100)	2.61(s,3H,CH_3), 6.31(s,4H, $2NH_2$ exchangeable with D_2O), 7.15-7.34 (m, 6H, Ar-H+pyridazine H+pyrazole H)
13	3240 (NH), 2228 (CN), 1683(C=O)	291 (M^+, 12), 194 (100)	2.73 (s, 3H, CH_3), 7.24-7.59 (m, 5H, Ar-H + pyridazine H), 11.41 (s, 1H, NH exchangeable with D_2O)
14	3280 (NH), 2215 (CN)	366 (M^+, 53), 274 (100)	1.96 (s, 3H, CH_3), 6.51 (s, 1H, NH exchangeable with D_2O), 7.13-7.45 (m, 10H, Ar-H + pyridazine H)
15	3274 (NH), 2210 (CN), 1680,1704 (2C=O)	319 (M^+, 12), 189 (100)	2.52 (s, 3H, CH_3), 6.85-7.32 (m, 5H, Ar-H + pyridazine H), 11.20 (s, 1H, NH exchangeable with D_2O)

1-(4-Cyanophenyl)-5-cyanomethyl-7-methyl-triazolo[3`,4`:3,2]pyrazolo-[4,3-c]pyridazine (5): A mixture of 2 (0.27 g, 0.001 mol) and cyano acetyl hydrazine (0.1 g, ~0.001 mol) in ethanol (30 ml) was refluxed for 8 h. The obtained precipitate was filtered off, dried and crystallized from ethanol to give 5 as brown powder.

1-(4-Cyanophenyl)-3-phenylmercapto-7-methyl-pyrazolo[4,3-c]pyridazine (6): A mixture of 2 (0.27 g, 0.001 mol) and thiophenol (0.11 g, 0.001 mol) in acetic acid (30 ml) was refluxed for 5 h. After cooling, the mixture was poured into water, the solid product was collected by filtration and crystallized from pet. ether (60:80) to give 6 as yellow powder.

1-(4-Cyanophenyl)-3-hydrazino-7-methyl-pyrazolo[4,3-c]pyridazine (7): A mixture of 2 (0.27 g, 0.001 mol) and hydrazine hydrate (0.4 g, 0.001 mol) in ethanol (30 ml) was refluxed for 6 h. The reaction mixture was evaporated under reduced pressure, the residue was triturated with diethyl ether, the obtained solid was filtered off, dried and crystallized from n-butanol to give 7 as green powder.

1-(4-Cyanophenyl)-8-methyl-tetrazolo [4`,5`:2,3] pyrazolo[4,3-c]pyridazine (8): A mixture of 2 (0.001 mol) and sodium azide (0.3 g, 0.005 mol) in acetic acid (30 ml) was refluxed for 4 h. The obtained solid was collected by filtration, dried and crystallized from ethanol to give 8 as brown powder.

Synthesis of 1-(4-Cyanophenyl)-3-(3,5-dimethylpyrazol-2-yl)-7-methyl-pyrazolo[4,3-c]pyridazine (9), 1-(4-Cyanophenyl)-3-(5-methyl-4H-3-oxo-pyrazol-2-yl)-7-methyl-pyrazolo[4,3-c]pyridazine (10), 1-(4-Cyanophenyl)-3-(3-amino-1H-5-oxo-pyrazol-2-yl)-7-methyl-pyrazolo[4,3-c]pyridazine (11) and 1-(4-Cyanophenyl)-3-(3,5-diamino-pyrazol-2-yl)-7-methylpyrazolo[4,3-c]pyridazine (12): General procedure: A mixture of 7 (0.27 g, 0.001 mol) and active methylene reagents, namely, acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate or malononitrile (0.001 mol) in ethanol (30 ml) was refluxed for 3 h. The reaction mixture was concentrated under reduced pressure, the obtained solid product was filtered off, dried and crystallized from the proper solvent to give 9-12, respectively.

1-(4-Cyanophenyl)-4H-5-oxo-8-methyl-1,2,4-triazolo[3`,4`:3,2]-pyrazolo-[4,3-c]pyridazine (13): A mixture of 7 (0.27 g, 0.001 mol) and ethyl chloroformate (0.11 g, 0.001 mol) in pyridine (30 ml) was refluxed for 10 h. After cooling, the reaction mixture was poured into diluted hydrochloric acid. The obtained solid was collected by filtration, washed with water, dried and crystallized from ethanol to give 13 as yellow powder.

