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A Convenient Synthesis of A Series Of Pyrazolo[3,4-*D*]Pyrimidines As Potential Antimicrobial And Antioxidant Agents

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ABSTRACT

A variety of new pyrazolo[3,4-d] pyrimidines has been synthesized as potential antimicrobial and antioxidant agents in which the key intermediate, 5-amino-4-cyano-1-phenyl-3-(pyridin-3-yl)-1H-pyrazole, was used. The process was proven to be simple, efficient, high yielding and more greener compared to traditional method. The antimicrobial and antioxidant activities for the synthesized compounds were evaluated. Some of the tested compounds displayed promising antimicrobial and antioxidant activities and one compound showed antioxidant activity close to that obtained with ascorbic acid.

Keywords: 5-Amino pyrazole; Pyrazolopyrimidine; Microwave technique; Antioxidant activity; Antimicrobial activity.

INTRODUCTION

Pyrazole template is considered as an important chemical entity of various physiological significances and pharmaceutical utility. Pyrazoles have drown greater attention due to their wide range of therapeutic activities including anticonvulsant [1], antidepressant [1, 2], antitumor [3-5], antimicrobial [6, 7], ACE inhibitor [8], antiviral [9] and anti-inflammatory [10, 11].

The biological and medicinal activities of pyrazolopyrimidines have stimulated considerable interest in the synthesis of derivatives of such fused ring system. They are known to exhibit pharmacological activities such as CNS depressant [12], neuroleptic [13] and tuberculostatic [14]. Pyrazolo[3,4-*d*]pyrimidines were indentified as a general class of adenosine receptors [15-17]. Recently, pyrazolopyrimidines have considerable importance as potent and selective phosphodiesterase 5 inhibitors (PDE5) that can be used for treating male erectile dysfunction [18, 19].

With this in mind and in continuation to our program directed towards the synthesis of new fused heterocycles moieties have interesting biological studies [20, 21] we wish to report here a facile synthesis of some pyrazolo[3,4-*d*]pyrimidines under microwave irradiation along with their antimicrobial and antioxidant activities. The reported process gave products in high yield and purity in a short reaction time compared with the classical conventional process [22-24].

MATERIALS AND METHODS

General: Melting points were recorded on a Gallenkamp melting point apparatus and are reported uncorrected. The infrared spectra were recorded on Perkin–Elmer FTIR 1430 spectrophotometer using the KBr disk technique. The ^{1}H NMR spectra were recorded on a Bruker AC spectrometer (300 MHz) at 25 °C in DMSO-d_6 with TMS as an internal standard and chemical shifts are reported in ppm as δ values. Reactions were conducted under microwave irradiation in closed vessels under magnetic stirring in a Synthos 3000 (Anton Paar) microwave with dual magnetrons system and with maximum power of 1000 W. Mass spectra were measured on a Finnigan MAT 8222 EX mass spectrometer at 70 eV and absorbance was measured using a Jenway 6305 spectrophotometer. Microanalyses were performed on Perkin-Elemer 2400 Elemental Analyzer at Microanalytical center at Cairo University. Reaction progress was monitored by thin layer chromatography (TLC) using benzene/acetone (2/1 by volume) as eluent. The strains for the biological activity were obtained from the Culture Collection of Bacteriology Laboratory, Microbiology Unit, Faculty of Science, Tanta University. Compound **1** was prepared according to the literature procedure [20].

Method A

Synthesis of 5-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo-4-carboxaldehyde (2): To a solution of aminopyrazole **1**, 2.36 g, 10 mmol in 30 mL dimethylformamide was added phosphorous oxychloride ,10 mL, 30 mmol in small portions at 10−15 °C with good stirring. After complete addition, the reaction mixture was refluxed in water bath at 60−70 °C for 3 h. The brown past was dissolved in water and neutralized with Na_2CO_3 solution (10%, 100 ml). The solid obtained was collected by filtration, dried and recrystallized from benzene to give **2**. m.p. 150−152 °C, yield 80%, IR (KBr) ν_{max}/cm^{-1} = 1519 (C=N), 1648 (C=O), 3160 (Ar−H); ^{1}H NMR (DMSO-d_6): δ ppm = 4.3 (s, exch., 2H, NH_2), 7.40−7.71 (m, 5H, Ar−H), 8.20 (s, 1H, H^5 of pyridine), 8.52 (s, 1H, H^4 of pyridine), 8.86 (s, 1H, H^6 of pyridine), 9.12 (s, 1H, H^2 of pyridine), 9.62 (s, 1H, CHO); Anal. Calcd. for $C_{15}H_{12}N_4O$ (264.28): C, 68.17; H, 4.58; N, 21.20; Found: C, 68.62; H, 4.22; N, 21.49; MS *m/z* 264 (M^+).

