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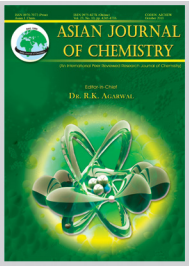

Synthesis and Antimicrobial Evaluation of Some New Pyrazole, Fused Pyrazolo[1,5-a]-pyrimidine and Pyrazolo[1,5-d]pyrimido[4,5-d][1,2,3]triazine Derivatives

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A variety of 3-arylazo-5-amino and 7-amino pyrazolo[1,5-a] pyrimidines were obtained via reacting **1** with cinnamonitriles and methoxymethylene malononitrile. Diazotized **1** coupled with active methylene reagents to yield pyrazolo[5,1-c][1,2,4]triazines. The products were screened for their antifungal and antibacterial activity properties and showed promising results. The mechanism of the studied reactions is discussed. The structures of the compounds prepared were elucidated on the basis of their elemental analyses, spectral data and alternate synthesis.

Key Words: Arylazo, Pyrazolopyrimidine, Pyrazolotriazinethione, Pyrazolotriazine, Antimicrobial activities.

INTRODUCTION

Polyfunctionally substituted azoloazines are interesting as potential biodegradable agrochemicals[1]. The synthetic analogues of purines are widely used in the medical science and in clinical medicine[2-5] and effective biological activities[6-8]. Pyrazolopyrimidine derivatives have been found to possess antitumor and antileukemia activities[9-11]. The derivatives of these ring systems have useful properties as antimetabolites in biochemical reactions[12]. The considerable biological activity of pyrazolopyrimidines[13] as adenosine cyclic monophospahte, phosphodieterase inhibitors[14], antischistosomal agents[15] and antimetabolites[16] is perhaps beyond this interest. Thus, it has been found that the aminopyrazoles **1**, which prepared according to the literature procedure[17] was used to synthesize several new pyrazoloazines *via* reaction with non-symmetrical double bond system and with different active methylene reagents, as well as with isothiocyanates. In addition, some of the newly synthesized compounds were screened for their antibacterial and antifungal activities

EXPERIMENTAL

All melting points were determined on an Electrothermal Gallenkamp apparatus and are uncorrected. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz). The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt.

The starting materials pyrazole **1** was prepared as reported in literature[17].

General procedure for the synthesis of 5-amino-2-methyl-7-aryl-3-arylazo-pyrazolo[1,5-a]pyrimidine-6-carbonitriles (3a-d): A mixture of the 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) (2.36 g, 10 mmol) and the appropriate arylidenemalononitrile **2a-d** (10 mmol) was refluxed in pyridine (30 mL) for 4 h then left to cool. The reaction mixture was poured onto ice cooled water, neutralized with hydrochloric acid. The solid product was collected, washed with water, dried and finally recrystallized from ethanol to afford the corresponding compounds **3a-d**.

5-Amino-3-(4-chlorophenylazo)-2-methyl-7-phenyl-pyrazolo[1,5-a]pyrimidine-6-carbonitrile (3a): Yellow solid; m.p. 293 ºC (from ethanol); yield 75 %; IR (KBr, ν_{max}, cm^{-1}) : 3436, 3301 (NH_2), 3058 (CH-arom.), 2923 (CH-aliph.), 2214 (CN); ^{1}H NMR (DMSO-d_6) δ/ppm: 2.51 (s, 3H, CH_3), 3.37 (s, 2H, NH_2), 7.51-7.89 (m, 9H, ArH); MS m/z (%): 388 (M^++1, 34); Anal. calcd. For $C_{20}H_{14}N_7Cl$ (m.w. 387.83): C, 61.94; H, 3.64; N, 25.28 %. Found: C, 61.78; H, 3.79; N, 25.33.

5-Amino-3-(4-chlorophenylazo)-7-(4-methoxyphenyl)-2-methylpyrazolo[1,5-a]pyrimidine-6-carbonitrile (3b): Yellow solid; m.p. 164 ºC (from ethanol); yield 65 %; IR (KBr,

ν_{max}, cm^{-1}): 3343, 3267 (NH_2), 3048 (CH-arom.), 2927 (CH-aliph.), 2211 (CN); ^1H NMR (DMSO-d_6) δ/ppm: 2.38 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.87 (s, 2H, NH_2), 7.04-7.84 (m, 8H, ArH); MS m/z (%): 418 (M^++1, 14); Anal. calcd. for $C_{21}H_{16}N_7OCl$ (m.w. 417.85): C, 60.36; H, 3.86; N, 23.46 %. Found: C, 60.22; H, 3.79; N, 23.41.

