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Facile Synthesis of New Pyrazolopyrimidine Derivatives of Potential Biosignificant Interest

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ABSTRACT. An easy and efficient route for the synthesis of some imidazo[1,2-*c*]pyrazolo[4,3-*e*]pyrimidines **3-6**, imidazo [1,2-*c*]pyrazolo[4,3-*e*]triazine **8**, pyrazolo[4,3-*e*]triazolo[1,5-*c*]pyrimidines **12-15** and pyrazolo-[3′,4′:4,5]pyrimido[1,6-*b*]triazines **16, 17** was described through the reaction of readily available 5-aminopyrazole-4-carbonitrile **1** with different reagents. The *in vitro* antimicrobial activity of some synthesized compounds was examined. Most of the tested compounds proved to be active as antibacterial and antifungal agents.

Key words: Imidazole pyrimidine, Pyrazole and triazine

INTRODUCTION

Pyrazolopyrimidine and related heterocyles are found to possess wide applications in the field of medicine and agriculture. They are biologically active isomeric purine analogues and have useful properties as antimetabolites in purine biochemical reactions.[1-3] They exhibit diversified pharmacological activities like tuberculostatic[4] antimicrobial activities,[5] neuroleptic,[6] CNS depressant,[7] antihypertensive[8] and antileishmanial.[9] Moreover, the pyrazole ring has shown to be the basic moiety for a number of dyes, drugs and agrochemicals.[10-14] Prompted by the varied biological activities of these heterocycles and as a part of our program aimed at developing new selective and environmentally friendly methodologies for the preparation of heterocyclic compounds,[15-17] we conducted the presented research to evaluate the potential of their antimicrobial activity. Herein, we found that 5-amino-1-(4-phenylphthalazin-1-yl)-1*H*-pyrazole-4-carbonitrile (**1**) is a highly versatile and useful building block for the synthesis of a wide range of pyrazolopyrimidine derivatives.

EXPERIMENTAL

Melting points were uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer 298 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Gemini 200 MHz instrument using TMS as internal reference with chemical shifts expressed as δ ppm. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 instrument (70 eV EI mode).

5-Amino-1-(4-phenylphthalazin-1-yl)-1H-pyrazole-4-carbonitrile (1)

A mixture of 1-hydrazinyl-4-phenylphthalazine (2.4 g, 6 mmol) and ethoxymethylenemalononitrile (0.64 g, 6 mmol) in absolute ethanol (40 mL) was heated at 80 °C for 4 h in a water bath. The reaction mixture was cooled, poured into ice-cold water and the precipitated product was filtered off and recrystallized from ethanol to give **1**. Yield, 63%; m.p. 186-188 °C; IR (KBr): υ 3410, 3200 (NH_2), 2215 cm^{-1} (CN); ^{1}H NMR (DMSO-d_6): δ 6.40 (brs, 2H, NH_2), 7.11-8.15 (m, 10H, ArH); Ms: m/z=312 (M^+); Anal. calcd for $C_{18}H_{12}N_6$ (312.33): C, 69.22; H, 3.87; N, 26.91%. Found: C, 69.38; H, 3.99; N, 26.76%.

4-(4,5-Dihydro-1H-imidazol-2-yl)-1-(4-phenylphthalazin-1-yl)-1H-pyrazol-5-amine (2)

To a mixture of **1** (1.6 g, 5 mmol) and ethylenediamine (4 mL) in absolute ethanol (15 mL), carbon disulfide (1 mL) was added dropwise. The reaction mixture was heated in a water bath for 10 h. After cooling, the reaction mixture was poured into ice-cold water and the solid precipitate was filtered off, washed with water and recrystallized from ethanol to give **2**. Yield, 60%; m.p. 196-198 °C; IR (KBr): υ 3430-3160 cm^{-1} (multiple bands, NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 3.30, 3.50 (2t, 4H, $2CH_2$), 6.30 (brs, 2H, NH_2), 7.13-8.10 (m, 10H, ArH), 8.50 (s, 1H, NH, exchangeable); ^{13}C NMR: δ=125.1, 127.2, 128.1, 129.3, 131.1, 134.2, 135.5 (C- of phenylphhthalzinyl moiety), 30.5, 44.3 (2 CH_2), 76.3 (C-2 of imidazolyl moiety), 100.2 (C-4), 139.5 (C-3), 140.2 (C-5); Anal. calcd for $C_{20}H_{17}N_7$ (355.40): C, 67.59; H, 4.82; N, 27.59%. Found: C, 67.70;

H, 4.98; N, 27.38%.