Synthesis of 5-amino-4-cyano-1-phenyl-3-(pyridin-3-yl)-1H-pyrazole 5: A solution of hydroxylamine hydrochloride (2.0 g, 290 mmol) in 5 mL water was treated with sodium hydroxide solution ,4 M, 8 mL to reach pH = 8. A solution of **2** ,2.64 g, 100 mmol, in 40 mL ethanol was added and the reaction mixture was heated under reflux for 2 h. The mixture was left to cool down, poured into 100 mL ice-cold water and acidified with aqueous HCl (20%, 4M, 10 ml). The solid formed was collected by filtration, dried and recrystallized from ethanol to give **5**. m.p. 238−241 °C, yield 64%, IR (KBr) ν_{max}/cm^{-1} = 2207 (C≡N), 3093 (Ar−H), 3414 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.81 (s, exch., 2H, NH_2), 7.40−7.71 (m, 5H, Ar−H), 8.20 (s, 1H, H^5 of pyridine), 8.53 (s, 1H, H^4 of pyridine), 8.85 (s, 1H, H^6 of pyridine), 9.12 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{15}H_{11}N_5$ (261.28): C, 68.95; H, 4.24; N, 26.80; Found: C, 69.33; H, 4.29; N, 26.36; MS *m/z* 261 (M^+).

Method B

Synthesis of 3-pyridinecarboxaldehyde phenyl hydrazone 3: To a solution of phenylhydrazine (5.04 g, 47 mmol) in 20 mL ethanol was added pyridine-3-carboxaldehyde (5 g, 47 mmol) and the reaction mixture was stirred for 1 h. The yellow precipitate was collected by filtration, dried and recrystallized from ethanol to give **3**. m.p. 210−213 °C, yield 88%, IR (KBr) ν_{max}/cm^{-1} = 1519 (C=N), 3168 (Ar−H); ^{1}H NMR (DMSO-d_6): δ ppm = 7.00 (s, exch., 1H, NH), 7.40 (s, 1H, CH), 7.57−7.79 (m, 5H, Ar−H), 8.20 (s, 1H, H^5 of pyridine), 8.57 (s, 1H, H^4 of pyridine), 8.82 (s, 1H, H^6 of pyridine), 9.12 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{12}H_{11}N_3$ (197.24): C, 73.07; H, 5.62; N, 21.30; Found: C, 73.72; H, 5.32; N, 21.59; MS *m/z* 197 (M^+).

Synthesis of 1-(bromo(pyridin-3-yl)methylene)-2-phenylhydrazine (4): Slurry of *N*-bromsuccinimide (8.18 g, 45 mmol) in 20 mL ethyl acetate was cooled to 0−5 °C and added to a solution of compound (**3**; 9 g, 45 mmol) in 20 mL methylene chloride while maintaining temperature below 25 °C. The reaction mixture was stirred at 0−5 °C overnight. The solid formed was collected by filtration and dissolved in ethyl acetate, filtrated and the solvent was evaporated under reduced pressure to give **4** as red oil in 83% yield.

Synthesis of 5-amino-4-cyano-1-phenyl-3-(pyridin-3-yl)-1H-pyrazole (5): A mixture of malononitrile (2.36 g, 30 mmol) in an ethanolic sodium ethoxide solution (sodium metal (0.81 g, 30 mmol) in 30 mL absolute ethanol) and **4** (9.9 g, 30 mmol) was stirred for 24 h at room temperature. The solid formed was collected by filtration, dried and recrystallized from ethanol to give **5** in 74% yield.

Synthesis of compounds 6 and 7 : A mixture 5-aminopyrazole-4-carbonitrile (**5**; 3.90 g, 15 mmol), triethyl orthoformate or 20 mL triethyl orthoacetate was heated under reflux for 7 h. The mixture then evaporated under reduced pressure and the residue obtained was treated with ethanol. The solid formed was collected by filtration, wash with ethanol, dried and recrystallized from ethanol to give **6** and **7**, respectively.

Ethyl N-4-cyano-1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-5-ylformimidate (6): m.p. 163−165 ºC, yield 74%, IR (KBr) ν_{max}/cm^{-1} = 1620, 1633 (C=N), 2208 (C≡N), 3093 (Ar−H); ^{1}H NMR (DMSO-d_6): δ ppm = 1.32 (t, 3H, *J* = 7.5 Hz, CH_3), 4.33 (q, 2H, *J* = 7.5 Hz, CH_2), 7.40−7.71 (m, 5H, Ar−H), 8.20 (s, 1H, H^5 of pyridine), 8.58 (s, 1H, H_4 of pyridine), 8.86 (s, 1H, H^6 of pyridine), 9.12 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{18}H_{15}N_5O$ (317.34): C, 68.13; H, 4.76; N, 22.07; Found: C, 68.63; H, 4.92; N, 22.66; MS *m/z* 317 (M^+).

Ethyl N-4-cyano-1-phenyl-3-(pyridin-3-yl)-1H-pyrazol-5-ylacetimidate (7) : m.p. 186−188 ºC, yield 80 %; IR (KBr) ν_{max}/cm^{-1} = 1625, 1634 (C=N), 2207 (C≡N), 3093 (Ar−H); ^{1}H NMR (DMSO-d_6): δ ppm = 1.17 (s, 3H, CH_3), 1.32 (t, 3H, *J* = 7.5 Hz, CH_3), 4.33 (q, 2H, *J* = 7.5 Hz, CH_2), 7.52−7.71 (m, 5H, Ar−H), 8.09 (s, 1H, H^5 of pyridine), 8.52 (s, 1H, H^4 of pyridine), 8.89 (s, 1H, H^6 of pyridine), 9.04 (s,1H, H^2 of pyridine); Anal. Calcd. for $C_{19}H_{17}N_5O$ (331.37): C, 68.87; H, 5.17; N, 21.13; Found: C, 69.29; H, 4.99; N, 21.32; MS *m/z* 331 (M^+).