1-(4-Cyanophenyl)-5-anilino-8-methyl-1,2,4-triazolo[3`,4`:3,2]pyrazolo-[4,3-c]pyridazine (14): A mixture of compound 7 (0.27 g, 0.001 mol), phenyl isothiocyanate (0.14 g, 0.001 mol) and sodium hydroxide (~0.1 g, 0.002 mol) in methanol (30 ml) was refluxed for 4 h. The reaction mixture was poured into water, the obtained precipitate was filtered off, washed with water, dried and crystallized from ethanol to give 14 as reddish brown powder.

1-(4-Cyanophenyl)-5,6-dioxo-4H-9-methyl-1,2,4-triazino[3`,4`:3,2]pyrazolo[4,3-c]pyridazine (15): A mixture of 7 (0.27 g, 0.001 mol) and diethyl oxalate (0.15 g, 0.001 mol) in ethanol (30 ml) was refluxed for 8 h. The reaction mixture was concentrated under reduced pressure, the solid product was collected by filtration, dried and crystallized from ethanol to give 15 as green powder.

Antimicrobial Screening

***Microorganisms species*:**

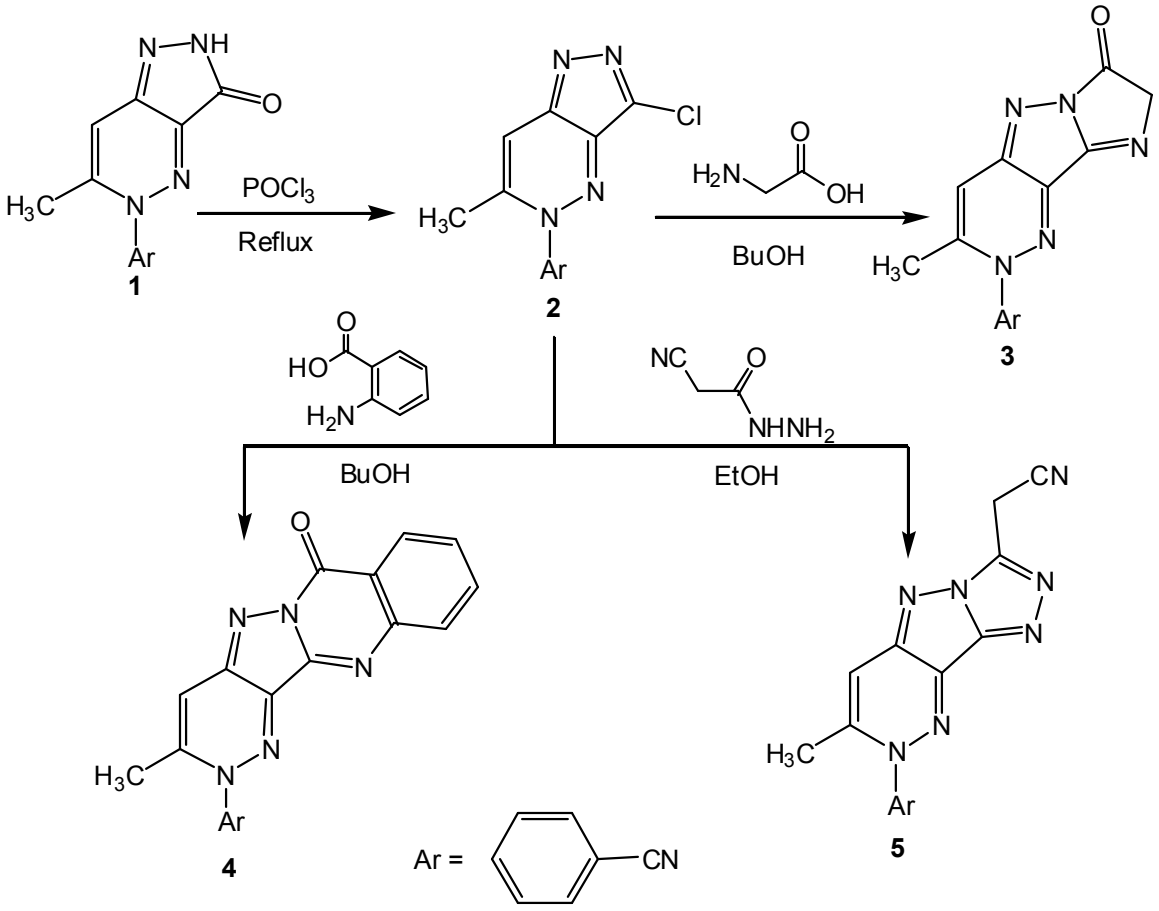
1. Bacteria
   a. Gram-negative bacteria, *Eschericha coli., Salmonella typhi*
   b. Gram-positive bacteria, *Bacillus subtitles, Staphylococcus aureus*, *Streptococcus*
2. Fungi: *Aspergillus Niger*
3. Yeast: *Candida albicans*, Sacchromyces