7-(4-Phenylphthalazin-1-yl)-6,7-dihydro-2H-imidazo [1,2-c]pyrazolo[4,3-e]pyrimidine-5(3H)-thione (3)

A mixture of **2** (0.7 g, 2 mmol) and carbon disulfide (5 mL) in dry pyridine was heated in a water bath for 20 h. The reaction mixture was cooled and poured into ice-HCl. The formed solid filtered off, washed with water and recrystallized from DMF to give **3**. Yield, 57%; m.p. 280-282 °C; IR (KBr): υ 3210 (NH), 1265 cm^{-1} (CS); ^{1}H NMR ($CDCl_3$): δ 3.70-3.90 (2t, 4H, $2CH_2$), 7.01-8.12 (m, 10H, ArH), 8.51 (s, 1H, NH, exchangeable); Anal. calcd for $C_{21}H_{15}N_7S$ (397.46): C, 63.46; H, 3.80; N, 24.67%. Found: C, 63.25; H, 3.59; N, 24.83%.

7-(4-Phenylphthalazin-1-yl)-3,7-dihydro-2H-imidazo [1,2-c]pyrazolo[4,3-e]pyrimidine (5)

A mixture of **2** (0.7 g, 2 mmol), triethyl orthoformate (4 mL) and glacial acetic acid (3 mL) was heated under reflux for 4 h. On cooling to room temperature, the solid precipitate that formed was filtered off and recrystallized from dioxane to give **4**. Yield, 71%; m.p. 241-243 °C; IR (KBr): υ 1610-1600 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.60-3.90 (2t, 4H, $2CH_2$), 7.11-8.10 (m, 11H, ArH); Anal. calcd for $C_{21}H_{15}N_7$ (365.39): C, 69.03; H, 4.14; N, 26.83%. Found: C, 69.20; H, 4.25; N, 26.65%.

5-Phenyl-7-(4-phenylphthalazin-1-yl)-3,5,6,7-tetrahydro-2H-imidazo[1,2-c]pyrazolo[4,3-e]pyrimidine (6)

A mixture of **2** (0.7 g, 2 mmol) and benzaldehyde (0.2 mL) in absolute ethanol (20 mL) containing concentrated HCl (0.3 mL) was stirred at 70 °C for 6 h. After cooling the mixture was neutrallized with aqueous sodium carbonate solution. The solid product obtained was filtered off and recrystallized from *n*-butanol to give **6**. Yield, 59%; m.p. 220-222 °C; IR (KBr): υ 3215 (NH), 1610-1605 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.40-3.62 (2t, 4H, $2CH_2$), 4.36 (s, 1H, CH), 7.15-8.11 (m, 15H, ArH), 8.40 (s, 1H, NH, exchangeable); Ms: m/z=443 (M^+); Anal. calcd for $C_{27}H_{21}N_7$ (443.50): C, 73.12; H, 4.77; N, 22.11%. Found: C, 73.26; H, 4.98; N, 22.01%.

7-(4-Phenylphthalazin-1-yl)-3,7-dihydro-2H-imidazo [1,2-c]pyrazolo[4,3-e][1,2,3]- triazine (8)

A concentrated solution of HCl (6 ml) was added to a solution of **2** (0.7g, 2 mmol) in acetic acid (6 ml). The mixture was cooled to 0-5 °C and sodium nitrite (1 g) was added gradually with stirring. The reaction mixture was left to stand in an ice bath for 2 h, then diluted with water, filtered off, washed with water and recrystallized from DMF to give **8**. Yield, 51%; m.p. 237-239 °C; IR (KBr): υ 1605-1600 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ 3.40, 3.70 (2t, 4H, $2CH_2$), 7.05-8.11 (m, 10H, ArH); Anal. calcd for $C_{20}H_{14}N_8$ (366.38): C, 65.56; H, 3.85; N, 30.58%. Found: C, 65.34; H, 3.61; N, 30.70%.