Synthesis of compounds 8 and 9

Method A: Amidines **6** or **7** (2 mmol) were added to a mixture of 15 mL methanol and 15mL aqueous ammonia solution (25%,). The reaction mixture was stirred for 3 h and the solid formed was collected by filtration, dried and recrystallized from ethanol/dimethylformamide (1:1) to give **8** and **9** respectively.

4-Amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d] pyrimidine (8): m.p. 260−262 ºC, yield 66%; IR (KBr) ν_{max}/cm^{-1} = 1627 (C=N), 3069 (Ar−H), 3457 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.08 (s, exch., 2H, NH_2), 7.42−7.84 (m, 5H, Ar−H), 8.20 (s, 1H, H^5 of pyridine), 8.55 (s, 1H, H^4 of pyridine), 8.89 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine), 8.35 (s,1H, pyrimidine); Anal. Calcd. for $C_{16}H_{12}N_6$ (288.31): C, 66.66; H, 4.20; N, 29.15; Found: C, 66.97; H, 4.34; N, 29.28; MS *m/z* 288 (M^+).

4-Amino- 6-methyl-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine (9): m.p. 160−163 ºC, yield 77%; IR (KBr) ν_{max}/cm^{-1} = 1563 (C=N), 2950 (Ar−H), 3435 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 2.41(s, 3H, CH_3), 6.92(s, exch., 2H, NH_2), 7.43−7.82(m, 5H, Ar−H), 8.24 (s, 1H, H^5 of pyridine), 8.51 (s, 1H, H^4 of pyridine), 8.90 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{17}H_{14}N_6$ (302.33): C, 67.54; H, 4.67; N, 27.80; Found: C, 67.99; H, 4.84; N, 28.13; MS *m/z* 302 (M^+).

Method B: For the preparation of compound **8** only, A mixture of 5-aminopyrazole-4-carbonitrile **5** (0.78 g, 3 mmol) and 1.5 mL formamide was heated under reflux for 6 h. The mixture was poured into ice cold

water. The precipitate was collected by filtration, dried and recrystallized from methanol to give **8** with yield 87 %. The products were consistent in all respects .

Synthesis of 4-amino-1,6-diphenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine (10): A mixture of 5-aminopyrazole-4-carbonitrile **5**, (0.51 g, 1.9 mmol), benzonitrile (0.19 g, 1.9 mmol) and triethylamine (0.5 mL) in 15 mL dioxane was refluxed for 8 h. The reaction mixture was poured into ice cold water. The precipitate was collected by filtration, dried and recrystallized from ethyl acetate to give **10**. m.p. 298−301 ºC, yield 85%; IR (KBr) ν_{max}/cm^{-1} = 2207 (C≡N), 3147 (Ar–H), 3366 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.90 (s, exch., 2H, NH_2), 7.40–7.81 (m, 10H, Ar–H), 8.20 (s, 1H, H^5 of pyridine), 8.57 (s, 1H, H^4 of pyridine), 8.84 (s, 1H, H^6 of pyridine), 9.012 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{22}H_{16}N_6$ (364.40): C, 72.51; H, 4.43; N, 23.06; Found: C, 72.499; H, 4.79; N, 23.32; MS *m/z* 364 (M^+).

Synthesis of 1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-4,6(5H,7H)-dithione (11): A mixture of 5-aminopyrazole-4-carbonitrile **5**, (0.50 g, 1.9 mmol) and carbon disulfide (1.5 mL) in 10mL,10% alcoholic potassium hydroxide was refluxed for 8 h. The reaction mixture was poured into water and neutralized with HCl (1 M, 10 mL). The precipitate was collected by filtration, washed several times with water, dried and recrystallized from DMF to give **11**. m.p. 271–273 ºC, yield 58 %; IR (KBr) ν_{max}/cm^{-1} = 1281 (C=S), 1625 (C=N), 3030 (Ar–H), 3423 (NH); ^{1}H NMR (DMSO-d_6): δ ppm = 7.59 (s, exch., 1H, NH), 7.65–7.89 (m, 5H, Ar–H), 7.90 (s, exch., 1H, SCNHCS), 8.25 (s, 1H, H^5 of pyridine), 8.56 (s, 1H, H^4 of pyridine), 8.85 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{16}H_{11}N_5S_2$ (337.42): C, 56.95; H, 3.29; N, 19.01; S, 19.01; Found: C, 57.29; H, 3.41; N, 19.27; S, 19.06; MS *m/z* 337 (M^+).

Synthesis of compounds 12−14: A mixture of 5-aminopyrazole-4-carbonitrile **5**, (0.39 g, 1.5 mmol), urea, thiourea or guanidine hydrochloride (1.5 mmol) in ethanolic sodium ethoxide was refluxed for 6 h. The reaction mixture was cooled and poured into cold water and the solid formed was collected by filtration, washed several times with water, dried and recrystallized from ethanol to give **12−14**, respectively.