Medium: The cap-assay method containing (g/l): peptone 6.0, yeast extract 3.0, meat extract 1.5, glucose 1.0 and agar 20.0 were used. The medium was sterilized and divided while hot (50-60°C) in 15 ml. Portions among sterile petri-diches of 9 cm diameter.

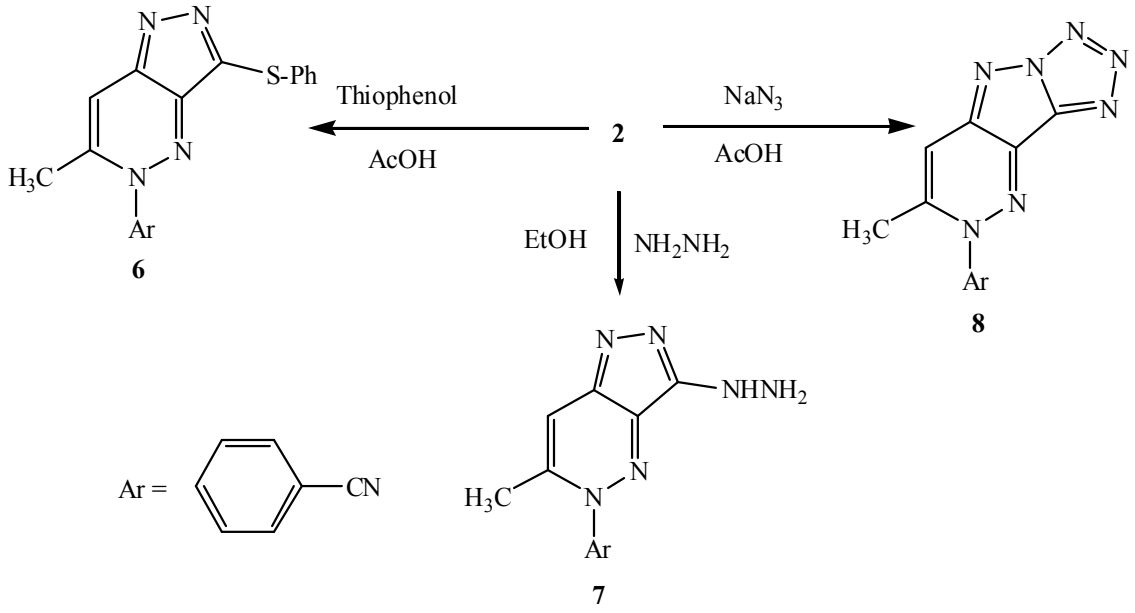
One ml of the spor suspension of each microorganism was spread all over the surface of the cold solid medium placed in the petri-dish.

