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Synthesis of some new annulated pyrazolo-pyrido (or pyrano) pyrimidine, pyrazolopyridine and pyranopyrazole derivatives

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The bifunctional pyrazolopyridine (**2**) and pyrano-pyrazole (**3**) derivatives were prepared by the reaction of 2--(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**1**) with *p*-methoxybenzaldehyde, malononitrile in the presence of ammonium acetate or piperidine, respectively. Compound **2** was used as the key intermediate to prepare the pyrazolo-pyrido-pyrimidine derivatives through its reaction with formic acid, formamide-formic acid-DMF, ammonium thiocyanate or reaction with triethyl orthoformate followed by cyclization with hydrazine hydrate. Reaction of **3** with triethyl orthoformate followed by cyclization with hydrazine hydrate gave the pyrazolo-pyrano-pyrimidine derivative **11**. Reaction of ethyl-3-oxo-2-[2-phenyl-diazenyl]butanoate and ethyl 2--[2-(4-chlorophenyl)diazenyl]-3-oxobutanoate with **1** to give the pyrazolone derivatives **13a** and **13b**, was also considered.

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A large number of pyrimidine derivatives are reported to exhibit antimycobacterial (1), antitumor (2), antiviral (3), anticancer (4), anti-inflammatory (4), analgesic (4), antifolate (5), antimicrobial (6), anti-fungal (7), antiproliferative (8) and antihistaminic (9) activities. They are also effective as antiplatelet agents with analgesic activity (10) and as a new drug for treatment of insomnia (11).

In view of these reports and as continuation of our recent studies (12, 13), the syntheses of a new series of compounds containing the pyrimidine moiety are now reported. The one-pot reaction of 2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**1**), *p*-methoxybenzaldehyde, malononitrile in the presence of ammonium acetate or piperidine afforded the bifunctional compounds **2** and **3**, which are used as key intermediates for the preparation of pyrimidine derivatives in their reactions with formic acid, formamide-formic acid-dimethylformamide, ammonium thiocyanate in acetic acid and triethyl orthoformate, followed by cyclization with hydrazine hydrate. Several compounds were screened for their antimicrobial activity.

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EXPERIMENTAL

All melting points are uncorrected. IR spectra (KBr) were recorded with a Shimadzu FT-IR 8201 PC spectrophotometer (Shimadzu, Japan). ^{1}H NMR were measured with a Varian UN 1009 S-60 T instrument (Varian-USA) using TMS as internal standard and mass spectra were measured with a Shimadzu GCMS-QP 100 EX mass spectrometer.

*Synthesis of 6-amino-1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1*H*-pyrazolo[3,4-b]pyridine-5-carbonitrile (**2**), 6-amino-1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano [2,3-c]pyrazole-5-carbonitrile (**3**) and 1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3,6-dimethyl-1*H*-pyrazolo[3,4-b]pyridine (**9**)*

To a mixture of 2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**1**) (0.01 mol), *p*-methoxybenzaldehyde (0.01 mol), malononitrile (0.01 mol) or ethyl acetoacetate (0.01 mol), ammonium acetate (0.03 mol) or a few drops of piperidine was added. The mixture was heated at 190 °C for 15 h. After cooling, the obtained solid was washed with water, dried and crystallized from butanol (products **2** and **9**) or ethanol (product **3**).

Compound **2**. – m.p. 228 °C. IR(KBr): ν_{max} 2206 (C≡N) and 1623 cm^{-1} (C=N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$), δ (ppm): 8.87–7.04 (7H, m, Ar-H), 3.33 (3H, s, OCH$_3$), 2.50 (3H, s, CH$_3$). Analysis of $C_{21}H_{15}N_7O_5$ (%): calcd. C 56.63, H 3.37, N 22.02; found C 56.36, H 3.58, N 21.80. Mass spectrum: ion peak at *m/z* 445 (6.52%).