5-Amino-7-(4-chlorophenyl)-3-(4-chlorophenylazo)-2-methylpyrazolo[1,5-a]pyrimidine-6-carbonitrile (3c): Yellow solid; m.p. 212 ºC (from ethanol); yield 60 %; IR (KBr, ν_{max}, cm^{-1}): 3405, 3298 (NH_2), 3047 (CH-arom.), 2995 (CH-aliph.), 2211 (CN); ^1H NMR (DMSO-d_6) δ/ppm: 2.53 (s, 3H, CH3), 3.59 (s, 2H, NH_2), 7.72-8.57 (m, 8H, ArH); MS m/z (%): 422 (M^+, 14); Anal. calcd. for $C_{20}H_{13}N_7Cl_2$ (m.w. 422.27): C, 56.89; H, 3.10; N, 23.22 %. Found: C, 56.45; H, 3.08; N, 23.10.

5-Amino-3-(4-chlorophenylazo)-2-methyl-7-(pyridin-3-yl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile (3d): Yellow solid; m.p. 288 ºC (from ethanol); Yield 62 %; IR (KBr, ν_{max}, cm^{-1}): 3340, (NH_2), 3017 (CH-arom.), 2923 (CH-aliph.), 2212 (CN); ^1H NMR (DMSO-d_6) δ/ppm: 2.53 (s, 3H, CH_3), 3.59 (s, 2H, NH_2), 7.72-8.57 (m, 8H, ArH); MS m/z (%) 389 (M^++1, 12); Anal. calcd. For $C_{19}H_{13}N_8Cl$ (m.w. 388.81): C, 58.69; H, 3.37; N, 28.82 %. Found: C, 58.54; H, 3.21; N, 28.04.

7-Amino-3-(4-chlorophenylazo)-2-methylpyrazolo [1,5-a]pyrimidine-6-carbonitrile (5): To a mixture of **1** (3.117 g, 10 mmol) and the 2-ethoxymethylene malononitrile compound **4** (1.081 g, 10 mmol) in ethanol (30 mL) was added piperidine (0.85 mL, 10 mmol). The mixture was refluxed for 3 h then left to cool. the solid formed was filtered and crystallized from ethanol to give pure compound **5** as yellow solid; mp > 300 ºC (from ethanol); yield 80 %; IR (KBr, ν_{max}, cm^{-1}): 3304, 3232 (NH_2), 3060 (CH-arom.), 2928 (CH-aliph.), 2219 (CN); ^1H NMR (DMSO-d_6) δ/ppm: 2.51 (s, 3H, CH_3), 3.31 (s, 2H, NH_2), 7.57-7.82 (m, 4H, ArH), 8.54 (s, 1H, pyrimidine H); MS m/z (%) 311 (M^+, 44); Anal. calcd. for $C_{14}H_{10}N_7Cl$ (m.w. 311.73): C, 53.94; H, 3.23; N, 31.45 %. Found: C, 53.10; H, 3.20; N, 31.35.

5-Amino-4(4-chlorophenylazo)-1-(2-cyanoethyl)-3-methyl pyrazole (7): A mixture of the 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (1) (2.36 g, 10 mmol) and the acrylonitrile (0.531 g, 10 mmol) was refluxed in pyridine (30 mL) for 4 h then left to cool. The reaction mixture was poured onto ice cooled water, neutralized with hydrochloric acid. The solid product was collected, washed with water, dried and finally recrystallized from ethanol to give pure **7** as pale yellow solid; m.p. 192 ºC (from ethanol); yield 72 %; IR (KBr, ν_{max}, cm^{-1}) : 3444, 3301 (NH_2) 3050 (CH-arom.), 2925 (CH-aliph.), 2225 (CN); ^1H NMR (DMSO-d_6) δ/ppm: 2.36 (s, 3H, CH_3), 3.31 (s, 2H, NH_2), 3.44 (t, 2H, CH_2), 4.70 (t, 2H, CH_2), 7.90-8.15 (m, 4H, ArH); MS m/z (%) 288 (M^+, 71); Anal. calcd. for $C_{13}H_{13}N_6Cl$ (m.w. 288.74): C, 54.08; H, 4.54; N, 29.11 %. Found: C, 54.10; H, 4.50; N, 29.10.