Ethyl N-4-cyano-1-(4-phenylphthalazin-1-yl)-1H-pyrazol-5-ylformimidate (9)

A mixture of **1** (3.1 g, 10 mmol), triethyl orthoformate (5 mL) and acetic anhydride (20 mL) was heated under reflux for 5 h. The solvent was removed under reduced pressure. The residue obtained was recrystallized from benzene to give **9**.Yield, 67%; m.p. 217-219 °C; IR (KBr): υ 2217 cm^{-1} (CN); ^{1}H NMR (DMSO-d_6): δ 1.25 (t, 3H, CH_3), 4.2 (q, 2H, CH_2), 7.05-8.13 (m, 11H, ArH and CH=N); Anal. calcd for $C_{21}H_{16}N_6O$ (368.39): C, 68.47; H, 4.38; N, 22.81%. Found: C, 68.30; H, 4.18; N, 22.97%.

4-Imino-1-(4-phenylphthalazin-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-amine (10)

A mixture of **9** (3.7 g, 10 mmol) and hydrazine hydrate (2 mL) in ethanol (30 mL) was stirred for 1 h, at room temperature. The precipitate which formed was filtered off, washed with water and recrystallized from dioxane to give **10**. Yield, 63%; m.p. 246-248 °C; IR (KBr): υ 3375-3210 cm^{-1} (multiple bands NH_2, NH); ^{1}H NMR (DMSO-d_6): δ 6.40 (brs, 2H, NH_2), 7.10-8.13 (m, 11H, ArH), 8.95 (s, 1H, NH, exchangeable); Anal. calcd for $C_{19}H_{14}N_8$ (354.37): C, 64.40; H, 3.98; N, 31.62%. Found: C, 64.25; H, 3.75; N, 31.86%.

General procedure for the preparation of 11a,b

A mixture of **10** (0.7 g, 2 mmol) and aromatic aldehydes *viz* benzaldehyde and *p*-chlorobenzaldehyde (2 mmol) in absolute ethanol (25 mL) was heated under reflux for 5 h. The precipitate formed was filtered off, washed with water and recrystallized from ethanol to give **11a,b**.

***N*-Benzylidene-4-imino-1-(4-phenylphthalazin-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-amine (11a)**: Yield, 59%; m.p. 207-209 °C; IR (KBr): υ 3210 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 7.11-8.10 (m, 17H, ArH and CH=N), 8.70 (s, 1H, NH, exchangeable); Anal. calcd for $C_{26}H_{18}N_8$ (442.47): C, 70.58; H, 4.10; N, 25.32%. Found: C, 70.63; H, 4.21; N, 25.48%.

***N*-(4-Chlorobenzylidene)-4-imino-1-(4-phenylphthalazin-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-5(4H)-amine (11b)**: Yield, 61%; m.p. 231-233 °C; IR (KBr): υ 3215 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 7.14-8.12 (m, 16H, ArH and

CH=N), 8.65 (s, 1H, NH, exchangeable); Anal. calcd for $C_{26}H_{17}ClN_8$ (476.92): C, 65.48; H, 3.59; N, 23.50%. Found: C, 65.24; H, 3.40; N, 23.64%.

General procedure for the preparation of 12a-e

A mixture of **10** (0.7 g, 2 mmol) and triethyl orthoformate or acetyl chloride or benzoyl chloride (2 mmol) in dry benzene (25 mL) was heated under reflux for 4 h. The solid formed was filtered off and recrystallized from dioxane to give **12a-c**. While a mixture of **10** (0.7 g, 2 mmol) and ethyl cyanoacetate or diethyl oxalate (2 mmol) in absolute ethanol (25 mL) was heated under reflux for 5 h. The solid formed was filtered off and recrystallized from dioxane to give **12d,e** respectively.

***7-(4-Phenylphthalazin-1-yl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (12a)*:** Yield, 70%; m.p. 251-253 °C; IR (KBr): υ 1610-1600 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.10-8.05 (m, 12H, ArH); Anal. calcd for $C_{20}H_{12}N_8$ (364.36): C, 65.93; H, 3.32; N, 30.75%. Found: C, 65.78; H, 3.20; N, 30.87%.