Compound **9**. – m.p. 235 °C. IR(KBr): ν_{max} 1730 (CO), 1618 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$), δ (ppm): 8.86–7.73 (7H, m, Ar-H), 7.06 (1H, s, =CH), 3.33 (3H, s, OCH$_3$), 2.51 (3H, s, CH$_3$), 2.50 (3H, s, CH$_3$). Analysis for $C_{21}H_{16}N_6O_6$ (%): calcd. C 56.25, H 3.57, N 18.75; found C 56.47, H 3.63, N 18.88. Mass spectrum: ion peak at *m/z* at 419 (100%).

Compound **3**. – m.p. 160 °C. IR(KBr): ν_{max} 2218 (C≡N) and 1619 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$), δ(ppm): 8.83–7.15 (7H, m, Ar-H), 3.88 (1H, s, -CH pyran), 3.36 (3H, s, OCH$_3$), 2.50 (3H, s, CH$_3$). Analysis for $C_{21}H_{17}N_5O_5$ (%): calcd. C 60.14, H 4.06, N 16.71; found C 60.35, H 3.97, N 16.43.

*Synthesis of 1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1,8-dihydro-5*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidin-5-one (**4**)*

A solution of **2** (0.01 mol) and formic acid (10 mL) was refluxed for 10 h. The solvent was removed under reduced pressure and the separated solid was recrystallized from ethanol to give **4**.

m.p. 273 °C. IR(KBr): ν_{max} 3240 (NH), 1685 (CO) and 1620 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d$_6$), δ(ppm): 8.58 (1H, s, CH pyrimidine), 7.96–7.21 (7H, m, Ar-H), 3.71 (3H, s, OCH$_3$), 2.34 (3H, s, CH$_3$). Analysis of $C_{22}H_{15}N_7O_6$ (%): calcd. C 55.81, H 3.17, N 20.72; found C 55.68, H 3.32, N 20.58.

*Synthesis of 1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1*H*-pyrazolo-[4′,3′:5,6]pyrido[2,3-d]pyrimidin-5-amine (**5**)*

To a mixture of formamide (10 mL), formic acid (5 mL) and dimethyl formamide (5 mL), compound **2** (0.01 mol) was added and the reaction mixture was refluxed for 12 h. The solid that separated on cooling was filtered off and purified by boiling several times with ethanol to give **5**.

m.p. 328 °C. IR(KBr): ν_{max} 3370 (NH) and 1622 (C=N) cm^{-1}. Analysis for $C_{22}H_{16}N_8O_5$ (%): calcd. C 55.93, H 3.39, N 23.73; found C 56.23, H 3.21, N 23.54.

Synthesis of N*-[1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-7-thioxo-7,8-dihydro-1*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidin-5-yl]thiourea (**6**)*

To a solution of **2** (0.01 mol) in acetic acid (15 mL), ammonium thiocyanate (0.03 mol) was added and the reaction mixture was refluxed for 10 h. The solid that separated on cooling and dilution with water was filtered off and purified by boiling several times with ethanol to give **6**.

m.p. > 350 °C. IR(KBr): ν_{max} 3330 (NH) and 1620 (C=N) cm^{-1}. Analysis for $C_{23}H_{17}N_9O_5S_2$ (%): calcd. C 49.02, H 3.02, N 22.38, S 11.37; found C 48.82, H 3.25, N 22.61, S 11.42. Mass spectrum: ion peak at *m/z* 563 (2.62%).

*Synthesis of 1-(2,4-dinitrophenyl)-6-[(ethoxymethylene)amino]-4-(4-methoxyphenyl)-3-methyl-1*H*-pyrazolo[3,4-b]pyridine-5-carbonitrile (7) and 1-(2,4-dinitrophenyl)-6-[(ethoxymethylene)amino]-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (**10**)*

To a mixture of triethyl orthoformate (0.01 mol) and acetic anhydride (20 mL), compound **2** or **3** (0.01 mol) was added and the reaction mixture was refluxed for 5 h. The solvent was removed under reduced pressure and the separated solid was recrystallized from butanol (products **7** and **10**).