4-Amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-6-ol 12: m.p. 176–178 ºC, yield 60%; IR (KBr) ν_{max}/cm^{-1} = 1620 (C=N), 2923 (Ar–H), 3310 (OH), 3341 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 5.06 (s, exch., 1H,OH), 6.82 (s, exch., 2H, NH_2), 7.42–7.82 (m, 5H, Ar–H),), 8.20 (s, 1H, H^5 of pyridine), 8.53 (s, 1H, H^4 of pyridine), 8.84 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{16}H_{12}N_6O$ (304.31): C, 63.15; H, 3.97; N, 27.62; Found: C, 63.50; H, 4.20; N, 27.85; MS *m/z* 304 (M^+).

4-Amino- 1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-6-thiol 13: m.p. 210−212 ºC, yield 77 %; IR (KBr) ν_{max}/cm^{-1} = 1620 (C=N), 2924 (Ar–H), 3198 (SH), 3335 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 4.30 (s, exch., 1H, SH), 6.81(s, exch., 2H, NH_2), 7.42−7.88 (m, 5H, Ar–H),), 8.22 (s, 1H, H^5 of pyridine), 8.60 (s, 1H, H^4 of pyridine), 8.91 (s, 1H, H^6 of pyridine), 9.02 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{16}H_{12}N_6S$ (320.37): C, 59.98; H, 3.78; N, 26.23; S, 10.01; Found: C, 60.22; H, 3.96; N, 26.47; S, 10.26; MS *m/z* 420 (M^+).

1-Phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidine-4,6-diamine 14: m.p. 185−187 ºC, yield 62%; IR (KBr) ν_{max}/cm^{-1} = 1620 (C=N), 3206 (Ar−H), 3347 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.93 (s, exch., 4H, $2NH_2$), 7.43–7.75 (m, 5H, Ar–H),), 8.24 (s, 1H, H^5 of pyridine), 8.60 (s, 1H, H^4 of pyridine), 8.90 (s, 1H, H^6 of pyridine), 9.16 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{16}H_{13}N_7$ (303.32): C, 63.36; H, 4.32; N, 32.32; Found: C, 63.69; H, 4.56; N, 32.58; MS *m/z* 403 (M^+).

Synthesis of 4-amino-1-phenyl-3-(pyridin-3-yl)-6-(trichloromethyl)-1H-pyrazo[3,4-d]pyrimidine (15) : A mixture of 5-aminopyrazole-4-carbonitrile **5**, (0.50 g, 1.9 mmol), trichloacetonitrile (0.25g, 1.9 mmol) and triethylamine (0.5ml) in 15 mL dioxane was refluxed for 8 h. The mixture was poured into ice water and the precipitate was collected by filtration, washed several times with water, dried and recrystallized from ethyl acetate to give **15**. m.p. 270–273 ºC, yield 65%; IR (KBr) ν_{max}/cm^{-1} = 701 (C-Cl), 1654 (C=N), 3093 (Ar–H), 3416, 3313 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.84(s, exch., 2H, NH_2), 7.44–7.76 (m, 5H, Ar–H), 8.20 (s, 1H, H^5 of pyridine), 8.51 (s, 1H, H^4 of pyridine), 8.81 (s, 1H, H^6 of pyridine), 9.14 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{17}H_{11}Cl_3N_6$ (405.67): C, 50.33; H, 2.73; Cl, 26.22, N, 20.72; Found: C, 50.67; H, 2.96; Cl, 26.44, N, 20.98; MS *m/z* 405 (M^+).

general procedure of the synthesis of compounds 16a-e: To a solution of 5-aminopyrazole-4-carbonitrile **5**, 0.50 g, 1.9 mmol) in 20 mL dimethylformamide containing 0.5 mL piperidine as a catalyst was added substituted *β*-ketonitrile (1.9 mmol). The reaction mixture was refluxed for 6–8 h. The solvent was evaporated under reduced pressure and the oil residue obtained was treated with petroleum ether (40–60 ºC) and recrystallized from ethyl acetate/ethanol (2/1 by volume) to give **16a-e.**

1-Pyridyl-2-(4-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-l)ethanone (16a): m.p. 276–278 ºC, yield 60%; IR (KBr) ν_{max}/cm^{-1} = 1643 (C=N), 1653 (C=O), 3086 (Ar–H), 3415, 3310 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.93(s, exch., 2H, NH_2), 7.42–7.73 (m, 5H, Ar–H), 8.22 (s, 2H, H^5 of pyridine), 8.51 (s, 2H, H^4 of pyridine), 8.91 (s, 2H, H^6 of pyridine), 9.20 (s, 2H, H^2 of pyridine); Anal. Calcd. for $C_{23}H_{17}N_7O$ (407.43): C, 67,80; H, 4.21; N, 24.06; Found: C, 68,22; H, 4.49; N, 24.29; MS *m/z* 407 (M^+).

1-Phenyl-2-(4-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-l)ethanone 16b: m.p. 284–286 ºC, yield 80%; IR (KBr) ν_{max}/cm^{-1} = 1639 (C=N), 1653 (C=O), 3081 (Ar–H), 3415, 3310 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.90(s, exch., 2H, NH_2), 7.41–7.80 (m, 10H, Ar–H), 8.22 (s, 1H, H^5 of pyridine), 8.52 (s, 1H, H^4 of pyridine), 8.87 (s, 1H, H^6 of pyridine), 9.10 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{24}H_{18}N_6O$ (406.44): C, 70.92; H, 4.46; N, 20.68; Found: C, 71.22; H, 4.61; N, 20.89; MS *m/z* 406 (M^+).

