

Facile heterocyclic synthesis and antimicrobial activity of polysubstituted and condensed pyrazolopyranopyrimidine and pyrazolopyranotriazine derivatives

HEND N. HAFEZ^{1,2}
ABDULRAHMAN G. ALSHAMMARI¹
ABDEL-RHMAN B. A. EL-GAZZAR^{1,2}

¹ Al-Imam Mohammad Ibn Saud
Islamic University (IMSIU)
Faculty of Science
Department of Chemistry
P.O. Box 90950 Riyadh 11623
Kingdom of Saudi Arabia

² Photochemistry Department
(Heterocyclic and Nucleosides Unit)
National Research Centre
Cairo, Egypt

Reaction of 6-amino-3-methyl-4-(substituted phenyl)-1,4-dihydropyran[2,3-*c*]pyrazole-5-carbonitrile (**1**) with triethylorthoformate followed by treatment with hydrazine hydrate, formic acid, acetic acid, phenylisocyanate, ammonium thiocyanate and formamide afforded the corresponding pyranopyrimidine derivatives **2–6**. Cyclocondensation of **1** with cyclohexanone afforded pyrazolopyranoquinoline **7**. One-pot process of diazotation and de-diazochlorination of **1** afforded pyrazolopyranotriazine derivative **8**, which upon treatment with secondary amines afforded **9** and **10a–c**. Condensation of **2** with aromatic aldehyde gave the corresponding Schiff bases **11a,b**, the oxidative cyclization of the hydrazone with appropriate oxidant afforded 11-(4-fluorophenyl)-2-(4-substituted phenyl)-10-methyl-8,11-dihydropyrazolo-[4',3':5,6]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (**12a,b**). Structures of the synthesized compounds were confirmed by spectral data and elemental analysis. All synthesized compounds were evaluated for antibacterial and antifungal activities compared to norfloxacin and fluconazole as standard drugs. Compounds **9**, **10c**, **12a** and **15** were found to be the most potent antibacterial agents, with activity equal to that of norfloxacin. On the other hand, compound **5** exhibited higher antifungal activity compared to fluconazole.

Keywords: pyrazolopyranopyrimidine, pyrazolopyranotriazine, pyrazolopyranoquinoline, antimicrobial activity

Accepted December 22, 2014

Antibiotic resistance, which results from inappropriate and irrational use of antimicrobial medicines, provides favorable conditions for resistant bacteria to emerge. This resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem. Hence, the design of new compounds to deal with resistant bacteria has become one of the most important goals of antibacterial research today.

* Correspondence; e-mail: profelgazzar@yahoo.com

In continuation of our study of the chemistry of heterocyclic β -enamino-carbonitrile (1) and the synthesis of fused pyrane ring systems of pharmacological importance (2), pyrane and its fused derivatives have attracted great interest owing to their antimicrobial (3–5), antiviral (6), antitumor (7), antiproliferative (8), molluscicidal (9), and anti-inflammatory activities (10). Also, pyrane derivatives are well known antihistaminic agents (11). Moreover, it has been noticed that introduction of an additional ring to the pyrimidine core tends to exert a profound influence in conferring novel biological activities in these molecules (12–14). Pyrimidine and their fused derivatives play an essential role in several biological processes and have considerable chemical and pharmacological importance. In particular, pyrimidine nucleus can be found in a broad variety of antibacterial and antitumor agents, as well as in agrochemical and veterinary products (15, 16). Some substituted pyrano[2,3-*c*]pyrazoles have been found to be effective antiplatelet molecules (17), which effect K^+ -induced calcium-dependent aortal contraction. Several pyrano[2,3-*c*]pyrazol-4-ones have demonstrated affinity toward A1 and A2a adenosine receptors (18). Also, 6-amino-5-cyano-dihydro-pyrano[2,3-*c*]pyrazoles have been identified as a screening hit for human Chk1 kinase inhibitors (19). We herein report the use of 6-amino-3-methyl-4-(4-fluorophenyl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (1) as starting material for the synthesis of a new series of pyrazolopyranopyrimidines (2–6) and their derivatives **11a,b**, pyrazolopyranoquinoline (7) and pyrazolopyranotriazine (8).

EXPERIMENTAL

All melting points were measured on an Electrothermal 9100 series digital melting point apparatus (Shimadzu, Japan). Microanalytical data were provided by a Vario Elemental apparatus (Shimadzu, Japan). Elemental analyses of all compounds were within ± 0.4 % of the theoretical values. Physicochemical data are given in Table I. The IR spectra (KBr) were recorded on a Perkin Elmer 1650 spectrometer (USA). ^1H spectra were recorded on a JEOL EX-300 and JEOL ECA-500 (Japan). Chemical shifts were expressed in ppm relative to SiMe_4 as internal standard in $\text{DMSO-}d_6$ as a solvent. Mass spectra were recorded on a 70 eV Finnigan SSQ 7000 spectrometer (Thermo-Instrument System Incorporation, USA) (Table II). The purity of the compounds was checked on aluminium plates coated with silica gel (Merck, Germany). Chemicals and solvents were purchased from Sigma-Aldrich (USA). Norfloxacin and fluconazole were supplied by Pasteur laboratory (Giza, Egypt). Compound 1 was synthesized according to the reported procedure (20).

Syntheses

*6-Amino-3-methyl-4-fluorophenyl-1,4-dihydro-pyrano[2,3-*c*]pyrazole-5-carbonitrile (1).* – Ethyl-3-(2-carbamothioylhydrazineylidene)-butanoate (2.03 g, 10 mmol) was added into sodium ethoxide solution (20 mL) followed by 2-(4-fluorobenzylidene)-malononitrile (1.71 g, 10 mmol). The reaction mixture was heated under reflux for 6 h. The compound obtained was crystallized as yellow powder.

*4-(4-Fluorophenyl)-5-imino-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidin-6(5H)-amine (2).* – Compound 1 (0.01 mol) was added to a mixture of triethylorthoformate (0.01 mol) and acetic anhydride (20 mL), and the reaction mixture was refluxed for

5 h. The solvent was removed under reduced pressure. The separated solid was refluxed with hydrazine hydrate (0.01 mol) in absolute ethanol (50 mL). The reaction mixture was refluxed for 2 h, concentrated, cooled, and the solid product that separated out was filtered off and recrystallized as yellow powder.

4-(4-Substituted phenyl)-3-methyl-4,8-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine 5(1H)-ones (**3a,b**). *General procedure.* – A mixture of **1** (0.01 mol) and appropriate acid (formic and/or acetic acid) was refluxed. The solvent was removed under reduced pressure and the separated solid was recrystallized from appropriate solvent in a good yield to give **3a,b** respectively.