5-Acetylamino-4-(4-chlorophenylazo)-3-methyl pyrazole (9). Method A: A solution of 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) (2.36 g, 10 mmol) in acetic acid (10 mL) was refluxed for 8 h then left to cool. The solid that precipitated was collected by filtration, washed with water, dried and finally crystallized from ethanol to give the respective 5-acetylamino-4-(4-chlorophenylazo)-3-methyl pyrazole (**9**).

Method B: A solution of 5-amino-4(4-chlorophenylazo)-1-(2-cyanoethyl)-3-methyl pyrazole (**7**) (1.444 g, 5 mmol) in acetic acid (10 mL) was refluxed for 8 h then left to cool. The solid that precipitated was collected by filtration, washed with water, dried and finally crystallized from ethanol to give pure compound **9** as yellow solid; mp 258 ºC (from ethanol); yield 62 %; IR (KBr, ν_{max}, cm^{-1}) : 3296,3205 (NH), 2921 (CH-arom.), 1694 (CO); ^1H NMR (DMSO-d_6) δ/ppm: 2.13 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.55-7.85 (m, 4H, ArH), 10.77 (s, 1H, pyrazole H), 12.97 (s, 1H, NH); MS m/z (%) 277 (M^+, 100); Anal. calcd. For $C_{12}H_{12}N_5OCl$ (m.w. 277.71): C, 51.90; H, 4.36; N, 25.22 %. Found: C, 51.88; H, 4.31; N, 25.10.

Synthesis of 3-(4-chlorophenylazo)-2,5-Dimethyl-7-hydroxy-pyrazolo[1,5-a] pyrimidine (10): To a mixture of 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) (2.36 g, 10 mmol) and ethyl acetoacetate (1.3 g, 10 mmol) in ethanol, few drops of piperidine are added. The reaction mixture was refluxed for 8 h. The crude product was filtered off, washed with water, dried and finally crystallized from ethanol to give pure compound **10** as yellow solid; mp 255 ºC (from ethanol); yield 54 %; IR (KBr, ν_{max}, cm^{-1}): br. 3480-3340 (OH), 3060 (CH-arom.); ^1H NMR (DMSO-d_6) δ/ppm: 2.48 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 7.22-8.01 (m, 5H, ArH), 10.15 (s, 1H, OH); MS m/z (%) 302 (M^++1, 27); Anal. calcd. for $C_{14}H_{12}N_5OCl$ (m.w. 301.73): C, 55.73; H, 4.01; N, 23.21 %. Found: C, 54.92; H, 3.60; N, 22.88.

Synthesis of 1-Benzoyl-3-(5-methyl-4-(4-chlorophenyl-azo)-2*H*-pyrazole-yl)thiourea (11): To a solution of benzoyl isothiocyanate (1.632 g, 10 mmol) in dry acetone (50 mL) 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) (2.36 g, 10 mmol) was added. The mixture was refluxed for 2 h; the solvent was evaporated in vacuo. The reaction was titrated with ethanol, the solid product was filtered off, washed with ether and dried to afford the title compound **11** as yellow crystals solid; m.p. 224 ºC (from Dioxane); yield 66 %; IR (KBr, ν_{max}, cm^{-1}) : 3455, 3426, 3321 (NH), 3090 (CH-arom.), 2990 (CH-aliph.), 1671 (CO), 1208 (C=S); ^1H NMR (DMSO-d_6) δ/pmm: 2.10 (s, 3H, CH_3), 7.09-8.05 (m, 9H, ArH), 11.30 (s, 1H, pyrazole H), 11.56 (S, 1H, NH), 11.75 (s, 1H, NH); MS m/z (%) 398 (M^+, 20); Anal. calcd. for $C_{18}H_{15}N_6OSCl$ (m.w. 398.87): C, 54.20; H, 3.79; N, 21.07 %. Found: C, 54.10; H, 3.68; N, 21.02.