RESULTS AND DISCUSSION

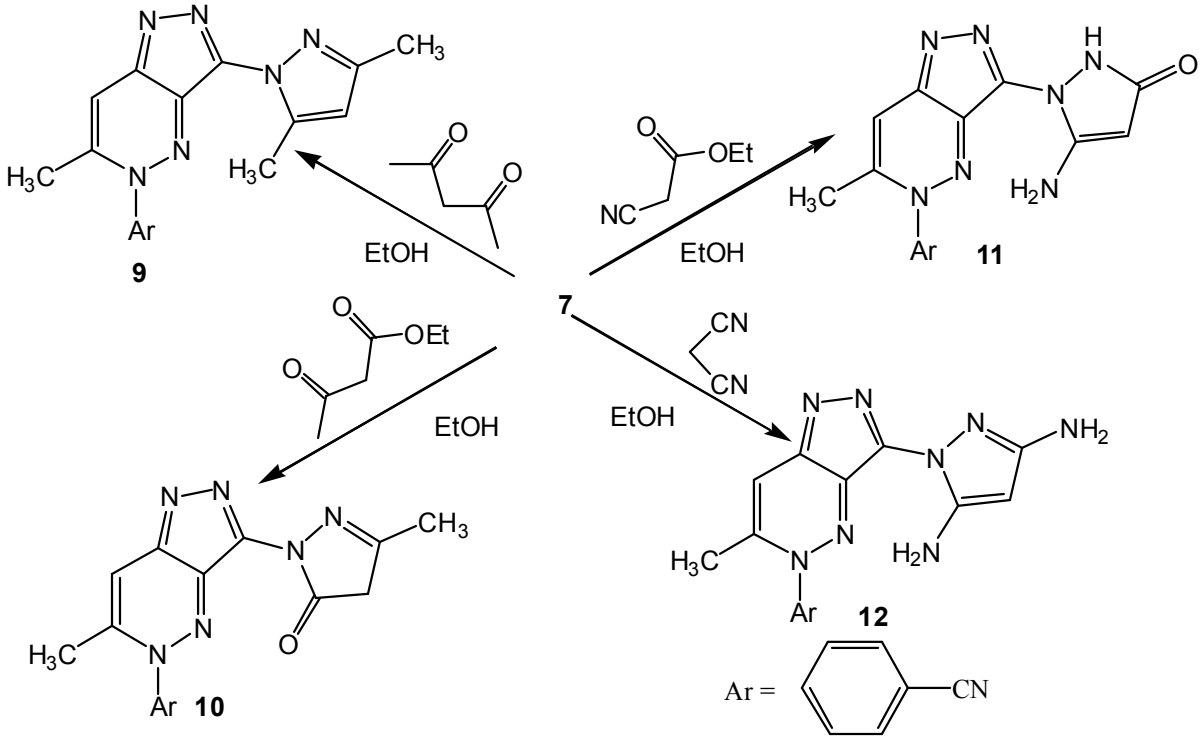
The starting material 3-oxo-1-(4-cyanophenyl)-4*H*-7-methyl-pyrazolo[4,3-*c*]pyridazine (1) was prepared according to Nagawade et al [17]. Treatment of compound 1 with refluxing $POCl_3$ produced 3-chloro-1-(4-cyanophenyl) -7-methyl-pyrazolo [4,3-*c*] pyridazine (2),



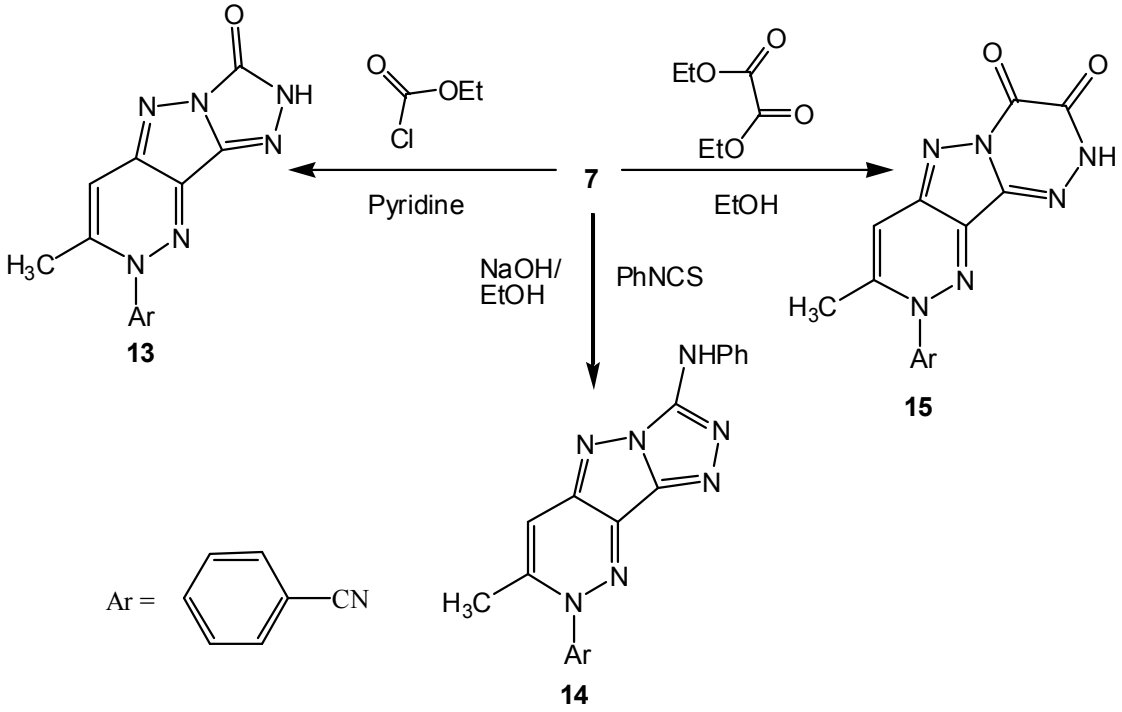
Scheme 1:



Scheme 2:



Scheme 3:



Scheme 4:

Table 3: Antimicrobial activities of the tested compounds

Tested Compounds and positive drugs	Inhibition Zone (mm)							
	Microorganism							
	Bacteria					Fungi	Yeast	
	Gram-negative		Gram-positive					
	E.coli	*S.typhi*	*B. Subtitls*	*S. aureus*	*Streptococcus*	*A.niger*	*C. albicans*	Sacchro-myces
Control (DMSO)	-	-	-	-	-	-	-	-
Streptomycin	+++	+++	+++	+++	+++	-	-	-
Erythromycin	+	-	+++	++	++	-	-	-
Fusidic Acid	-	-	-	-	-	+++	+++	+++
4	+++	++	+++	+++	++	+	+++	+++
6	+++	+++	+++	++	++	+	+++	++
7	-	++	+	-	-	-	++	-
10	-	-	+	-	++	+	+	+
15	+	++	-	+	-	+	++	++

+++ Highly sensitive (inhibition zone = 21-40 mm).

++ Fairly sensitive (inhibition zone = 16-20 mm).

+ Slightly sensitive (inhibition zone = 10-15 mm).

- No sensitive

which was reacted with bifunctional reagents, namely, glycine, anthranilic acid or cyano acetyl hydrazine in refluxing alcohol to yield the corresponding substituted pyrazolopyridazine derivatives 3-5, respectively (Scheme 1).

Also, compound 2 was reacted with thiophenol in refluxing acetic acid or hydrazine hydrate in refluxing ethanol to give 3-phenyl mercapto- and 3-hydrazino-pyrazolopyridazine derivatives 6 and 7, respectively. While, 2 was reacted with sodium azide in refluxing acetic acid to afford tetrazolopyrazolo-pyridazine derivative 8 (Scheme 2).

Compound 7 was reacted with active methylene reagents, namely, acetyl acetone, ethyl acetoacetate, ethyl cyanoacetate or malononitrile in refluxing ethanol to produce the corresponding 3-(substituted pyrazolo)-pyrazolo[4,3-c]pyridazine derivatives 9-12, respectively (Scheme 3).

On the other hand, compound 7 was reacted with ethyl chloroformate or phenyl isothiocyanate in the presence of base as a catalyst to afford the corresponding triazolopyrazolopyridazine derivatives 13 and 14, respectively. Also, compound 7 was reacted with diethyl oxalate in refluxing ethanol to produce triazinopyrazolopyridazine derivative 15 (Scheme 4).

Biological Activity: Some of the synthesized compounds 4, 6, 7, 10 and 15 were evaluated for its antimicrobial activity against five bacterial strains *E. Coli, S. typhi, B. subtilis, S. aureus and Staph.aureus* and one fungal strain *Aspergillus niger* and also two strains of yeast *Candida albicans* and Sacchromyces at 50-6 μg cm^{-1} concentration, according to modified Kirby-Bauer's disk diffusion method [18]. MIC values of tested compounds were determined by tube dilution technique. The solvents DMSO/DMF were used as negative controls and Streptomycin®, Erythromycin® and Fusidic acid were used as standards. Calculated average diameters (for triplicate sets) of the zones of inhibition (in mm) for test samples were compared with that produced by the standard drugs. Almost, all the tested compounds were found to exhibit antimicrobial activities. Analysis of antimicrobial data suggested that compounds 4 and 6 possessed higher significant antibacterial and antifungal activities than some known standard drugs. The results of antimicrobial screening were recorded as average diameter of inhibition zone in mm and summarized in Table 3.