N-[4-(4-fluorophenyl)-3-methyl-7-oxo-6-phenyl-4,6,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5(1H)-ylidene]benzamide (**4**). – Phenyl isocyanate (0.01 mol) and triethylamine (0.5 mL) were added to a solution of **1** (0.01 mol) in ethanol (20 mL). The reaction mixture was refluxed for 3 h, cooled and the resulting solid was filtered off and recrystallized to give **4** as yellow powder.

N-[4-(4-fluorophenyl)-3-methyl-7-thioxo-1,4,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidine-5-yl]thiourea (**5**). – Ammonium thiocyanate (0.03 mol) was added to a solution of **1** (0.01 mol) in acetic acid (15 mL) and the reaction mixture was refluxed for 10 h. The solid that separated upon cooling and dilution with water was filtered off and purified to give **5** as pale brown powder.

4-(4-Fluorophenyl)-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-5-amine (**6**). – Compound **1** (0.01 mol) was added to a mixture of formamide (10 mL), formic acid (5 mL) and dimethylformamide (5 mL). The reaction mixture was refluxed for 12 h. The solid that separated on cooling was filtered off as pale yellow powder.

4-(4-Fluorophenyl)-3-methyl-1,4,6,7,8,9-hexahydropyrazolo[4',3':5,6]pyrano[2,3-b]quinolin-5-amine (**7**). – Cyclohexanone (2.8 mmol) was added to a solution of **1** (2 mmol) in a mixture of dichloroethane/THF (2:1). After stirring for 5 min at room temperature, aluminum chloride (4 mmol) was added, and the mixture was heated under reflux for 2 h. The mixture was cooled to room temperature and aluminum chloride (4 mmol) was added. The mixture was refluxed 2 h. The solvent was removed under reduced pressure. An aqueous solution of sodium hydroxide (50 mL, 10 %) was added. After stirring for 30 min, the precipitate was filtered, washed twice with 25 mL of water and with 10 mL of ether, dried at room temperature until constant mass, purified in 5 mL of acetonitrile, and filtered while hot as white powder.

4-Chloro-5-(4-fluorophenyl)-6-methyl-5,8-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d][1,2,3]-triazine (**8**). – A solution of sodium nitrite (11.4 mmol) in water (7 mL) was added for 15 min to a suspension of the foregoing compound **1** (8.1 mmol) at 0–5 °C in concentrated hydrochloric acid (16 mL). The resulting mixture was stirred at 0 °C for further 40 min and then allowed to stand at room temperature overnight. The reaction mixture was quenched in water (100 mL). The precipitate was washed twice with 15 mL of water and dried under room temperature, purified in 2 mL of acetonitrile, and filtered while hot to give **8** as orange powder.

5-(4-Fluorophenyl)-4-(pyrrolidine, morpholine, piperazine, and/or N-(methyl-piperazinyl)-6-methyl-5,8-dihydropyrazolo[4',3':5,6]pyrano[2,3-d][1,2,3]triazines (**9, 10a–c**). *General procedure.* – Compound **8** (1 mmol) and the corresponding amine (5 mmol) were fused in a sand bath for 6 h. The mixture was cooled at room temperature and poured into ethanol (30 mL)

under stirring. When a precipitate was formed, it was filtered off, washed twice with 15 mL of ethanol and twice with 8 mL of diethyl ether, and then dried at room temperature overnight.

5-(4-Fluorophenyl)-6-methyl-4-pyrrolidin-1-yl)-5,8-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]-[1,2,3]triazine (**9**) was obtained from **8** with pyrrole as pale yellow powder, 5-(4-fluorophenyl)-6-methyl-4-morpholin-4-yl)-5,8-dihydropyrazolo-[4',3':5,6]pyrano-[2,3-d][1,2,3]triazine (**10a**) was obtained from compound **8** with morpholine as yellow powder, 5-(4-fluorophenyl)-6-methyl-4-piperazin-1-yl)-5,8-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]-[1,2,3]triazine (**10b**) was obtained from **8** with piperazine as brown powder, and 5-(4-fluorophenyl)-6-methyl-4-methylpiperazin-1-yl)-5,8-dihydropyrazolo[4',3':5,6]pyrano [2,3-d][1,2,3]triazine (**10c**) was obtained from **8** with *N*-methylpiperazine as gray powder.

4-(4-Fluorophenyl)-*N*-[(1*Z*)-(4-substituted phenyl)methylene]-5-imino-3-methyl-1,4-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-6(5*H*)-amine (**11a,b**). *General procedure.* – A mixture of **2** (0.31 g, 1.0 mmol), with appropriate aldehyde (*p*-fluorobenzaldehyde or 4-methoxy benzaldehyde) (10 mmol), piperidine (0.5 mL) and dioxane (30 mL) was refluxed for 6 hours. The precipitate was filtered off and washed several times with cold EtOH. The solid was recrystallized from the appropriate solvent.

4-(4-Fluorophenyl)-*N*-[(1*Z*)-(4-fluorophenyl)methylene]-5-imino-3-methyl-1,4-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-6(5*H*)-amine (**11a**) was obtained from **2** with *p*-fluorobenzaldehyde as pale yellow powder, and 4-(4-fluorophenyl)-5-imino-*N*-[(1*Z*)-(4-methoxyphenyl)methylene]-3-methyl-1,4-dihydro-pyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-6(5*H*)-amine (**11b**) was obtained from **2** with 4-methoxy-benzaldehyde as pale brown powder

11-(4-Fluorophenyl)-2-(4-substituted phenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano-[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**12a,b**). *General procedure.* – Compounds **11a,b** where to come with alumina-supported calcium hypochlorite Ca(OCl)₂/Al₂O₃ = 1:1, ground mixture, stirred overnight and the reaction mixture was quenched in water (50 mL).

2,11-Bis(4-fluorophenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-*e*][1,2,4]-triazolo[1,5-*c*]pyrimidine (**12a**) was obtained from **11a** as pale gray powder, while 11-(4-fluorophenyl)-2-(4-methoxyphenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano-[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**12b**) was obtained from **11b** as pale yellow powder.

11-(4-Fluorophenyl)-10-methyl-8,11-dihydropyrazolo[4',3':5,6]pyrano[3,2-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidine-2(3*H*)-thione (**13**). – To a solution of **2** (0.31 g, 10 mmol in dry pyridine (30 mL), CS₂ (20 mmol) was added and the reaction mixture was heated under reflux for 8 h. After cooling, the reaction mixture was poured onto ice/HCl mixture and the solid that separated was washed with cold water and filtered off to give **13** as yellow powder.

2-[4-(4-Fluorophenyl)-5-imino-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-d]pyrimidin-6(5*H*)-yl]-1*H*-isoindole-1,3(2*H*)-dione (**14**). – To a solution of **2** (0.31 g, 10 mmol) in *n*-BuOH, 10 mmol of phthalic acid anhydride was added. The reaction mixture was left overnight and the solid that formed (**14**) was collected as pale brown powder.