1-(4-Tolyl)-2-(4-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)ethanone 16c: m.p. 319–322 ºC, yield 70%; IR (KBr) ν_{max}/cm^{-1} = 1644 (C=N), 1653 (C=O), 3086 (Ar–H), 3415, 3311 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 2.33 (s, 3H, CH_3), 6.92(s, exch., 2H, NH_2), 7.40–7.81(m, 9H, Ar–H), 8.22 (s, 1H, H^5 of pyridine), 8.54 (s, 1H, H^4 of pyridine), 8.91 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{25}H_{20}N_6O$ (420.47): C, 71.41; H, 4.79; N, 19.99; Found: C, 71.89; H, 4.99; N, 20.15; MS *m/z* 420 (M^+).

1-(4-Fluorophenyl)-2-(4-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)ethanone 16d: m.p. 307–309 ºC, yield 65%; IR (KBr) ν_{max}/cm^{-1} = 1640 (C=N), 1653 (C=O), 3089 (Ar–H), 3416, 3312 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.90 (s, exch., 2H, NH_2), 7.42–7.84 (m, 9H, Ar–H), 8.22 (s, 1H, H^5 of pyridine), 8.56 (s, 1H, H^4 of pyridine), 8.87 (s, 1H, H^6 of pyridine), 9.01 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{24}H_{17}FN_6O$ (424.43): C, 67.92; H, 4.04; F, 4.48; N, 19.80; Found: C, 68.29; H, 4.30; F, 4.62; N 20.09; MS *m/z* 423 ($M^+ - 1$).

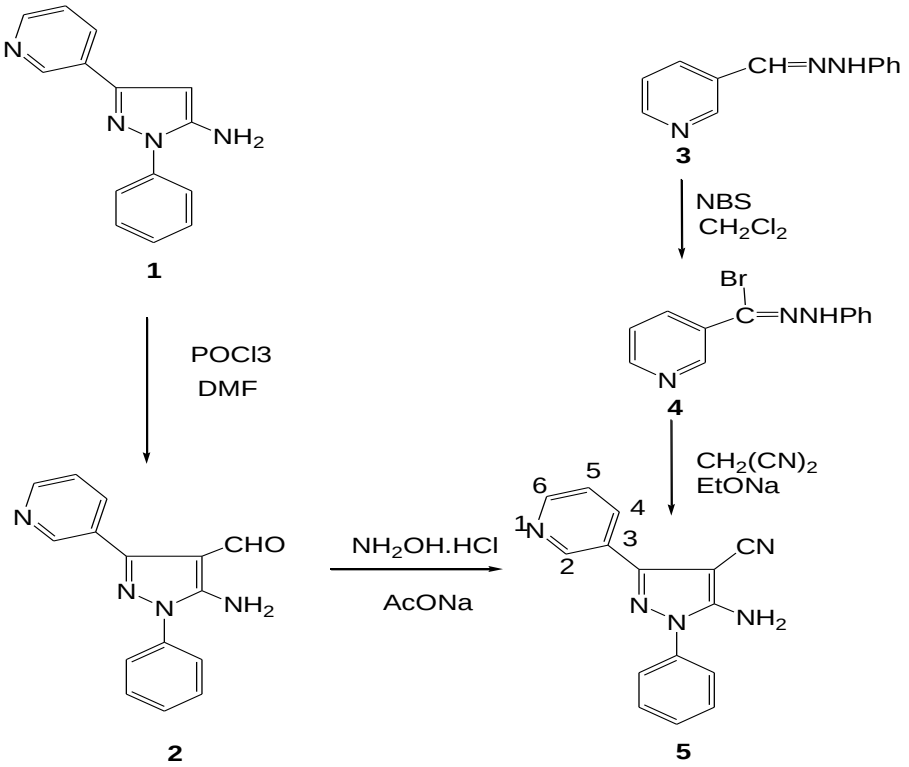
1-(4-Chlorophenyl)-2-(4-amino-1-phenyl-3-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)ethanone 16e: m.p. 293–296 ºC, yield 66%; IR (KBr) ν_{max}/cm^{-1} = 1641 (C=N), 1655 (C=O), 3088 (Ar–H), 3415, 3308 (NH_2); ^{1}H NMR (DMSO-d_6): δ ppm = 6.91(s, exch., 2H, NH_2), 7.42–7.82(m, 9H, Ar–H), 8.20 (s, 1H, H^5 of pyridine), 8.58 (s, 1H, H^4 of pyridine), 8.90 (s, 1H, H^6 of pyridine), 9.00 (s, 1H, H^2 of pyridine); Anal. Calcd. for $C_{24}H_{17}ClN_6O$ (440.88): C, 65.38; H, 3.89; Cl, 8.04; N, 19.06; Found: C, 65.72; H, 4.19; Cl, 8.30; N, 19.23; MS *m/z* 440 (M^+).

