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-3H-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-pyranopyrazoline 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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EXPERIMENTAL

All melting points are uncorrected. IR spectra (KBr) were recorded with a Shimadzu FT-IR 8201 PC spectrophotometer (Shimadzu, Japan). 1H 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-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (2), 6-amino-1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyran-2,3-c]pyrazole-5-carbonitrile (3) and 1-(2,4-dinitrophenyl)-4-(4-methoxyphenyl)-3,6-dimethyl-1H-pyrazolo[3,4-b]pyridine (9)**

To a mixture of 2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (1) (0.01 mol), p-methoxybenzaldehyde (0.01 mol), malononitrile (0.01 mol) or ethyl acetooacetate (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): v_max 2206 (C=N) and 1623 cm⁻¹ (C=N) cm⁻¹. 1H NMR (DMSO-d₆), δ (ppm): 8.87–7.04 (7H, m, Ar-H), 3.33 (3H, s, OCH₃), 2.50 (3H, s, CH₃). Analysis of C₂₁H₁₅N₇O₅ (%): 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): v_max 1730 (CO), 1618 (C=N) cm⁻¹. 1H NMR (DMSO-d₆), δ (ppm): 8.86–7.73 (7H, m, Ar-H), 7.06 (1H, s, =CH), 3.33 (3H, s, OCH₃), 2.50 (3H, s, CH₃). Analysis for C₂₁H₁₆N₆O₆ (%): 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 419 (100%).

**Compound 3.** – m.p. 160 °C. IR(KBr): v_max 2218 (C=N) and 1619 (C=N) cm⁻¹. 1H NMR (DMSO-d₆), δ (ppm): 8.83–7.15 (7H, m, Ar-H), 3.88 (1H, s, =CH pyran), 3.36 (3H, s, OCH₃), 2.50 (3H, s, CH₃). Analysis for C₂₁H₁₇N₅O₅ (%): 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-5H-pyrazolo[4',3':5,6]pyridof[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): v_max 3240 (NH), 1685 (CO) and 1620 (C=N) cm⁻¹. 1H NMR (DMSO-d₆), δ (ppm): 8.58 (1H, s, CH pyrimidine), 7.96–7.21 (7H, m, Ar-H), 3.71 (3H, s, OCH₃), 2.34 (3H, s, CH₃). Analysis of C₂₂H₁₅N₇O₆ (%): 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-1H-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): v_max 3370 (NH) and 1622 (C=N) cm⁻¹. Analysis for C_{22}H_{16}N_{8}O_{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-1H-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): v_max 3330 (NH) and 1620 (C=N) cm⁻¹. Analysis for C_{23}H_{17}N_{9}O_{5}S_{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-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile (7) and 1-(2,4-dinitrophenyl)-6-{(ethoxymethylene)amino}-4-(4-methoxyphenyl)-3-methyl-1,4-dihydropyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidin-6(5H)-amine (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): v_max 2210 (C≡N) and 1618 (NH) cm⁻¹. ¹H NMR (DMSO-d₆), δ(ppm): 8.50 (1H, s, N=CH), 8.21–7.13 (7H, m, Ar-H), 4.60 (2H, q, OCH₂CH₃), 3.54 (3H, s, OCH₃), 2.31 (3H, s, CH₃), 1.26 (3H, t, OCH₂CH₃). Analysis for C_{24}H_{19}N_{7}O_{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): v_max 2210 (C≡N) and 1610 (C≡N) cm⁻¹. Analysis for C_{24}H_{20}N_{6}O_{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-6H-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]pyrido[2,3-d]pyrimidin-6(5H)-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): $\nu_{\max}$ 3365 (NH$_2$) and 1610 (C=N) cm$^{-1}$. Analysis for C$_{22}$H$_{17}$N$_9$O$_5$ (%): 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): $\nu_{\max}$ 3370 (NH$_2$) and 1615 (C=N) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$), $\delta$(ppm): 9.62 (1H, s, N=CH), 8.00–7.38 (7H, m, ArH), 5.81 (1H, s, CH)$_3$, 3.51 (3H, s, OCH$_3$), 2.17 (3H, s, CH$_3$). Analysis for C$_{22}$H$_{18}$N$_8$O$_6$ (%): 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-dinitrophenyl)-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): $\nu_{\max}$ 3321 (NH), 1647 (CO) and 1617 (C=N) cm$^{-1}$. Analysis for C$_{16}$H$_{12}$N$_6$O$_5$ (%): 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): $\nu_{\max}$ 3320 (NH), 1647 (CO) and 1614 (C=N) cm$^{-1}$. $^1$H NMR (DMSO-d$_6$), $\delta$(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$_6$O$_5$ (%): 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.
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 1

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 fumigatus (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 1. Antimicrobial activity of some compounds

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Fungi</th>
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<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus cereus</td>
<td>Serratia marcescens</td>
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<tr>
<td></td>
<td>(NCTC-7447)</td>
<td>(ATCC-14579)</td>
<td>(IMRU-70)</td>
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<tr>
<td>11</td>
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</tbody>
</table>

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.
REFERENCES


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

SAIED ABDULLAH EL-ASSIERY, GALAL HOSNI SAYED* i AHMED FOUDA

Bifunkcionalni derivati pirazolopiridina (2) i piranopirazola (3) pripravljeni su reakcijom 2-(2,4-dinitrofenil)-5-metil-2,4-dihidro-3H-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 ciklizacijom s hidrazin hidratom dobiveni su derivati pirazoloo-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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