***2-Methyl-7-(4-phenylphthalazin-1-yl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (12b)*:** Yield, 73%; m.p. 265-267 °C; IR (KBr): υ 1605-1600 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 3H, CH_3), 7.05-8.12 (m, 11H, ArH); Anal. calcd for $C_{21}H_{14}N_8$ (378.39): C, 66.66; H, 3.73; N, 29.61%. Found: C, 66.78; H, 3.96; N, 29.40%.

***2-Phenyl-7-(4-phenylphthalazin-1-yl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine* (12c):** Yield, 69%; m.p. 227-229 °C; IR (KBr): υ 1610-1605 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.12-8.10 (m, 16H, ArH); Anal. calcd for $C_{26}H_{16}N_8$ (440.46): C, 70.90; H, 3.66; N, 25.44%. Found: C, 70.72; H, 3.51; N, 25.56%.

***2-[7-(4-Phenylphthalazin-1-yl)-7H-pyrazolo[4,3-e][1,2,4]-triazolo[1,5-c]pyrimidin-2-yl]acetonitrile (12d)*:** Yield, 74%; m.p. 246-248 °C; IR (KBr): υ 2218 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$): δ 4.3 (s, 2H, CH_2), 7.10-8.12 (m, 11H, ArH); Anal. calcd for $C_{22}H_{13}N_9$ (403.40): C, 65.50; H, 3.25; N, 31.25%. Found: C, 65.69; H, 3.41; N, 31.12%.

***Ethyl 7-(4-phenylphthalazin-1-yl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine-2-carboxylate (12e)*:** Yield, 63%; m.p. 266-268 °C; IR (KBr): υ 1730 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 4.40 (q, 2H, CH_2), 7.12-8.15 (m, 11H, ArH); Anal. calcd for $C_{23}H_{16}N_8O_2$ (436.43): C, 63.30; H, 3.70; N, 25.68%. Found: C, 63.42; H, 3.93; N, 25.51%.

Ethyl 2-oxo-7-(4-phenylphthalazin-1-yl)-2H-pyrazolo [4,3-e][1,2,4]triazolo[1,5-c]-pyrimidine-3(7H)-carboxylate (14)

A mixture of **10** (0.7 g, 2 mmol) and ethyl chloroformate (5 mL) in dry benzene was heated under reflux for 8 h. On cooling, the solid obtained was recrystallized from dioxane to give **14**. Yield, 65%; m.p. 263-265 °C; IR (KBr): υ 1730, 1680 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, CH_3), 4.35 (q, 2H, CH_2), 7.12-8.11 (m, 11H, ArH); Ms: m/z=452 (M^+); Anal. calcd for $C_{23}H_{16}N_8O_3$ (452.42): C, 61.06; H, 3.56; N, 24.77%. Found: C, 61.20; H, 3.70; N, 24.54%.

7-(4-Phenylphthalazin-1-yl)-3,7-dihydro-2H-pyrazolo [4,3-e][1,2,4]triazolo[1,5-c]-pyrimidine-2-thione (15)

A mixture of **10** (0.7g, 2 mmol), carbon disulfide (3 mL) and potassium hydroxide (0.3 g) in ethanol (25 mL) was heated under reflux for 15 h. The solvent was removed under reduced pressure and the residue was acidified with acetic acid. The formed precipitate was filtered off and recrystallized from benzene to give **15**. Yield, 54%; m.p. 272-274 °C; IR (KBr): υ 3240 (NH), 1260 cm^{-1}(CS); ^{1}H NMR (DMSO-d_6): δ 7.10-8.10 (m, 11H, ArH), 8.45 (s, 1H, NH, exchangeable); Anal. calcd for $C_{20}H_{12}N_8S$ (396.43): C, 60.59; H, 3.05; N, 28.27%. Found: C, 60.71; H, 3.20; N, 28.10%.