ACKNOWLEDGEMENT

Appreciation is expressed to Dr. Y.M. El-Ayouty, Botany Department, Faculty of Science, Zagazig University, Egypt, for antimicrobial activity screening.

REFERENCES

1. Amr, A.E., N.A. Abdel-Latif and M.M. Abdalla, 2006. Acta Pharm., 56: 203-218.

2. Amr, A.E., N.A. Abdel-Latif and M.M. Abdalla, 2006. Bioorg. Med. Chem., 14: 373-384.
3. Amr, A.E. 2005. Z. Naturforsch., 60b: 990-998.
4. Amr, A.E., H.H. Sayed and M.M. Abdulla, 2005. Arch. Pharm. Chem. Life Sci., 338: 433-440.
5. Abo-Ghalia, M.H. and A.E. Amr, 2004. Amino Acids, 26: 283-289.
6. Hammam, A.G., A.F.M. Fahmy, A.E. Amr and A.M. Mohamed, 2003. Ind. J. Chem., 42B: 1985-1993.
7. El-Goda, F.E., A.R. Maarouf and E.R. El-Bendary, 2003. Saudi Pharm. J., 11: 111-117.
8. El-Emary, T.I., 2006. J. Chin. Chem. Soc., 53: 391-401.
9. Mansour, A.K., M.M. Eid and N.S.A.M. Khalil, 2003. Molecules, 8: 744-755.
10. Mohamed, M.S., A.E. Rashad, M.E.A. Zaki and S.S. Fatahala, 2005. Acta Pharm., 55: 237-249.
11. Rashad, A.E., A.H. Shamroukh, M.A. Ali and F.M. Abdel-Motti, 2007. Heteroatom Chem., 18: 274-282.
12. Shamroukh, A.H., M.E.A. Zaki, E.M.H. Morsy, F.M. Abdel-Motti and F.M.E. Abdel-Megeid, 2007. Arch. Pharm., 340: 236-243.
13. Piaz, V.D., M.C. Castellana, C. Vergelli, M.P. Giovannoni, A. Gavaldà, V. Segarra, J. Beleta, H. Ryder and J.M. Palacios, 2002. J. Enzyme Inhibition and Med. Chem., 17: 227-233.
14. Sweeney, Z.K., S.F. Harris, N.N. Arora, H.H. Javanbakht, Y.Y. Li, J.J. Fretland, J.P. Davidson, J.R. Billedeau, S.K. Gleason, D.D. Hirschfeld, J.J. Kennedy-Smith, T.T. Mirzadegan, R.R. Roetz, M.M. Smith, S.S. Sperry, J.M. Suh, J.J. Wu, S.S. Tsing, A.G. Villasen~or, A.A. Paul, G.G. Su, G.G. Heilek, J.Q. Hang, A.S. Zhou, J.A. Jernelius, F.F.J. Zhang and K.K. Klumpp, 2008. J. Med. Chem., 51(23): 7449-7458.
15. Takayoshi, K., W. Masaichi, O. Makoto, S. Kentaro, N. Masahiro and F. Takashi, 2006. Bioorg. Med. Chem. Lett., 16: 55-58.
16. Braña, M.F., M. Cacho, M.L. García, E.P. Mayoral, B. López, B. de Pascual-Teresa, A. Ramos, N. Acero, F. Llinares, D. Muñoz-Mingarro, O. Lozach and L. Meijer, 2005. J. Med. Chem., 48(22): 6843-6854.
17. Nagawade, R.R., N.N. Khanna, S.S. Bhagwat and D.B. Shinde, 2005. Eur. J. Med. Chem., 40: 1325-1330.
18. Bauer, A.W., M.M. Kirby, J.C. Sherris and M. Turck, 1996. Am. J. Clin. Pathol., 45: 493-496.