Compound **7**. – m.p. 180 °C. IR(KBr): ν_{max} 2210 (C≡N) and 1618 (NH) cm^{-1}. ^{1}H NMR (DMSO-d_6), δ(ppm): 8.50 (1H, s, N=CH), 8.21–7.13 (7H, m, Ar-H), 4.60 (2H, q, O**CH$_2$**CH$_3$), 3.54 (3H, s, OCH$_3$), 2.31 (3H, s, CH$_3$), 1.26 (3H, t, OCH$_2$**CH$_3$**). Analysis for $C_{24}H_{19}N_7O_6$ (%): calcd. C 57.49, H 3.79, N 19.56; found C 57.79, H 4.02, N 19.48.

Compound **10**. – m.p. 120 °C. IR(KBr): ν_{max} 2210 (C≡N) and 1610 (C=N) cm^{-1}. Analysis for $C_{24}H_{20}N_6O_7$ (%): calcd. C 57.14, H 3.97, N 16.67; found C 57.31, H 4.26, N 16.85.

*Synthesis of 1-(2,4-dinitrophenyl)-5-imino-4-(4-methoxyphenyl)-3-methyl-1,5-dihydro-6*H*-pyrazolo[4′,3′:5,6]pyrido[2,3-d]pyrimidin-6-amine (**8**) and 1-(2,4-dinitrophenyl)-5-imino-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrazolo[4′,3′:5,6]pyrano[2,3-d]pyrimidin-6(5*H*)-amine (**11**)*

To a solution of **7** or **10** (0.01 mol) in absolute ethanol (50 mL), hydrazine hydrate (0.01 mol) was added. The reaction mixture was refluxed for 2 h, concentrated, cooled,

and the solid product that separated out was filtered off and recrystallized from ethanol (products **8** and **11**).

Compound **8**. – m.p. 258 °C. IR(KBr): ν_{max} 3365 (NH_2) and 1610 (C=N) cm^{-1}. Analysis for $C_{22}H_{17}N_9O_5$ (%): calcd. C 54.21, H 3.49, N, 25.87; found C 53.92, H 3.63, N 25.59.

Compound **11**. – m.p. 158 °C. IR(KBr): ν_{max} 3370 (NH_2) and 1615 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6), δ(ppm): 9.62 (1H, s, N=CH), 8.00–7.38 (7H, m, ArH), 5.81 (1H, s, CH), 3.51 (3H, s, OCH_3), 2.17 (3H, s, CH_3). Analysis for $C_{22}H_{18}N_8O_6$ (%): calcd. C 53.88, H 3.67, N 22.86; found C 53.69, H 3.73, N 23.12.

*Synthesis of 4-[2-phenyldiazenyl]-2-(2,4-dimitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (**13a**) and 4-[2-(4-chlorophenyl)diazenyl]-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (**13b**)*

A mixture of 2,4-dinitrophenylhydrazine (0.01 mol) in ethanol (50 mL) ethyl 3-oxo-2-[2-phenyldiazenyl]butanoate (**12a**) or ethyl 2-[(4-chlorophenyl)diazenyl]-3-oxobutanoate (**12b**) (0.01 mol) was added and the reaction mixture was refluxed for 5 h. The solid that separated on cooling was crystallized from ethanol (products **13a** and **13b**).