(2*Z*)-2-[[4-(4-fluorophenyl)-5-imino-3-methyl-1,4-dihydropyrazolo[4',3':5,6]-pyrano[2,3-d]pyrimidin-6(5*H*)-yl]imino]-1,2-dihydro-3*H*-indol-3-one (**15**). – A solution of **2** (0.31 g, 10 mmol) in dioxane (10 mL) was stirred with isatin (15 mmol) for 24 hours at room temperature. The product that separated out (**15**) as orange powder was filtered off.

Pharmacological screening

Antimicrobial activity. – Antimicrobial activities of the newly synthesized compounds were tested *in vitro* for activity against the following bacteria: Gram-positive bacteria *Streptococcus lactis* NCTC-1030, *Staphylococcus aureus* NCTC-4493, *Enterococcus faecalis* NCTC-4737, Gram-negative bacteria *Escherichia coli* ATCC-1416, *Pseudomonas aeruginosa* ATCC-2642, *Klebsiella pneumoniae* ATCC-1081, and fungal strains *Candida albicans* ATCC-14154, *Aspergillus flavus* ATCC-23554 and *Ganoderma lucidum* ATCC-33455. All microorganisms were purchased from the American Type Culture Collection (Manassas, USA). The newly synthesized compounds (**2–15**) were dissolved in DMSO and tested for antimicrobial activity with the agar disk diffusion technique (**21**), using a 1-cm microplate-well diameter and a solution of 100 $\mu\text{g mL}^{-1}$ of the test compound. Compound-impregnated disks were placed on an agar plate containing a standard suspension of microorganisms. The plate was incubated for 24 h at 37 °C. Diameters of the zones of inhibition were measured with calipers or automated scanners and were compared with those of the standards. Norfloxacin (0.16 $\mu\text{mol mL}^{-1}$) and fluconazole (6.5 $\times 10^{-3}$ $\mu\text{mol mL}^{-1}$) were used as reference drugs for antibacterial and antifungal activity, respectively.

For determination of minimum inhibitory concentrations (*MIC*) (**22**) by the serial plate dilution method, 5 mg of each test compound was dissolved in 1 mL of dimethylsulfoxide (DMSO) to prepare the stock solution. Serial dilutions were prepared from the stock solution. The plates were incubated at 37 °C for 24 h. *MIC* was the lowest concentration ($\mu\text{mol mL}^{-1}$) of the test compound that resulted in no visible growth on the plates. DMSO was used as a solvent control to ensure that the solvent had no effect on bacterial growth. The results of antimicrobial activities are summarized in Tables I and II.

RESULTS AND DISCUSSION

Chemistry

The bifunctional compound **1** was used for the synthesis of pyrazolopyranopyrimidine derivatives by the reaction with different reagents. Thus, the treatment of 6-amino-3-methyl-4-(substituted-phenyl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (**1**), which is the starting material (**23**) for the synthesis of polycyclic fused ring systems, with triethylorthoformate followed by hydrazine hydrate afforded three fused rings, namely, 4-(4-fluorophenyl)-5-imino-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidin-6(5*H*)-amine (**2**). Also, the reaction of **1** with formic acid and/or acetic acid afforded pyranopyrimidine derivatives **3a,b**. In addition, the interaction of **1** with phenylisocyanate consumed two moles of the reagent to furnish *N*-[(5*Z*)-4-(4-fluorophenyl)-3-methyl-7-oxo-6-phenyl-4,6,7,8-tetrahydropyrazolo[4',3':5,6]-pyrano[2,3-*d*]pyrimidine-5(1*H*)-ylidene]benzamide (**4**). Heating of **1** with ammonium thiocyanate in boiling acetic acid gave pyranopyrimidinethione derivatives (**5**). The treatment of **1** with formamide in the presence of formic acid and dimethylformamide gave compound **6**. The structure of **6** was confirmed *via* inspection of elemental analysis and spectral data. Its ¹H NMR spectrum revealed δ at 5.00 (s, pyran-*H*), 7.19 (d, 2H, Ar-*H*), 7.80 (d, 2H, Ar-*H*), 8.10 (s, CH pyrimidine) and 10.29, 11.60 ppm (2 NH). Compound 4-(4-fluorophenyl)-3-methyl-1,4,6,7,8,9-hexahydropyrazolo-[4',3':5,6]pyrano[2,3-*b*]quinolin-5-amine (**7**) was obtained in good yield (see

Table I. Physical and analytical data of newly synthesized compounds

Compd.	Mol. formula (M_r)/ solvent	M. p. (°C) (yield, %)	Analysis (calcd./found, %)			Compd.	Mol. formula (M_r)/ solvent	M. p. (°C) (yield, %)	Analysis (calcd./found, %)		
			C	H	N				C	H	N
1	C ₁₄ H ₁₁ FN ₄ O (270.26) (Ethanol)	210–212 (72)	62.21 62.19	4.10 4.00	20.73 20.70	10a	C ₁₈ H ₁₇ FN ₆ O ₂ (368.37) (Dioxane)	229–231 (60)	58.69 58.66	4.65 4.64	22.81 22.78
2	C ₁₅ H ₁₃ FN ₆ O (312.30) (Ethanol)	231–232 (60)	57.68 57.65	4.19 4.17	26.91 26.90	10b	C ₁₈ H ₁₈ FN ₇ O (367.38) (Dioxane)	241–242 (65)	58.85 58.84	4.94 4.92	22.69 22.68
3a	C ₁₅ H ₁₁ FN ₄ O ₂ (298.27) (Dioxane)	272–274 (75)	60.40 60.38	3.71 3.70	18.78 18.76	10c	C ₁₉ H ₂₀ FN ₇ O (381.40) (Dioxane)	253–254 (60)	59.83 59.81	5.29 5.29	25.71 25.70
3b	C ₁₆ H ₁₃ FN ₄ O ₂ (312.29) (Dioxane)	260–262 (78)	61.53 61.50	4.20 4.19	17.94 17.91	11a	C ₂₂ H ₁₆ F ₂ N ₆ O (418.4) (DMF)	250–252 (70%)	63.15 63.12	3.85 3.83	20.08 20.05
4	C ₂₈ H ₂₀ FN ₅ O ₃ (493.48) (Ethanol)	255–257 (59)	68.15 68.13	4.08 4.05	14.19 14.19	11b	C ₂₃ H ₁₉ FN ₆ O ₂ (430.43) (DMF)	256–258 (80)	64.17 64.15	4.44 4.41	19.52 19.50
5	C ₁₆ H ₁₃ FN ₆ OS ₂ (297.29) (Ethanol)	310–312 (70)	49.47 49.44	3.37 3.35	21.64 21.60	12a	C ₂₂ H ₁₄ F ₂ N ₆ O (416.38) (Dioxane)	220–221 (73)	63.46 63.43	3.39 3.36	20.18 20.14
6	C ₁₅ H ₁₂ FN ₅ O (546.1) (isopropanol)	224–226 (69)	60.59 60.58	4.07 4.05	23.55 23.53	12b	C ₂₃ H ₁₇ FN ₆ O ₂ (428.42) (Dioxane)	280–282 (75)	64.48 64.45	4.00 4.00	19.62 19.60
7	C ₂₀ H ₁₉ FN ₄ O (350.38) (Acetonitrile)	268–270 (85)	68.56 68.55	5.47 5.45	15.99 15.97	13	C ₁₆ H ₁₁ FN ₆ OS (354.36) (Ethanol)	260–262 (61)	54.23 54.22	3.13 3.09	23.72 23.70
8	C ₁₄ H ₉ ClFN ₃ O (317.70) (Acetonitrile)	199–201 (73)	52.92 52.90	2.85 2.84	22.04 22.01	14	C ₂₃ H ₁₅ FN ₆ O ₃ (442.40) (EtOAc)	267–269 (56)	62.44 62.40	3.42 3.41	18.99 18.96
9	C ₁₈ H ₁₇ FN ₆ O (352.37) (Ethanol)	169 (52)	61.35 61.33	4.86 4.83	23.85 23.84	15	C ₂₃ H ₁₆ FN ₇ O ₂ (441.42) (methanol)	296–298° (70)	62.57 61.54	3.65 3.63	22.21 22.20