Microwave irradiation technique

For the microwave irradiation technique the reaction mixture was capped in closed vessels and irradiated in a microwave oven at mentioned temperature and time (Table 1). The reaction mixture was worked up as usual and the solid obtained was recrystallized from the appropriate solvent.

RESULTS AND DISCUSSION

Chemistry: The synthetic process to produce the target compounds is outlined in Schemes 1-3. The key intermediate, 5-amino-4-cyno-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole (**5**), was synthesized *via* two routes. The first route involves formylation of aminopyrazole (**1**) by Vilsmeier-Haack reaction [25] to give 5-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo-4- carboxaldehyde (**2**), followed by treatment with hydroxylamine hydrochloride and sodium acetate to give **5** in 64% yield. The second one started by bromination of pyridine-3-carboxaldehyde hydrazone (**3**) with *N*-bromosuccinimide to give 1-(bromo(pyridin-3-yl)methylene)-2-phenyl hydrazine (**4**) followed by the reaction with malononitrile in sodium ethoxide solution to give **5** in 74% yield (Scheme 1).

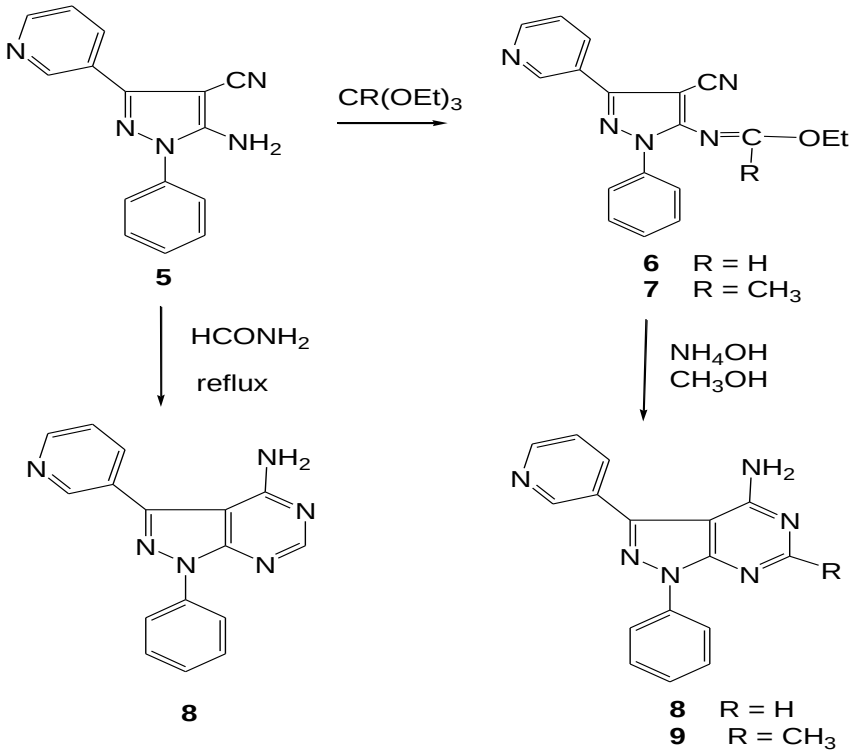


Scheme 1: Synthesis of 5-amino-4-cyno-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazole **5**

Compound **5** was allowed to react with various reagents to obtain new pyrazolo[3,4-*d*]pyrimidines **8-16** by microwave irradiation and convention heating techniques. The condensation of **5** with triethyl orthoformate or triethyl orthoacetate gave the corresponding ethoxymethyleneamino derivatives **6** and **7**, which could be cyclized by stirring at room temperature in methanolic ammonia to produce 4-aminopyrazolopyrimidins **8** and **9**, respectively. Also, 4-amino-pyrazolopyridimidine **8** was successfully synthesized by heating compound **5** with formamide (Scheme 2).

Reaction of **5** with benzonitrile in sodium ethoxide solution for 24 h gave 4-amino-1,6-diphenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**10**) in 85% yield. Also, reaction of **5** with carbon disulphide in the presence of alcoholic potassium hydroxide (10 %) afforded 1-phenyl-3-(pyridine-3-yl)-1*H*-