8-(4-Phenylphthalazin-1-yl)-2,8-dihydropyrazolo[3′,4′: 4,5]pyrimido[1,6-b][1,2,4]triazin-3(4H)-one (16)

***Method a*:** A mixture of **10** (0.7 g, 2 mmol) and ethyl chloroacetate (2 mmol) in ethanolic sodium ethoxide solution [prepared from sodium metal (46 mg) and absolute ethanol (20 ml) was heated under reflux for 6 h. The reaction mixture was cooled, poured onto ice (25 g). The formed precipitate was filtered off and recrystallized from dioxane to give **16**. Yield, 63%.

***Method b*:** A mixture of **10** (0.7 g, 2 mmol) and chloroacetyl chloride (2 mmol) in dry dioxane (25 mL) was allowed to stand overnight at room temperature. The formed precipitate was filtered off and recrystallized from dioxane to give **16**. Yield, 60%; m.p. 270-272 °C; IR (KBr): υ 3220 (NH), 1670 cm^{-1}(CO); ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 2H, CH_2), 7.13-8.14 (m, 11H, ArH), 8.50 (s, 1H, NH, exchangeable); Anal. calcd for $C_{21}H_{14}N_8O$ (394.39): C, 63.95; H, 3.58; N, 28.41%. Found: C, 63.76; H, 3.45; N, 28.59%.

8-(4-Phenylphthalazin-1-yl)-4,8-dihydropyrazolo[3′,4′: 4,5]pyrimido[1,6-b][1,2,4]triazin-2,3-dione (17)

To a solution of **10** (0.7g, 2 mmol) in dry benzene (25 mL), oxalyl chloride (3 mL) was added and the reaction mixture was heated under reflux for 10 h. On cooling the formed solid product was filtered off and recrystallized from DMF to give **17**. Yield, 55%; m.p. 226-228 °C; IR

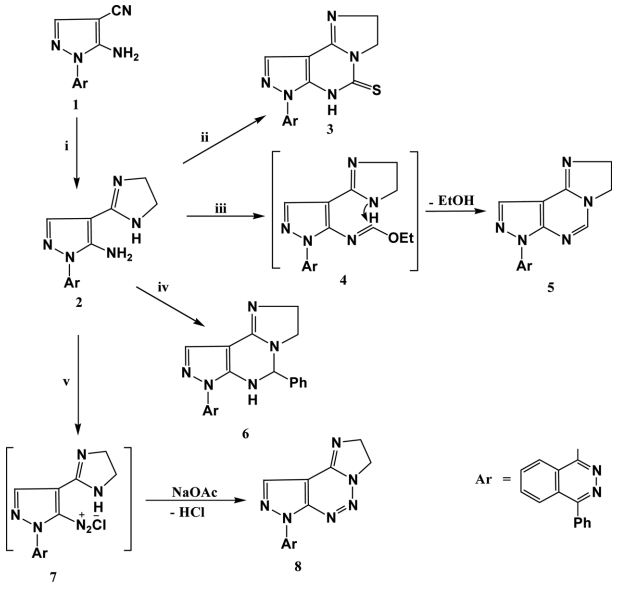
(KBr): υ 3230 (NH), 1675-1670 cm^{-1} (CO); Ms: m/z = 408 (M^+); Anal. calcd for $C_{21}H_{12}N_8O_2$ (408.37): C, 61.76; H, 2.96; N, 27.44%. Found: C, 61.54; H, 2.81; N, 27.56%.

RESULTS AND DISCUSSION

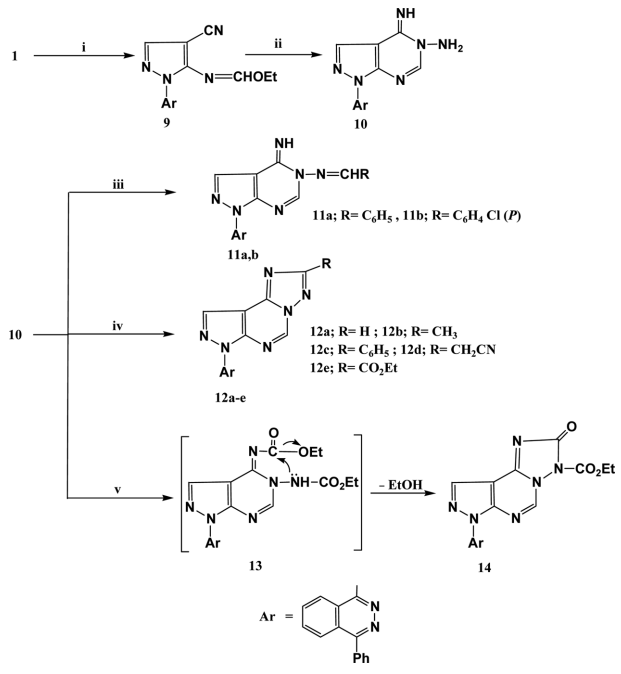
The condensation of 1-hydrazinyl-4-phenylphthalazine with ethoxymethylene-malononitrile in absolute ethanol afforded the target compound, 5-amino-1-(4-phenylphthalazin-1-yl)-1*H*-pyrazole-4-carbonitrile (**1**), *Scheme* 1.