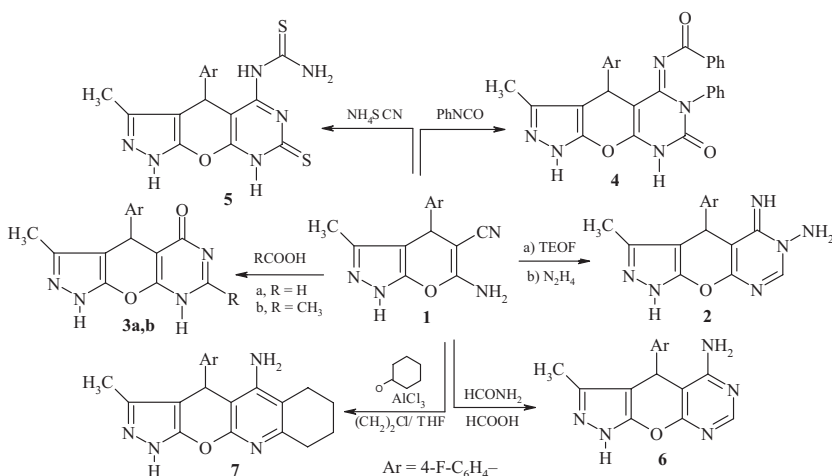
Table II. Spectral data of newly synthesized compounds

Compd.	IR (KBr) (ν_{\max} , cm^{-1})	MS (m/z)	^1H , ^{13}C NMR (DMSO- d_6) (δ , ppm)
1	3370, 3173, 3050, 2922, 2202, 1640	MS 270 (M^+ , 72 %)	2.20 (s, 3H, CH_3), 4.94 (s, pyran- <i>H</i>), 7.23 (d, 2H, Ar- <i>H</i>), 7.90 (d, 2H, Ar- <i>H</i>), 10.24, 10.52 (2brs, D_2O exchangeable-NH)
2	3370, 1615	MS 312 (M^+ , 86 %)	2.17 (s, 3H, CH_3), 5.05 (s, pyran- <i>H</i>), 7.23 (d, 2H, Ar- <i>H</i>), 7.72 (d, 2H, Ar- <i>H</i>), 8.01 (s, 1H, pyrimidine- <i>CH</i>). 10.05, 10.26 (2brs, D_2O exchangeable-NH)
3a	3300, 1690, 1620	MS 298 (M^+ , 83 %)	2.23 (s, 3H, CH_3), 5.01 (s, pyran- <i>H</i>), 8.58 (1H, s, pyrimidine- <i>CH</i>), 7.23 (d, 2H, Ar- <i>H</i>), 7.78 (d, 2H, Ar- <i>H</i>), 8.09 (s, 1H, pyrimidine- <i>CH</i>). 10.20, 10.62 (2brs, D_2O exchangeable-NH)
3b	3300, 1690, 1620	MS 312 (M^+ , 74 %)	2.20 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 5.05 (s, pyran- <i>H</i>), 7.23 (d, 2H, Ar- <i>H</i>), 7.78 (d, 2H, Ar- <i>H</i>), 10.20, 10.62 (2brs, D_2O exchangeable-NH)
4	3379, 3247, 1735, 1689	MS 508 (M^+ , 65 %)	2.20 (s, 3H, CH_3), 4.99 (s, pyran- <i>H</i>), 6.90–7.01 (m, 5H, Ar- <i>H</i>), 7.37–7.90 (m, 5H, Ar- <i>H</i>), 8.09 (d, 2H, Ar- <i>H</i>), 8.20 (d, 2H, Ar- <i>H</i>), 8.35 (s, 1H, pyrimidine- <i>CH</i>), 10.65, 11.53, 14.03, (3s, 3H, D_2O exchangeable 3NH)
5	3424, 2923, 1230, 1235	MS 388 (M^+ , 85 %)	2.23 (s, 3H, CH_3), 4.95 (s, pyran- <i>H</i>), 7.23 (d, 2H, Ar- <i>H</i>), 7.90 (d, 2H, Ar- <i>H</i>), 10.24, 10.52, 11.43, 12.03, (4brs, D_2O exchangeable-NH)
6	3370, 1622	MS 297 (M^+ , 80 %)	2.19 (s, 3H, CH_3), 5.00 (s, pyran- <i>H</i>), 7.19 (d, 2H, Ar- <i>H</i>), 7.8 0 (d, 2H, Ar- <i>H</i>), 8.10 (s, 1H, pyrimidine- <i>CH</i>), 10.29, 11.60 (2brs, D_2O exchangeable-NH)
7	3320, 3205	MS 350 (M^+ , 75 %)	1.76 (m, 4H, 2CH_2), 2.20 (s, 3H, CH_3), 2.45 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 4.98 (s, pyran- <i>H</i>), 6.22 (s, 2H, NH_2), 7.20 (d, 2H, Ar- <i>H</i>), 7.80 (d, 2H, Ar- <i>H</i>), 10.22, (br, 1NH, D_2O exchangeable)
8	3310, 1622	MS 317 (M^+ , 70 %)	2.21 (s, 3H, CH_3), 5.12 (s, pyran- <i>H</i>), 7.15 (d, 2H, Ar- <i>H</i>), 7.60 (d, 2H, Ar- <i>H</i>), 10.35 (br, 1NH, D_2O exchangeable)
9	3315	MS 352 (M^+ , 53 %)	2.19 (s, 3H, CH_3), 2.58 (m, 4H, 2CH_2), 3.56 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 4.98 (s, pyran- <i>H</i>), 7.21 (d, 2H, Ar- <i>H</i>), 7.80 (d, 2H, Ar- <i>H</i>), 10.34, (br, 1NH, D_2O exchangeable)
10a	3310	MS 368 (M^+ , 65 %)	2.15 (s, 3H, CH_3), 3.47 (m, 4H, 2CH_2), 3.76 (m, 4H, 2CH_2), 5.01 (s, pyran- <i>H</i>), 7.15 (d, 2H, Ar- <i>H</i>), 7.74 (d, 2H, Ar- <i>H</i>), 10.40 (br, 1NH, D_2O exchangeable)
10b	3315	MS 367 (M^+ , 70 %)	2.17 (s, 3H, CH_3), 2.48 (brs, 4H, piperazinyl 2NCH_2), 3.29 (brs, 4H, piperazinyl 2NCH_2), 5.05 (s, pyran- <i>H</i>), 7.08 (d, 2H, Ar- <i>H</i>), 7.68 (d, 2H, Ar- <i>H</i>), 10.25, (br, 1NH, D_2O exchangeable)
10c	3312	MS 381 (M^+ , 67 %)	2.13 (s, 3H, CH_3), 2.33 (s, 3H, piperazinyl NCH_3), 2.53 (brs, 4H, piperazinyl 2NCH_2), 3.36 (brs, 4H, piperazinyl 2NCH_2), 5.00 (s, pyran- <i>H</i>), 7.01 (d, 2H, Ar- <i>H</i>), 7.80 (d, 2H, Ar- <i>H</i>), 10.09, (br, 1NH, D_2O exchangeable)
11a	3325, 1625	MS 418 (M^+ , 90 %)	2.30 (s, 3H, CH_3), 5.10 (s, pyran- <i>H</i>), 6.80 (s, 2H, NH_2), 7.25 (d, 2H, Ar- <i>H</i>), 7.80 (d, 2H, Ar- <i>H</i>), 7.57 (d, 2H, Ar- <i>H</i>), 7.94 (d, 2H, Ar- <i>H</i>), 8.39 (s, 1H, pyrimidine- <i>CH</i>), 8.90 (s, 1H, $\text{N}=\text{CH}$), 10.09 (br, 1NH, D_2O exchangeable)