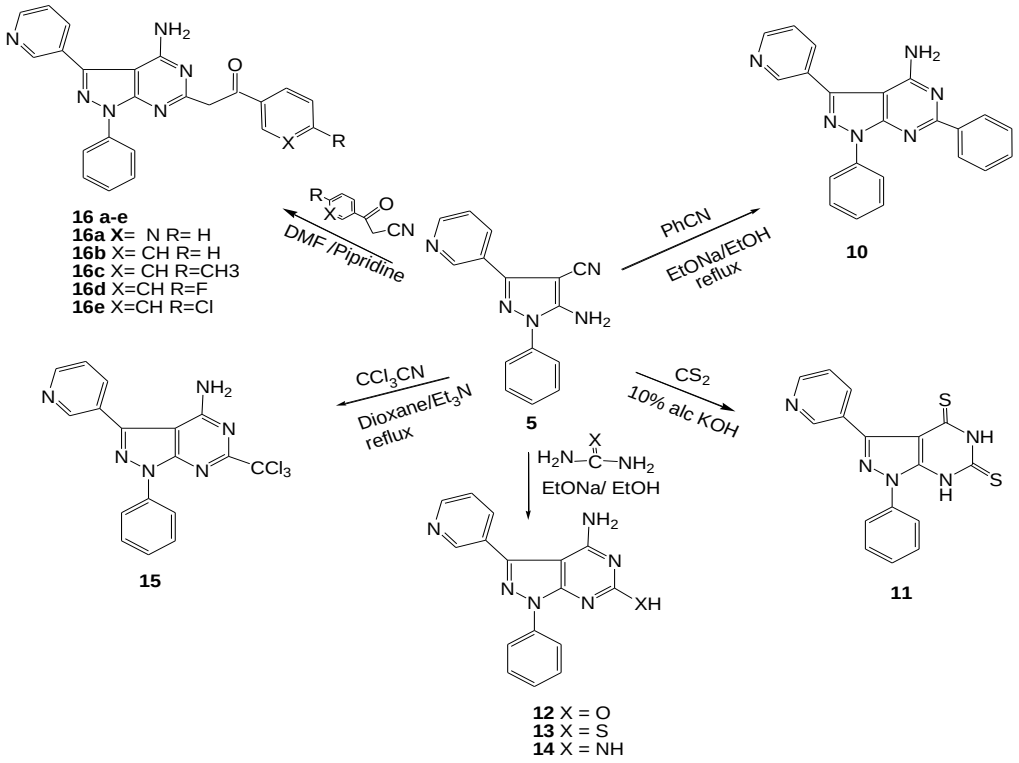
pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dithione (**11**) in 58% yield. On the other hand, when compound **5** was refluxed with urea, thiourea and guanidine hydrochloride in ethanolic sodium ethoxide, 4-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-ol (**12**), 4-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol (**13**) and 1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine(**14**) were obtained, respectively, in good yields.



Scheme 2: Synthesis of 4-amino-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**8**) and 4-amino-6-methyl-1-phenyl-3-(pyridin-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**9**)

Reaction of **5** with trichloacetonitrile in boiling dioxane in the presence of triethylamine as a basic catalyst for 8 h gave 4-amino-1-phenyl-3-(pyridin-3-yl)-6-(trichloromethyl)1*H*-pyrazolo[3,4-*d*]pyrimidine (**15**) in 65% yield. Finally, compound **5** has been reacted with some *β*- ketonitriles in boiling dimethylformamide in the presence of piperidine as a catalyst for 10 h gave 1-aryl-2-(4-amino-1-phenyl-3-(pyridin-3yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl)ethanone **16a-e** (Scheme 3). The structures of products **2–16** was established by elemental analyses and compatible spectroscopic data.

Of noteworthy observation that compounds **8–16** (Schemes 2 and 3) were prepared by conventional heating and microwave irradiation techniques. The comparison of the experimental results was listed in Table 1. It is clear that the reaction time was reduced from 6–24 h to just only 15–30 min and the yield was increased from 58–85 to 81–94%.



Scheme 3: Synthesis of different pyrazolo[3,4-*d*]pyrimidine derivatives **10**−**16**

Table 1: Comparison between the time and yield for compounds **8-16**.

Product	Time		Yield (%)	
	Microwave (120–130 ºC, min)	Conventional heating (reflux, h)	Microwave	Conventional heating
8	15	6	88–90	66
9	20	8	90–92	77
10	30	24	94	85
11	20	8	87–89	58
12	25	8	90–92	60
13	25	8	92–94	77
14	20	6	88–91	62
15	20	8	83	65
16a	15	6	91–92	60
16b	20	8	86–88	80
16c	20	8	82	70
16d	15	6	81–84	62
16e	15	6	93–95	66

APPLICATIONS

Pharmacology

Antimicrobial evaluation: The antimicrobial properties of the synthesized compounds were tested *in vitro* by cut plug method according to Pridham et al. [26]. The antimicrobial properties were tested against Gram-negative bacteria (*Escherichia coli and Klibsella*) and Gram-positive bacteria (*Bacillus subtilis, Bacillus cereus, Pseudomonas vulgarus and Staphylococcus aureus*) along with the non-filamentous

fungus (*Candida albicans*) as pathogenic bacterial strains. Three different broadly used antibiotics (Amoxycilline, Chloramphenicol and Tetracycline) were used as references.

Antibacterial assay: An aliquot of 0.1 mL of each bacterial strain was inoculated and spread on nutrient agar while 0.1 mL of the yeast was spread on sabaroud agar slopes. Antimicrobial activity of the synthesized compounds was tested *in vitro* against different types of bacteria and one fungal strain by the cut plug method [26]. The assay plates were inoculated with 100 mL containing the diluted inoculums (107 CFU mL^{-1}) of each tested organism that were spread on the corresponding media. After solidification, the wells were made and 10 mg of the synthesized chemicals were dissolved in 1 ml DMSO and inserted in the wells. Nutrient agar plated was incubated at 37 °C for 24 h, while plates were incubated at 25 °C for 48 h. The zones of inhibition around the wells were measured and the average based on 3 replies was recorded. For reference drugs 100 mg mL^{-1} of Amoxicillin, Chloramphenicol and Tetracycline were used as antibacterial and antifungal drugs, respectively.