The IR spectrum of **1** showed absorption bands in the regions 3410, 3200 and 2215 cm^{-1} for amino and nitrile groups. The ^1H NMR spectrum of **1** showed signals at δ 6.40 ppm for amino group and aromatic multiplet in the region at δ 7.11-8.15 ppm. In addition, their mass spectrum revealed the corresponding molecular ion peak for the molecular formula $C_{18}H_{12}N_6$. The reaction of **1** with ethylenediamine in the presence of a catalytic amount of carbon disulfide furnished 4-(4,5-dihydro-1*H*-imidazol-2-yl)-1-(4-phenylphthalazin-1-yl)-1*H*-pyrazol-5-amine (**2**). The structure of **2** was deduced from their elemental analyses and spectral data. Its IR spectrum showed multiple absorption bands in the region 3430-3160 cm^{-1} due to NH_2 and NH groups and devoid any absorption band due to nitrile group.

Poly-functionalized heterocyclic compounds play important role in the drug industry and represent 68% of the drugs on the market.[18] Therefore; it is not surprising that synthesis of poly-functionalized heterocyclic compounds has received significant attention. In that direction, we carried out the reaction of **2** with carbon disulfide in dry pyridine to afford the imidazo[1,2-*c*]-pyrazolo[4,3-*e*]pyrimidine derivative **3**. Alternative approaches for other imidazopyrazolopyrimidine derivatives **5**,**6** can be achieved through the reaction of **2** with triethyl orthoformate and/or benzaldehyde, respectively. When the pyrazole derivative **2** was treated with sodium nitrite in acetic acid, it yielded one isolable product which was analyzed correctly for $C_{20}H_{14}N_8$. The structure of the isolated product was assigned as 7-(4-phenylphthalazin-1-yl)-3,7-dihydro-2*H*-imidazo[1,2-*c*]pyrazolo[4,3-*e*][1,2,3]triazine (**8**) based on its elemental analysis and spectral data, *Scheme* 1. Its IR spectrum was free of amino and NH absorption bands in the region 3450-3000 cm^{-1}. In further exploratory studies, we observed that the condensation of **1** with triethyl orthoformate in refluxing acetic anhydride yielded formimidate derivative **9**, which underwent further cyclization upon treatment with hydrazine hydrate at room temperature affording 4-imino-1-(4-phenylphthalazin-1-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-5(4*H*)-amine (**10**). The structure of **10** was established on the basis of IR spectrum which showed the absence of any absorption band for nitrile group, *Scheme* 2. The reactivity of compound **10** towards some carbon electrophiles was also investigated. Thus, when compound **10** was treated with aromatic aldehydes *viz* benzaldehyde