Table II. continued

Compd.	IR (KBr) (ν_{\max} , cm^{-1})	MS (m/z)	^1H , ^{13}C NMR (DMSO- d_6) (δ , ppm)
11b	3315,1615	MS) 430 (M^+ , 85 %)	2.35 (s, 3H, CH_3), 3.59 (s, 3H, OCH_3), 5.06 (s, pyran- H), 6.82 (s, 1H, NH), 7.30 (d, 2H, Ar- H), 7.78 (d, 2H, Ar- H), 7.60 (d, 2H, Ar- H), 7.99 (d, 2H, Ar- H), 8.35 (s, 1H, pyrimidine- CH), 9.25 (s, 1H, $\text{N}=\text{CH}$), 10.05 (br, 1NH, D_2O exchangeable)
12a	3320, 1630	(MS) 416 (M^+ , 90 %)	2.22 (s, 3H, CH_3), 5.10 (s, pyran- H), 7.20 (d, 2H, Ar- H), 7.85 (d, 2H, Ar- H), 7.48 (d, 2H, Ar- H), 8.00 (d, 2H, Ar- H), 8.30 (s, 1H, pyrimidine- CH) 10.20 (br, 1NH, D_2O exchangeable)
12b	3318, 1625	(MS) 428 (M^+ , 90 %)	2.20 (s, 3H, CH_3), 3.60 (s, 3H, OCH_3), 5.05 (s, pyran- H), 7.22 (d, 2H, Ar- H), 7.87 (d, 2H, Ar- H), 7.44 (d, 2H, Ar- H), 8.05 (d, 2H, Ar- H), 8.35 (s, 1H, pyrimidine- CH), 10.23 (br, 1NH, D_2O exchangeable)
13	1453, 3400	(MS) 353 (M^+ , 60 %)	2.19 (s, 3H, CH_3), 5.05 (s, pyran- H), 7.30 (d, 2H, Ar- H), 7.80 (d, 2H, Ar- H), 8.15 (s, 1H, pyrimidine- CH), 10.90, 11.52 (2brs, 2H, D_2O exchangeable-2NH)
14	1685, 1737, 3118	(MS) 442 (M^+ , 60 %)	2.30 (s, 3H, CH_3), 4.98 (s, pyran- H), 7.14–7.20 (m, 2H, Ar- H and pyrimidine- H), 7.54–7.81 (m, 3H, Ar- H), 7.99 (d, 2H, Ar- H), 8.10 (d, 2H, Ar- H), 9.05, 10.11 (2brs, 2H, D_2O exchangeable-NH)
15	3479, 1700, 1619	(MS) 441 (M^+ , 75 %)	2.20 (s, 3H, CH_3), 4.95 (s, pyran- H), 7.37–7.86 (m, 4H, Ar- H), 8.07 (d, 2H, Ar- H), 8.16 (d, 2H, Ar- H), 8.30 (s, 1H, pyrimidine- CH), 10.65, 14.20 (2s, 2H, D_2O exchangeable 2NH)

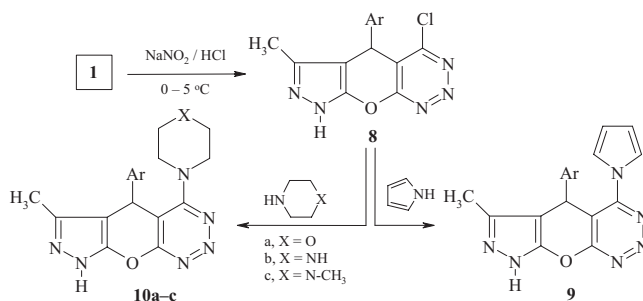
Scheme 1) by the Friedlander reaction on pyranopyrazole 1 (24) by heating compound 1 with cyclohexanone in dichloroethane, aluminium chloride and THF as solvent and reflux of reaction mixture for 15 h.