7-Methyl-4-phenyl-8-(4-chlorophenylazo)-1*H*-pyrazolo-[1,5-a][1,3,5]triazine-2-thione (12): A solution of 1-Benzoyl-3-(5-methyl-4-(4-chlorophenylazo)-2*H*-pyrazoleyl)-thiourea (**11**) (1.994 g, 5 mmol) in acetic acid (15 mL) was refluxed for 8 h then left to cool. The solid that precipitated was collected by filtration, washed with water, dried and finally crystallized from ethanol to give pure compound **12** as yellow solid; mp 239 ºC (from ethanol); yield 65 %; IR (KBr, ν_{max}, cm^{-1}) : 3428, 3344 (NH), 3061 (CH-arom.), 1264 (C=S); ^1H NMR (DMSO-d_6) δ/ppm: 2.17 (s, 3H, CH_3), 7.45-8.14 (m, 9H, ArH), 12.41 (br., 1H, pyrimidine NH); MS m/z (%) 380 (M^+, 20); Anal. calcd. for $C_{18}H_{13}N_6SCl$ (m.w. 380.85): C, 56.77; H, 3.44; N, 22.07 %. Found: C, 56.73; H, 3.42; N, 22.02.

Synthesis of 4-amino-7-methyl-8-(4-chlorophenylazo)-pyrazolo[5,1-c][1,2,4]triazine-3-carbonitrile (13): A solution of malonnitrile (0.66 g, 10 mmol) in 50 mL ethanol was stirred with (1.4 g, 10 mmol) sodium acetate trihydrate for 15 min.

The mixture was chilled in an ice bath at 0 ºC. While the solution was cooling, the 4-(4-chlorophenylazo)-3-methylpyrazole-5- diazonium chloride was prepared by the diazotization of 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) (2.36 g, 10 mmol) in 6 mL 6 M hydrochloric acid with 10 mL cold 1 M sodium nitrite solution in the usual way keeping the temperature below 5 ºC. The diazonium chloride solution was added to the reaction solution dropwise under stirring. Then further stirring for 2 h, the reaction mixture was left for 3 h in a refrigerator. The precipitated solid was filtered off, washed with water and ethanol and dried. The product was recrystallized to give compound **13** as pure pale yellow crystals; m.p. > 300 ºC (from ethanol); yield 64 %; IR (KBr, ν_{max}, cm^{-1}): 3500, 3340 (NH_2), 3075 (CH-arom.), 2227 (CN); ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.35 (s, 2H, NH_2), 7.60-7.88 (m, 4H, ArH); MS m/z (%) 312 (M^+, 50); Anal. calcd. for $C_{13}H_9N_8Cl$ (m.w. 312.72): C, 49.93; H, 2.90; N, 35.83 %; Found: C, 49.91; H, 2.86; N, 35.80.

Synthesis of 3-(4-chlorophenylazo)-2-methyl-9-phenylpyrazolo[1,5-d]pyrimido[4,5-d][1,2,3]triazin-8-amine (14): Compounds **3a** (1.94 g, 5 mmol) were added to a solution of hydrazine hydrate (10 mmol) in ethanol (20 mL) and the mixture was refluxed for 4 h. On cooling, a precipitate was formed. This product was filtered and washed with water and drying. The product was then recrystallized from ethanol to give pure compound **14** as yellow solid; m.p. 245 ºC (from ethanol); yield 85 %; IR (KBr, ν_{max}, cm^{-1}) : 3408, 3320 (NH_2), 2980 (CH-aliph.); ^1H NMR (DMSO-d_6) δ/ppm: 2.25 (s, 3H, CH_3), 5.55 (s, 2H, NH_2) 7.45-8.64 (m, 9H, ArH); MS m/z (%) 417 (M^+ + 2, 75); Anal. calcd. for $C_{20}H_{14}N_9Cl$ (m.w. 415.84): C, 57.77; H, 3.39; N, 30.31 %; Found: C, 57.55; H, 3.35; N, 30.25.

Synthesis of 3-(4-chloro-phenylazo)-2-methyl-9-phenylpyrazolo[1,5-d]pyrimido[4,5-d]pyrimidine-6,8-dithione (15): Compounds **3a** (1.94 g, 5 mmol) was dissolved in hot ethanolic KOH (prepared by dissolving (0.285 g, 5 mmol) in 100 mL of absolute ethanol) and the carbon disulphide (0.4 mL, 5 mmol) and the mixture was refluxed for 2 h. and then cooled. The reaction mixture was poured onto ice cooled water, neutralized with hydrochloric acid. The solid product was collected, washed with water, dried and finally recrystallized from ethanol to give pure 15 as yellow solid; mp 233 ºC (from ethanol); yield 60 %; IR (KBr, ν_{max}, cm^{-1}) : 3310, 3260 (NH), 1333 (C=S); ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 6.46-8.34 (m, 9H, ArH), 12.78 (br., 2H, pyrimidine 2NH); MS m/z (%) 464 (M^+ + 1, 75); Anal. calcd. For $C_{21}H_{14}N_7S_2Cl$ (m.w. 463.97): C, 54.36; H, 3.04; N, 21.13; S, 13.82 %, Found: C, 54.18; H, 3.01; N, 21.10; S 13.65.