***Scheme* 1.** i) $H_2N(CH_2)_2NH_2$, CS_2; ii) CS_2; iii) $HC(OEt)_3$; iv) PhCHO; v) $NaNO_2$/HCl, AcOH.

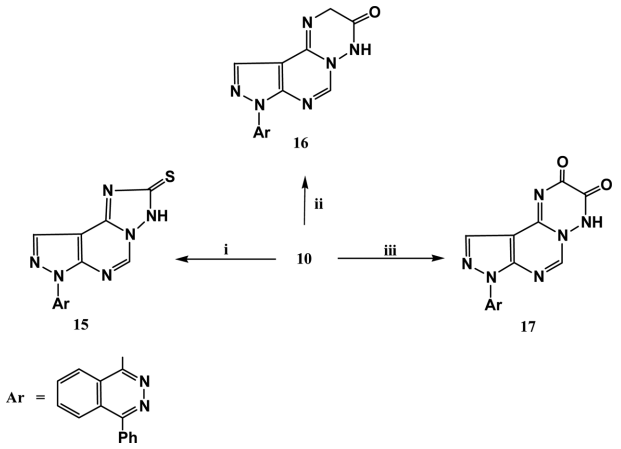


***Scheme* 2.** i) $HC(OEt)_3$, AC_2O; ii) $N_2H_4.H_2O$; iii) RCHO; iv) $HC(OEt)_3$ or CH_3COCl or PhCOCl or $NCCH_2CO_2Et$ or $(COOEt)_2$; v) 2 $ClCO_2Et$.

and *p*-chlorobenzaldehyde it afforded the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **11a**,**b**, respectively. The next goal was the synthesis of derivatives of another heterocyclic systems of pyrazolo[4,3-e][1,2,4] triazolo [1,5-*c*]pyrimidines and pyrazolo[3′,4′:4,5]pyrimido[1,6-*b*]triazin-3-(4*H*)-ones. Thus, the reaction of **10** with triethyl orthoformate or acetyl chloride or benzoyl chloride or ethyl cyanoacetate or diethyl oxalate afforded the substituted triazolo[1,5-*c*]pyrimidine derivatives **12a-e**, respectively. Next, the treatment of **10** with two moles of ethyl chloroformate in dry benzene afforded the ester derivative **14**. Formation of **14** was assumed to proceed *via* the intermediate bis(ethoxycarbonyl) derivative **13**, which cyclized into **14** with elimination of ethanol molecule, *Scheme* 2.

Also, the treatment of **10** with carbon disulfide in alcoholic potassium hydroxide solution gave pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2-thione derivative **15**, *Scheme* 3.

As an extension of this synthetic route, the behavior of compound **10** for construction of polyfunctionally substituted bioactive triazine derivatives[19,20] was investigated. Thus, the interaction of compound **10** with ethyl chloroacetate in ethanolic sodium ethoxide solution afforded pyrimidotriazine derivative **16**. Compound **16** was alternatively obtained by an independent synthesis *via* treatment of compound **10** with chloroacetyl chloride in dry dioxane. Similarly, when compound **10** was subjected to react with an equimolar amount of chloroacetyl chloride in dry benzene, it furnished pyrimidotriazin-2,3-dione derivative **17**, *Scheme* 3. The structures of the synthesized compounds were assigned on the basis of elemental analysis and spectral data. (*cf.* experimental).



***Scheme* 3.** i) CS_2; ii) $ClCH_2CO_2Et$ or $ClCOCH_2Cl$; iii) ClCOCOCl.

ANTIMICROBIAL ACTIVITY

The antimicrobial activities of some synthesized compounds were determined in *vitro* using the hole plate and filter paper methods[21] against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) in addition to some fungal plant pathogen (Aspergillus flavus and Candida albicans). Also, a comparison between the activity of our synthesized compounds and standard drugs (Tetracycline, Amphotericin B) was carried out. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/mL solution. DMSO alone showed no inhibition zone. The inhibition zones of microbial growth produced by different compounds were measured in millimeters at the end of an incubation period of 48 h at 28 ºC. As can be seen from *Table* 1, good to improved antibacterial and antifungal activity was observed for most of the tested compounds against the selected micro-organisms used in the study.

In conclusion, we have been able to describe convenient protocols for the preparation of a number of annulated

***Table* 1.** The antimicrobial activity of tested compounds

Compound No	Staphylococcus aureus	Escherichia coli	Aspergillus flavus	Candida albicans
2	15	14	--	--
3	16	22	15	14
6	13	15	--	--
8	13	15	--	--
10	14	15	--	12
12a	17	18	19	10
12d	23	19	12	14
14	28	14	10	11
15	29	18	12	10
17	24	17	13	11
Tetracycline	28	32	--	--
Amphotericin B	--	--	15	17

pyrazolopyrimidine derivatives, which exhibited good to excellent antimicrobial activity against most of the tested strains.

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