Scheme 1

Amino-cyanopyranopyrazole **1** could also be used to synthesize pyrazolopyranotriazine **8**. The 4-chlorotriazine ring was prepared *via* a one-pot process of diazotation and dediazo-chlorination. The reactivity of chlorine atom in 4-chloro-5-(4-fluorophenyl)-6-methyl-5,8-dihydropyrazolo-[4,3':5,6]pyrano[2,3-*d*][1,2,3]triazine (**8**) appeared when compound **8** was subject to the action of secondary amine, namely, pyrrole, morpholine, piperazine and *N*-methylpiperazine. The chlorine atom was displaced to form the corresponding compounds **9** and **10a–c**, respectively (Scheme 2).

According to the literature (25, 26), compounds having azomethine linkage exhibit *E/Z* geometrical isomerism around C=N double bond and can exist as *cis/trans* amide conformers (26). Moreover, hydrazones were proven to exist in higher percentage in DMSO-*d*₆ solution in the form of geometrical *E* isomers. Thus, compound **2** on treatment with the appropriate aromatic aldehyde, namely *p*-fluorobenzaldehyde/anisaldehyde, by refluxing with ethanol in the presence of a catalytic amount of piperidine gave 4-(4-fluorophenyl)-*N*-[(1*E*)-(4-substitutedphenyl)-methyl-ene]-5-imino-3-methyl-1,4-dihydropyrazolo[4,3':5,6]pyrano[2,3-*d*]pyrimidin-6(5*H*)-amines (**11a,b**). Also, NMR spectra of compounds **11** recorded in DMSO-*d*₆ solution revealed that all compounds existed as *E* geometrical isomers. Oxidative cyclization of the resultant hydrazone derivatives **11a,b** to **12a,b** was achieved by using alumina-supported calcium hypochlorite (Ca(OCl)₂/Al₂O₃ = 1:1, ground mixture) as a new oxidant.

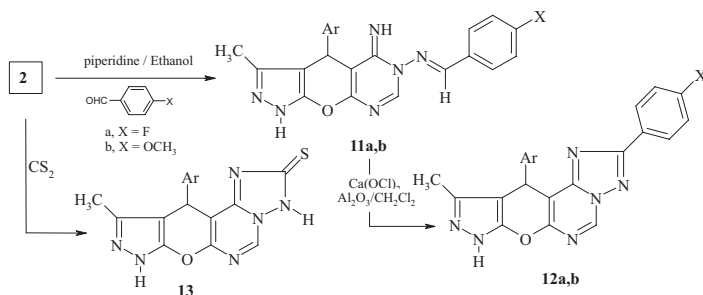


Scheme 2

For instance, a maximum yield of 73 % for **12a** and 75 % for **12b** in 2 h was achieved with the 1:3 molar ratio of hydrazone to calcium hypochlorite. The use of alumina-supported calcium hypochlorite as a heterogeneous oxidant in this reaction has the advantage of an enhanced reaction rate and yield (see Scheme 3).

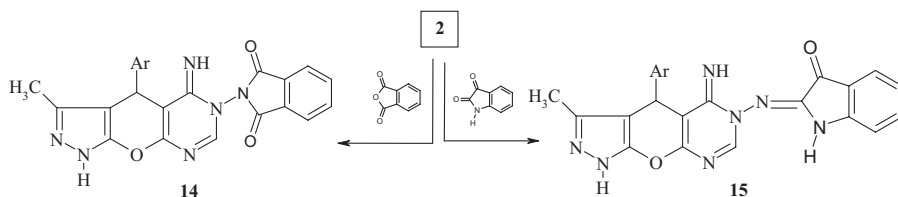
Furthermore, the hydrazide derivative **2** reacted with carbon disulfide and NaOH in ethanol to afford 11-(4-fluorophenyl)-10-methyl-8,11-dihydropyrazolo[4,3':5,6]pyrano[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2(3*H*)-thione (**13**) (Scheme 3).

In the present work, we wish to point out the reaction of hydrazino derivative **2** with phthalic anhydride. When compound **2** with phthalic anhydride in an oil bath, it yielded 2-phthalimidoamino derivative **14**. The reaction took place *via* acylation of hydrazino moiety by phthalic anhydride, followed by ring closure to give the desired product. On the other hand, stirring of compound **2** with isatin at room temperature yielded compound **15**



Scheme 3

(Scheme 4). The structure of the resultant compound was fully established by spectral data, which included IR, ^1H NMR and MS spectra. The ^1H NMR spectra of compound **15** revealed signals at δ : 2.20 (s, 3H, CH_3), 4.95 (s, pyran-H), 7.37–7.86 (m, 4H, Ar-H), 8.07 (d, 2H, Ar-H), 8.16 (d, 2H, Ar-H), 8.30 (s, 1H, pyrimidine CH), 10.65 and 14.20 ppm (2s, 2H, D_2O exchangeable 2NH).



Scheme 4

Antimicrobial screening and SAR

The minimum inhibitory concentration values (MICs) against different Gram-positive, Gram-negative bacteria and fungi were tested. The investigations showed significant inhibitory effects against bacteria with the majority of the compounds with MIC values of 1–21 $\mu\text{mol mL}^{-1}$ (Table III). The antibacterial data indicated that compounds **2**, **3a,b**, **4**, **5**, **6**, **11a,b**, **13** and **14** displayed good activity against all tested bacteria. This activity can be attributed to the presence of pyrimidine moiety. Compound **2** comprised iminopyrimidine with amino group attached at *N*-3, compounds **3a,b** are pyrimidone derivatives, while compounds **4**, **5** are **6** are 4-amino pyrimidine and pyrimidine thione derivatives. Compounds **11a,b** contain *N*-hydrazonyl, **13** triazolo thione and **14** *N*-isindol dione pyrimidine moieties. Evaluation of the antibacterial activity of synthesized compounds **7** and **8** revealed that compounds were effective against all tested bacteria due to the presence of tetrahydroquinoline moiety in **7** and 4-chlorotriazine in **8**. Regarding the antibacterial activities, pyrazolopyranotriazine derivatives **9** and **10a–c** were the most active compounds; their MIC values were 1–5 $\mu\text{mol mL}^{-1}$ against Gram-positive and 3–8 $\mu\text{mol mL}^{-1}$ against Gram-negative bacteria compared to the standard drug norfloxacin with respective MICs of 2–3 and

4–5 $\mu\text{mol mL}^{-1}$. The antimicrobial activity of compound **10c** comprising *N*-methylpiperazine attached to the triazine moiety at position 4 exhibited the higher activity against *S. lactis*, *E. faecalis*, *E. coli* and *K. pneumoniae*, but was equipotent against *S. aureus* and *P. aeruginosa* compared to norfloxacin, whereas compounds **9** and **10a,b** showed promising activity against bacteria which were equal or nearly close to MIC of norfloxacin. The activity of the latter compounds can be attributed to the presence of pyrolyl in **9**, morpholinyl in **10a** and piperazinyl moiety in **10b**, attached to the pyranotriazine, which increased the antimicrobial activity against Gram-positive and Gram-negative bacteria.