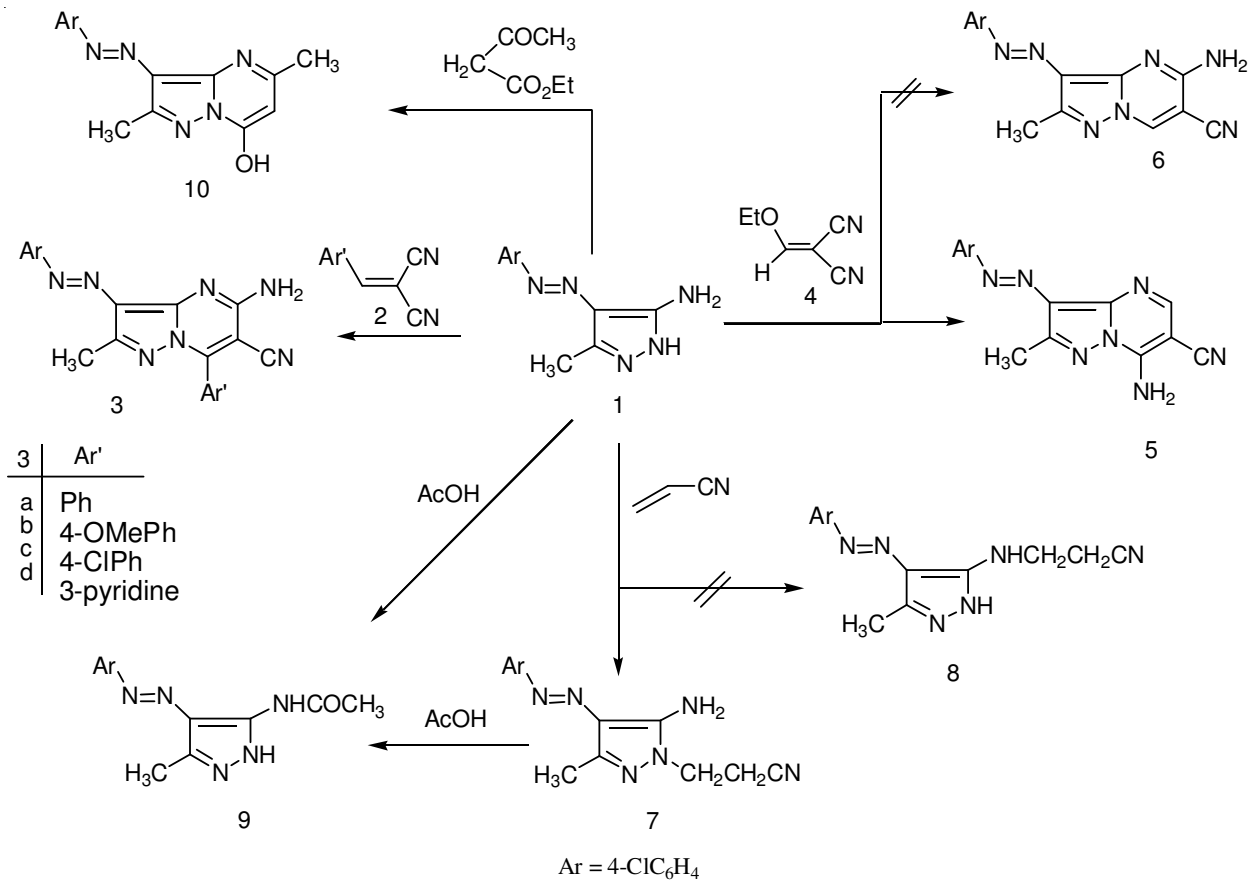
Biological activity: The antibacterial and antifungal activity was carried out in the Microbiology Division of Microanalytical Center of Cairo University, using the diffusion plate technique[18-20] a bottomless cylinder containing a measured quantity (1 ml, mg/mL) of the sample is placed on (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium, which has been heavily seeded with the spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism.