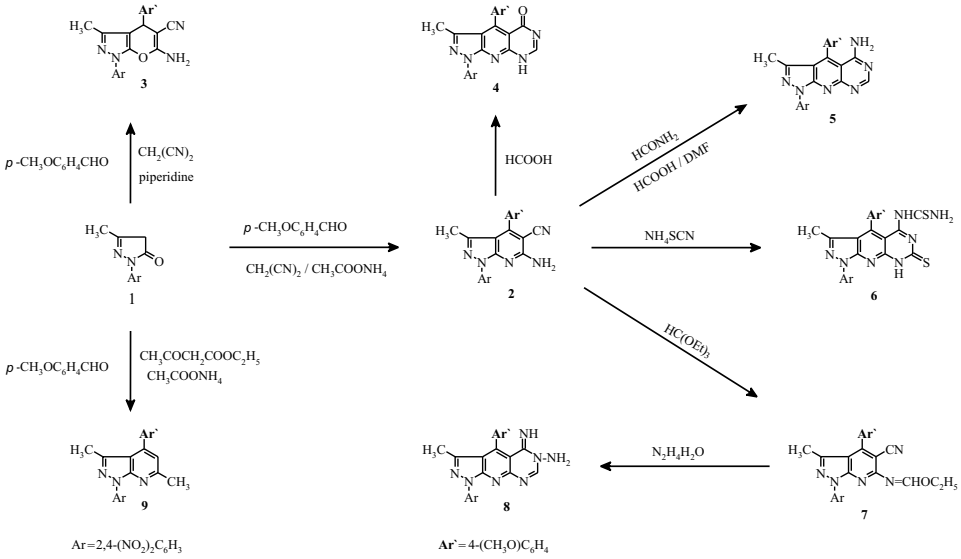
Compound **13a**. – m.p. 168 °C. IR(KBr): ν_{max} 3321 (NH), 1647 (CO) and 1617 (C=N) cm^{-1}. Analysis for $C_{16}H_{12}N_6O_5$ (%): calcd. C 52.17, H 3.26, N 22.83; found C 52.32, H 3.18, N 22.96.

Compound **13b**: m.p. 180 °C. IR(KBr): ν_{max} 3320 (NH), 1647 (CO) and 1614 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6), δ(ppm): 8.82–7.66 (7H, m, Ar-H), 5.05 (1H, s, -CH pyrazolone), 2.5 (3H, s, CH_3). Analysis for $C_{16}H_{11}ClN_6O_5$ (%): calcd. C 47.70, H 2.73, N 20.87; found C 47.89, H 2.78, N 21.12.

RESULTS AND DISCUSSION

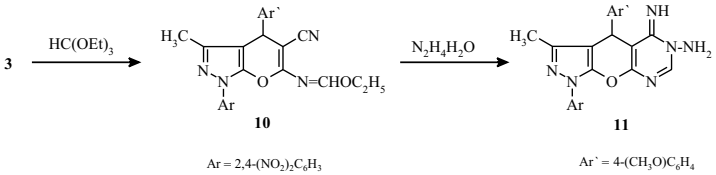
The one-pot reaction of pyrazolone **1** with *p*-methoxybenzaldehyde, in the presence of ammonium acetate afforded compound **2** through the adduct product of pyrazolone **1** and the first formed condensed product 2-(4-methoxybenzylidene)malononitrile followed by elimination of water and hydrogen. However, when the reaction was repeated in the presence of piperidine, the pyranopyrazole derivative **3** was obtained. In further reactions, the bifunctional compound **2** was used for preparation of pyrazolo-pyrido-pyrimidine derivatives. Thus, reaction of **2** with formic acid afforded compound **4**. Treatment of **2** with formamide in the presence of formic acid and dimethyl formamide gave compound **5**. On the other hand, heating of **2** with ammonium thiocyanate in boiling acetic acid gave compound **6**, while the reaction of **2** with triethyl orthoformate in boiling acetic anhydride gave the pyrazolo pyridine derivative **7**. Reaction of compound **7** with hydrazine hydrate gave the three fused ring **8**.

The present investigation also involved the reaction of pyrazolone **1** with anisaldehyde and ethyl acetoacetate in the presence of ammonium acetate. This reaction afforded pyrazolopyridine **9** through addition reaction, elimination of water, hydrogen and decarboethoxylation. The syntheses of compounds **2**–**9** are outlined in Scheme 1.

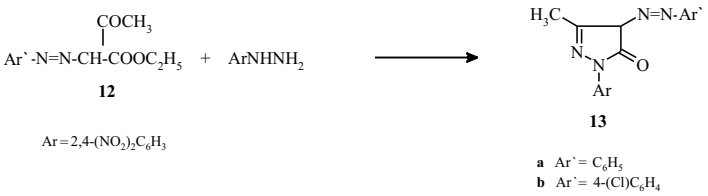


Scheme 1

Reaction of **3** with triethyl orthoformate afforded compound **10**. The structure of **10** was confirmed by its reaction with hydrazine hydrate to afford the pyrazolo pyranopyrimidine **11** (Scheme 2).