On the other hand compound **12a,b** displayed antibacterial activity with MIC equal or nearly close to MIC of norfloxacin, This is attributed to the presence of 4-fluoro substituent in the phenyl ring attached to [1,2,4]triazole in compound **12a**, while compound **12b** had 4-methoxyphenyl. Among the synthesized hydrazone derivatives, compound **15** displayed antibacterial activity comparable to that of norfloxacin against *E. faecalis*, *E. coli* and *P. aeruginosa*, and was equal or nearly close to MIC of norfloxacin against *S. lactis*, *S. aureus* and *K. pneumoniae*. This can be attributed to the presence of the indol-3-one attached to *N*-pyrimidinyl derivative.

Table III. Minimal inhibitory concentration (MIC, $\mu\text{mol mL}^{-1}$) of the synthesized compounds against bacteria

Compd.	Microorganism					
	<i>S. lactis</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
2	9	12	17	20	20	21
3a	14	12	14	18	14	14
3b	15	16	15	16	17	14
4	14	13	17	17	17	18
5	14	12	14	18	14	14
6	12	14	15	15	18	18
7	10	10	12	15	14	16
8	8	8	8	10	11	11
9	3	2	3	5	7	5
10a	4	3	5	4	4	3
10b	5	3	2	6	6	8
10c	1	2	2	3	5	3
11a	12	14	15	15	18	18
11b	10	15	17	14	15	15
12a	2	3	3	5	6	5
12b	5	4	5	8	6	8
13	11	10	10	11	12	16
14	12	13	13	10	12	14
15	3	3	2	3	4	4
Norfloxacin	2	2	3	4	5	4

Antifungal screening showed that most of the tested compounds possessed antifungal activity against all fungal strains (Table IV). Compound **5** exhibited higher activity against *C. albicans* and *G. lucidum* and was equal to MIC of fluconazole against *A. flavus*. This may be attributed to the presence of an intact thiourea NH-(C=S)-NH grouping attached to the thioxopyrimidine. On the other hand, compounds **8**, **9**, **10a,b**, **12a,b**, **14**, and **15** revealed good antifungal activity against the examined fungi compared to fluconazole as reference antifungal, while the other compounds (**2**, **3a,b**, **4**, **6**, **7**, **10c**, **11a,b** and **13**) exhibited moderate antifungal activities.

Table IV. Minimal inhibitory concentration (MIC, $\mu\text{mol mL}^{-1}$) of the synthesized compounds against fungi

Compd.	Microorganism		
	<i>C. albicans</i>	<i>A. flavus</i>	<i>G. lucidum</i>
2	27	22	21
3a	20	17	22
3b	23	24	21
4	21	24	26
5	2	2	2
6	18	24	22
7	17	17	21
8	14	12	12
9	11	12	14
10a	13	17	14
10b	8	7	8
10c	25	27	22
11a	24	21	23
11b	22	24	18
12a	10	10	14
12b	14	12	14
13	17	20	17
14	7	6	6
15	11	7	14
Fluconazole	3	2	3

CONCLUSIONS

The objective of the present study was to synthesize and investigate the antimicrobial activity of some novel pyrazolopyranopyrimidine, pyrazolopyranotriazine and pyrazolopyranotriazolo-pyrimidine derivatives. Compound 5-(4-fluorophenyl)-6-methyl-4-methylpiperazin-1-yl)-5,8-dihydropyrazolo[4',3':5,6]pyrano[2,3-*d*][1,2,3]triazine (**10c**) was found to have the most potent antibacterial activity slightly higher than that of norfloxacin. On

the other hand, compound *N*-[4-(4-fluoro-phenyl)-3-methyl-7-thioxo-1,4,7,8-tetrahydropyrazolo[4',3':5,6]pyrano[2,3-*d*]pyrimidine-5-yl]thiourea (**5**) exhibited higher potency of anti-fungal activity than fluconazole.

Acknowledgements. – The authors are grateful to the Micro-analytical Unit, Cairo University, Egypt, for micro-analytical data, IR, NMR and mass spectra. The authors are also grateful to the Fermentation Biotechnology and Applied Microbiology (Ferm-BAM) Center, Al-Azhar University, Faculty of Science, Cairo, Egypt. The authors extend their sincere appreciation to the Deanship of Scientific Research at the Al-Imam Mohammad Ibn Saud Islamic University for its funding of this research through the Research Group Project no. 341212.

REFERENCES

1. A. B. A. El-Gazzar and H. N. Hafez, Synthesis of 4-substituted pyrido[2,3-*d*]pyrimidin-4(1*H*)-one as analgesic and anti-inflammatory agents, *Bioorg. Med. Chem. Lett.* **19** (2009) 3392–3397; DOI: 10.1016/j.bmcl.2009.05.044.
2. F. M. A. El-Taweel, M. A. Sofan, M. A. Mashaly, M. A. Hanna and A. A. Elagamey, Synthesis of some new pyranoquinoline, pyridine and pyrone derivatives, *Pharmazie* **45** (1990) 671–673; DOI: 10.1002/chin.199149187.
3. A. M. El-Agrody, M. S. Abd El-Latif, N. A. El-Hady, A. H. Fakery and A. H. Bedair, Heteroaromatization with 4-hydroxycoumarin Part II: Synthesis of some new pyrano[2,3-*d*]pyrimidines, [1,2,4]triazolo[1,5-*c*]pyrimidines and pyrimido[1,6-*b*][1,2,4]triazine derivatives, *Molecules* **6** (2001) 519–527; DOI: 10.3390/60600519.
4. A. H. Bedair, N. A. El-Haddy, M. S. Abd El-Latif, A. H. Fakery and A. M. El-Agrody, 4-Hydroxycoumarin in heterocyclic synthesis. Part III: Synthesis of some new pyrano[2,3-*d*][1,2,4]triazolo[1,5-*c*]pyrimidine and pyrimido[1,6-*b*][1,2,4]triazine derivatives, *Farmaco* **55** (2000) 708–714; DOI: 10.1016/S0014-827X(00)00097-5.
5. A. M. El-Agrody, M. H. El-Hakim, M. S. Abd El-Latif, A. H. Fakery, E. M. El-sayed and K. A. El-Ghareab, Synthesis of pyrano[2,3-*d*]pyrimidine and pyrano[3,2-*e*][1,2,4]triazolo[2,3-*c*]pyrimidine derivatives with promising antibacterial activities, *Acta Pharm.* **50** (2000) 111–120.
6. A. G. Martinez and L. J. Marco, Friedlander reaction on 2-amino-3-cyano-4*H*-pyrans, synthesis of derivatives of 4*H*-pyran[2,3-*b*]quinoline, new tacrine analogues, *Bioorg. Med. Chem. Lett.* **7** (1997) 3165–3170; DOI: 10.1016/S0960-894X(97)10165-2.
7. F. Eiden and F. Denk, Synthesis and CNS activity of pyrane derivatives: 6,8-dioxabicyclo(3,2,1)octanes, *Arch. Pharm. (Weinheim)* **324** (1991) 353–354.
8. C. P. Dell and C. W. Smith, Antiproliferative derivatives of 4*H*-naphtho[1,2-*b*]pyran and process for their preparation, *Eur. Pat.* 537, 94, 9 21 Apr 1993; ref. *Chem. Abstr.* **119** (1993) 139102d.
9. F. M. Abdelrazek, P. Metz, N. H. Metwally and S. F. El-Mahrouky, Synthesis and molluscicidal activity of new cinnoline and pyrano[2,3-*c*]pyrazole derivatives, *Arch. Pharm. Chem. Life Sci.* **339** (2006) 456–460.
10. G. Bianchi and A. Tava, Synthesis of (2*R*)(+)-2,3-dihydro-2,6-dimethyl-4*H*-pyran-4-one, a homologue of pheromones of a species in the hepialidae family, *Agr. Biol. Chem.* **51** (1987) 2001–2002; DOI: 10.1271/bbb1961.51.2001.
11. K. Noda, A. Nakagawa, Y. Nakajima and H. Ide, Pyrido[2,3-*d*]-s-triazolo[4,3-*c*]pyrimidine derivatives, *Japan Kokai* 7785, 194, 15 Jul 1977; ref. *Chem. Abstr.* **88** (1978) P50908q.