RESULTS AND DISCUSSION

The required starting 5-amino-4-(4-chlorophenylazo)-3-methylpyrazole (**1**) was prepared by reacting 2-arylhydrazono-3-iminobutyronitrile with hydrazine hydrate as previously described[17]. The reaction of compound **1** with a molar equivalent of arylidine malonitrile (**2**) in refluxing pyridine for 4 h (evidenced by TLC) afforded the corresponding cyclized products, pyrazolo[1,5-a]pyrimidine derivatives (**3**) in good yields (**Scheme-I**). The structure of the latter derivatives **3a-d** were confirmed by their IR, ^1H NMR, mass spectra and elemental analysis. The IR spectrum of **3a** as example revealed bands at ν 2214 cm^{-1} characteristic for a CN group and two bands characteristic for NH_2 at ν 3436, 3301 cm^{-1}. Moreover, The ^1H NMR of the product **3a** showed a signal for CH_3 protons at δ 2.51 ppm, signal of NH_2 protons at δ 3.37 ppm, signal for aromatic protons at δ 7.51-7.89 ppm and no signal of NH at δ 10.88 ppm was observed as reported in literature[17]. Also the mass spectrum of the product isolated revealed a molecular ion peak (m/z) at 388 of $C_{20}H_{14}N_7Cl$ (387.83). As reported in literature[21], the formation of compound **3** may proceed *via* initial alkylation of the ring nitrogen in compound **1** to give open compound as intermediate which undergo cyclization to the final products **3**.

In a similar way, refluxing of compound **1** with ethoxymethylene malononitrile (**4**) in EtOH in the presence of piperidine for 3 h gave a single product as indicated by TLC analysis of the crude product. The structure of the product was established on the basis of its spectral (MS, IR and ^1H NMR) analyses. The mass spectrum of the product **5** revealed a molecular ion peak (m/z) at 311 of $C_{14}H_{10}N_7Cl$. Its infrared spectrum revealed the absence of the absorption bands due to NH group and showed a band at ν 2219 cm^{-1} characteristic for a CN group and two bands characteristic for NH_2 at ν 3304, 3232 cm^{-1}. Moreover, the ^1H NMR of the product **5** showed a signal for CH_3 protons at δ 2.51 ppm, signal of NH_2 protons at δ 3.31 ppm, signal for aromatic protons at δ 7.57-7.82 ppm and no signal of NH at δ 10.88 ppm was observed as reported in literature[17]. This product can thus be formulated as compound **5** or its isomeric compound **6**. The structure of compound **6** was ruled out as reported in literature[22]. And formation of compound **5** from **1** and ethoxymethylenemalon-onitrile (**4**) was believed to be formed *via* Michael type addition of compounds **1** on **4** followed by ethanol elimination to give the acyclic intermediate which is then underwent intramolecular cyclization and subsequent tautomerism to give[23] compound **5** (**Scheme-I**).

In the same manner, compound **1** was reacted with acrylonitrile to yield product, which was formulated as compound **7** or isomeric compound **8**. Structure compound **7** was established for product of cyanoethylation based on ^1H NMR which revealed two triplets at δ 3.4 and δ 4.70 for two CH_2 groups. If the reaction product was compound **8** one would expect the triplet at δ 4.70 to appear as multiplet as it would be coupled both with CH_2 and NH. Attempted cyclization of compound **7** into pyrazolopyrimidine failed. Refluxing compound **7** in acetic acid resulted in the formation of the acetylamino derivative **9**. These compounds were confirmed by elemental analysis and spectral data. Similarly when compound **1** was refluxing in acetic acid afforded the acetamide derivatives **9** dealkylation



Scheme-I Synthesis of pyrazole and fused pyrazolo[1,5-a]pyrimidine derivatives

of *N*-alkyl derivatives under similar conditions has been observed earlier derivative[23] **9**. (**Scheme-I**). A conclusive evidence for that the compound prepared have structure **9** provided by alternate synthesis of **9**. Thus, reaction of compound **1** with glacial acetic acid afforded the product **9** that proved identical in all respects (mp, mixed mp. and spectra) with that one obtained by refluxing of compound **7** in acetic acid (**Scheme-I**).