Scheme 2



Scheme 3

Interestingly, the reaction of ethyl 3-oxo-2-[2-phenyldiazenyl]butanoate (**12a**) and ethyl 2-[2-(4-chlorophenyl)diazenyl]-3-oxobutanoate (**12b**) with 2,4-dinitrophenylhydrazine in boiling ethanol afforded the pyrazolone derivatives **13a** and **13b**, respectively (Scheme 3).

Antimicrobial activity of the prepared compounds **2–6**, **8** and **11** was tested by the disk diffusion method (14). Whatman No. 1 filter paper disks were sterilized by autoclaving for one hour at 140 °C. The sterile disks were impregnated with the tested compounds (250 μg mL^{-1}). Agar plates were uniformly surface inoculated with fresh broth culture of *Staphylococcus aureus* and *Bacillus cereus* (as Gram positive strains), *Serratia marcescens* and *Proteus mirabilis* (as Gram negative strains), *Aspergillus fungytus* (as fungi). The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 5 °C for 1 h to permit good diffusion and were then transferred to an incubator at 28 °C for 24 h. The zones of inhibition were measured. The results of antimicrobial activity tests are listed in Table I. It is clear that compounds **5** and **6** possess high activity, while compounds **2–4**, **8** and **11** possess moderate activity against Gram positive strains.

Table I. Antimicrobial activity of some compounds[a,b]

Compd. No.	Gram positive bacteria		Gram negative bacteria		Fungi
	Staphylococcus aureus (NCTC-7447)	*Bacillus cereus* (ATCC-14579)	*Serratia marcescens* (IMRU-70)	*Proteus mirabilis* (NCTC-289)	*Aspergillus fungytus* (PP-29)
2	++	++	++	++	+
3	+++	++	+++	++	+
4	+++	++	+++	+++	++
5	+++	+++	+++	+++	++
6	+++	+++	+++	+++	++
8	++	+++	+++	++	+
11	+++	++	+++	+++	++

[a] c = 250 μg mL^{-1} (solvent: DMF)
[b] Reference substances: ampicillin for Gram positive and Gram negative bacteria, mycostatine for fungi.
+ Low activity (diameter 0.2–0.5 cm); ++ moderate activity (diameter 0.6–1.4 cm); +++ high activity (diameter 1.5–3.0 cm).

As far as Gram negative microorganisms are concerned, compounds **4–6** and **11** showed high activity and compounds **2**, **3** and **8** showed moderate activity.

Compounds **4–6** and **11** exerted moderate and compounds **2**, **3** and **8** low activity against fungi.

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S A Ž E T A K

Sinteza nekih anuliranih derivata pirazolopirido (ili pirano) pirimidina, pirazolopiridina i piranopirazola

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Bifunkcionalni derivati pirazolopiridina (**2**) i piranopirazola (**3**) pripravljeni su reakcijom 2-(2,4-dinitrofenil)-5-metil-2,4-dihidro-3*H*-pirazol-3-ona (**1**) s *p*-metoksibenzaldehidom, malononitrilom u prisutnosti amonijevog acetata ili piperidina. Iz spoja **2** pripravljeni su derivati pirazolopiridopirimidina reakcijom s mravljom kiselinom, smjesom formamida-mravlje kiseline-DMF, amonijevim tiocijanatom ili s trietil-ortoformatom, te naknadnom ciklizacijom s hidrazin hidratom. Reakcijom spoja **3** s trietil-ortoformatom te cilklizacijom s hidrazin hidratom dobiveni su derivati pirazolo-pirano-pirimidina (**11**). Reakcijom etil-3-okso-2-[2-fenil-diazenil]butanoata i etil 2-[2-(4-klorfenil)diazenil]--3-oksobutanoata sa spojem **1** sintetizirani su derivati pirazolona **13a** i **13b**.

Ključne riječi: derivati pirazolopiridina, derivati piranopirazola, sinteza

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