12. H. N. Hafez, H. A. S. Abbas and A. B. A. El-Gazzar, Synthesis and evaluation of analgesic, anti-inflammatory and ulcerogenic activities of some triazolo- and 2-pyrazolyl-pyrido[2,3-*d*]-pyrimidines, *Acta. Pharm.* **58** (2008) 359–378; DOI: 10.2478/v10007-008-0024-1.
13. A. B. A. El-Gazzar, H. A. R. Hossein and H. N. Hafez, Synthesis and biological evaluation of thieno[2,3-*d*]pyrimidines derivatives as anti-inflammatory, analgesic and ulcerogenic activity, *Acta. Pharm.* **57** (2007) 395–411; DOI: 10.2478/v10007-007-0032-6.
14. A. B. A. El-Gazzar, H. N. Hafez and G. M. Nawwar, New acyclic nucleosides analogues as potential analgesic, anti-inflammatory, anti-oxidant and anti-microbial derived from pyrimido[4,5-*b*]quinolines, *Eur. Med. Chem.* **44** (2009) 1427–1436; DOI: 10.1016/j.ejmech. 2008.09.030.
15. L. P. Prikazchikova, B. M. Khutova, I. F. Vladimirtsev, I. V. Boldyrev and N. I. Zhuravskaya, stimulating activity of mono- and diketone pyrimidine derivatives, *Fiziol. Akt. Veshchestva* **7** (1975) 84–87; ref. *Chem. Abstr.* **83** (1975) 127346m.
16. D. J. Brown, *Pyrimidines and Their Benzo Derivatives*, in *Comprehensive Heterocyclic Chemistry* (Eds. A. R. Katritzky and C. W. Rees), Vol. 3, Pergamon Press, Oxford 1984, p. 443.
17. S. M. Yu, S. C. Kuo, L. J. Huang, S. S. M. Sun, T. F. Huang and C. M. Teng, Vasorelaxation of rat thoracic aorta caused by two Ca²⁺-channel blockers, HA-22 and HA-23, *J. Pharm. Pharmacol.* **44** (1992) 667–671; DOI: 10.1111/j.2042-7158.1992.tb05491.x.
18. D. Catarzi, L. Cecchi, V. Colotta, G. Filacchioni, C. Martini, P. Tacchi and A. Lucacchini, Tricyclic heteroaromatic systems. Synthesis and A1 and A2a adenosine binding activities of some 1-aryl-1,4-dihydro-3-methyl[1]benzopyrano[2,3-*c*]pyrazol-4-ones, 1-aryl-4,9-dihydro-3-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-4-ones, and 1-aryl-1*H*-imidazo[4,5-*b*]quinoxalines, *J. Med. Chem.* **38** (1995) 1330–1336.
19. N. Foloppe, L. M. Fisher, R. Howes, A. Potter, A. G. S. Robertson and A. E. Surgenor, Identification of chemically diverse Chk1 inhibitors by receptor-based virtual screening, *Bioorg. Med. Chem.* **14** (14) (2006) 4792–4802; DOI: 10.1016/j.bmc.2006.03.021.
20. H. M. Al-Matar, K. D. Khalil, A. Y. Adam and M. H. Elnagdi, Green one pot solvent-free synthesis of pyrano[2,3-*c*]pyrazoles and pyrazolo[1,5-*a*]pyrimidines, *Molecules* **15** (2010) 6619–6629; DOI: 10.3390/molecules15096619.
21. S. R. Jain and A. Kar, The antibacterial activity of some essential oils and their combination, *Planta Med.* **20** (1971) 118–123; DOI: 10.1055/s-0028-109967.
22. H. Naeimi, Z. S. Nazifi, S. M. Amininezhad and M. Amouheidari, Synthesis, characterization and *in vitro* antimicrobial activity of some new Schiff bases and their complexes, *J. Antibiot.* **66** (2013) 687–689; DOI: 10.1038/ja.2013.73.
23. M. B. Madhusudana Reddy, V. P. Jayashankara and M. A. Pasha, Glycine-catalyzed efficient synthesis of pyranopyrazoles via one-pot multicomponent reaction, *Synth. Commun.* **40** (2010) 2930–2934; DOI: 10.1080/00397910903340686.
24. D. Thomae, G. Kirsch and P. Seck, Synthesis of selenophene analogues of the tacrine series: comparison of classical route and microwave irradiation, *Synthesis* **10** (2008) 1600–1606; DOI: 10.1055/s-2008-1067001.
25. N. Galic, B. Peric, B. Kojic-Prodic and Z. Cimerman, Structural and spectroscopic characteristics of aroylhydrazones derived from nicotinic acid hydrazide, *J. Mol. Struct.* **559** (2001) 187–194; DOI: 10.1016/S0022-2860(00)00703-1.
26. V. Klimesova, L. Zahajska, K. Waisser, J. Kaustova and U. Mollmann, Synthesis and antimycobacterial activity of 1, 2, 4-triazole 3-benzylsulfanyl derivatives, *Farmaco* **59** (2004) 279–288; DOI: 10.1016/j.farmac.2004.01.006.