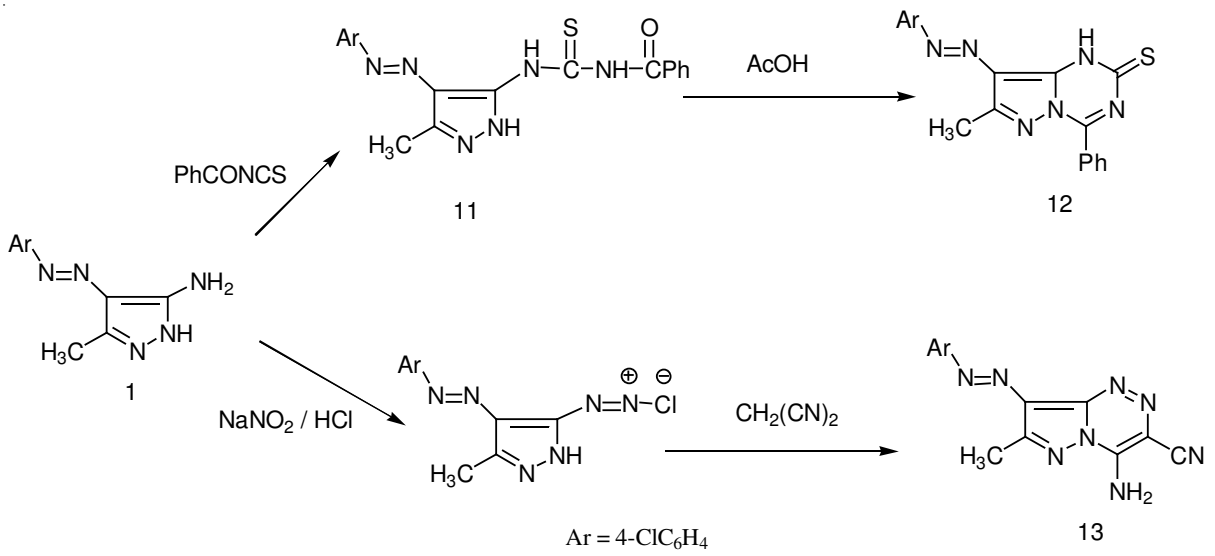
Our investigation was extended to include the reactivity of the aminopyrazole **1** towards active methylene reagents namely, ethyl acetoacetate when refluxed in EtOH containing a few drops of piperidine afforded products *via* water and ethanol elimination. The pyrazolopyrimidine derivative **10** were established as reaction products based on their correct elemental analysis and spectral data (**Scheme-I**). Compound **10** was assumed to be formed *via* initial condensation of the exocyclic amino function in **1** with the carbonyl group in ethyl acetoacetate to give the intermediate, which readily cyclized to the final isolable product[24] **10** (**Scheme-I**).

On the other hand, when aminopyrazole **1** was allowed to react with benzoyl isothiocyanate, compound **11** was isolated. Compound **11** was confirmed by elemental analysis and spectral data. Thus, the IR spectrum shows the appearance of peaks at ν 1671 cm^{-1} assigned to C=O group. The mass spectrum of compound **11** showed the molecular ion peak at m/z = 398 (M^+) corresponding to molecular formula $C_{18}H_{15}N_6OSCl$. Boiling of compound **11** in acetic acid afforded the pyrazolotriazinethione **12** (**Scheme-II**).

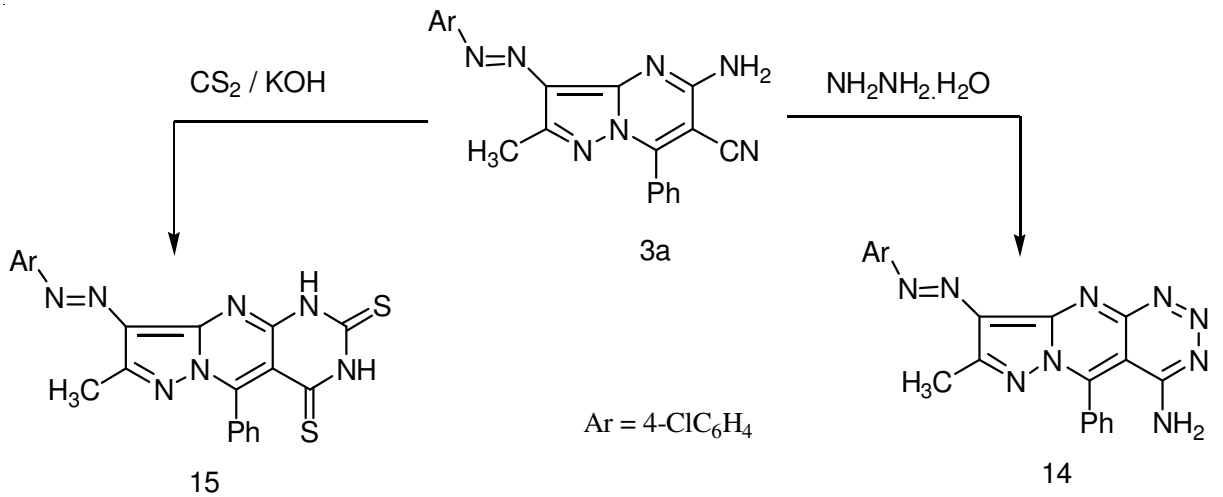
On additionally, coupling of active methylene reagent such as malononitrile with diazotized amino functional group in compound **1** gave the pyrazolotriazine derivatives **13**. Compound **13** was confirmed by elemental analysis and spectral data. Thus, the IR spectrum shows the appearance of peaks at ν 2227 cm^{-1} assigned to C=N groups and ν 3500, 3340 cm^{-1} assigned to NH_2 group. The mass spectrum of compound **13** showed the molecular ion peak at m/z = 312 (M^+) corresponding to molecular formula $C_{13}H_9N_8Cl$ (**Scheme-II**).

Furthermore, when compound **3a** were reacted with hydrazine hydrate in ethanol they produced the corresponding 3-(4-chlorophenylazo-2-methyl-9-phenylpyrazolo[1,5-d]-pyrimido[4,5-d]triazine-8-amine (**14**). The structures proposed for this product were established from their correct elemental analysis and spectral data. Their IR revealed the absence of nitrile absorption frequencies. Further confirmation of the structure of **3a** was achieved from their reaction with carbon disulfide. Thus, refluxing compound **3a** with carbon disulfide and potassium hydroxide in ethanol gave **15** (**Scheme-III**). The structure of the compound produced was established by elemental analysis and spectral data. The IR spectra revealed absorption bands corresponding to the ν two NH and C=S functions near 3310, 3260 and 1333 cm^{-1}, respectively. The ^{1}H NMR spectra exhibited appearance of two NH group at δ 12.78 ppm. Also the mass spectra of all prepared compounds were compatible with the proposed structure (**Scheme-III**).

Screening for antimicrobial activity: Most of the compounds were tested *in vitro* against gram negative bacteria



Scheme-II Synthesis of pyrazole, fused pyrazolo[1,5-a][1,3,5]triazine and pyrazolo[5,1-c]-[1,2,4]triazine



Scheme-III Synthesis of pyrazolo[1,5-d]pyrimido[4,5-d][1,2,3]triazine derivatives

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS

Compd. No	Inhibition zone diameter (cm)[a]							
	Gram (-)		Gram (+)		Fungi			
	(EC)	(PA)	(SA)	(BS)	(AF)	(AN)	(CA)	(CA)
Control (DMSO)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	+++	+++	+++	+++	++	++	+++	+++
9	++	++	++	+++	-	-	++	++
12	++	++	++	++	++	-	++	+
14	++	++	++	+	++	++	++	++
15	++	++	++	++	++	++	++	++
Tetracyclin	++++	++++	+++	++++	-	-	-	-
Ampicillin	-	-	-	-	+++	+++	+++	+++

a) + = Low activity, ++ = Moderate activity, +++ = High activity

[*Escherichia coli anaerobic* (EC)] and *Pseudomonas aeruginos* (PA)], gram positive bacteria [*Staphylococcus albus*(SA) and *Bacillus subtilis* (BS)] and antifungal activity against *Candida albicans* (CA), *Aspergillus niger* (AN), *Candida albicans* (CA) and *Aspergillus flavus* (AF). The reference antibiotics ampicillin and tetracycline were used as references to evaluate the potency of the tested compounds under the same condition. The solvent used was DMSO and the concentration of the sample used is 100 µg/mL. The test results are presented in Table-1. They revealed that all compounds exhibited moderate activity while compound **7** was very active against all tested bacteria species and *Candida albicans*.

Conclusion

A simple method for the synthesis of the title heterocyclic derivatives starting from the arylazopyrazolo **3** is demonstrated.

The structures of all new synthesized compounds were established from their spectral data and elemental analysis. Additionally, the antimicrobial activity of selected compounds was examined.

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