The tested compounds showed variation in their antimicrobial activities (Table 2). In general, the compounds showed a relatively moderate activity against Gram-positive and negative bacteria as well as against yeast. From the bioactivity data of the synthesized compounds, it was inferred that compound **13** with a SH group in the pyrimidine moiety and compound **15** with a CCl_3 group in the pyrimidine moiety showed the higher activity than the rest of the compounds. Compounds **8−12**, **14** and **16b-d** were found to have slight or moderate activity while compounds **16a** and **16e** were found to be inactive. It is worth mentioning that minor change in molecular configuration of the tested compounds profoundly influences the microbial activity.

The present study was focused on the determination of minimum inhibitory concentration (MIC) for compound **13** and **15**. MIC is important in diagnostic laboratories to confirm resistance of microorganism to an antimicrobial agent and also to monitor the reactivity of new antimicrobial agents. The MIC values for compounds **13** and **15** were given in Table 3.

Table 2: Diameter of inhibition zones (mm) of the compounds against different tested microorganisms

Compound	*Escherichia Coli*	*Klibsella*	*Bacillus subtilus*	*Pseudomonas vulgarus*	*Bacillus cereus*	*Staphylococcus aures*	*Candida albicans*
8	12	10	8	12	12	—	—
9	9	10	12	8	8	8	—
10	10	12	10	12	10	—	—
11	12	10	6	6	12	10	12
12	8	6	8	8	10	12	—
13	12	12	12	10	10	12	14
14	8	12	13	12	—	—	15
15	16	10	8	8	11	14	14
16a	—	—	—	—	—	—	—
16b	—	8	6	14	16	17	—
16c	—	—	—	10	—	—	—
16d	12	—	—	14	12	—	—
16e	—	—	—	—	—	—	—
Amoxycilline	20	18	20	24	22	12	25
Chloramphenicol	18	16	21	26	22	14	22
Tetracycline	18	18	18	30	24	14	22

The concentration used was 10 mg mL^{-1}. Control discs were performed in DMSO (dimethylsulfoxide) and no inhibition zones were observed (-ve resistance)

Table 3*:* Minimal inhibitory concentration (MIC) of the provided samples against test microorganisms (MIC) µg mL^{-1}

Compound	*Escherichia Coli*	*Klibsella*	*Bacillus subtilus*	*Pseudomonas vulgarus*	*Bacillus cereus*	*Staphylococcus aures*	*Candida albicans*
3	100	100	125	—	100	100	500
15	65	200	150	—	200	250	250
St	31.25	62.5	31.25	62.5	31.25	31.25	62.5

All the dilutions of both samples and standards were performed by double fold dilution

Antioxidant evaluation : Radical scavenging activities of compounds **8-16e** were assessed using model colorimetric test, depending on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Ascorbic acid was used as an antioxidant reference, and the results were grouped in table 4 and represented in figures 1 and 2.

Table 4: Decrease of DPPH absorbance (%) by the synthesized compounds

Compound No.	% of radical scavenging (mean ± SD)	IC50
8	57.57 ± 0.75	27.01
9	—	—
10	14.39 ± 0.76	150.78
11	71.72 ± 0.44	18.97
12	33.37 ± 0.76	67.22
13	—	—
14	37.84 ± 0.77	61.04
15	54.04 ± 1.15	28.81
16a	34.85 ± 0.75	83.53
16b	27.02 ± 1.15	80.24
16c	20.20 ± 1.58	115.69
16d	—	—
16e	65.66 ± 1.16	21.45
Ascorbic acid	82.77 ± 1.17	4.95

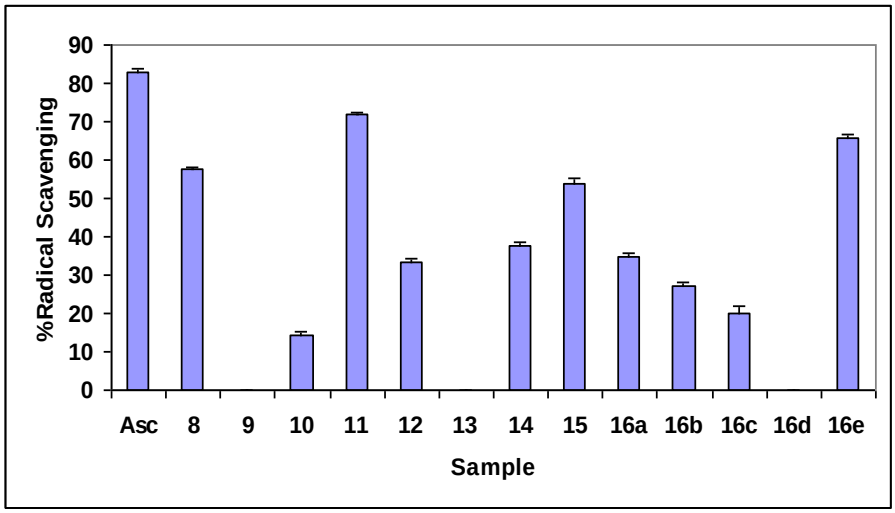


Fig. 1: Assessment of the antioxidant activity of the tested compounds in comparison with ascorbic acid.

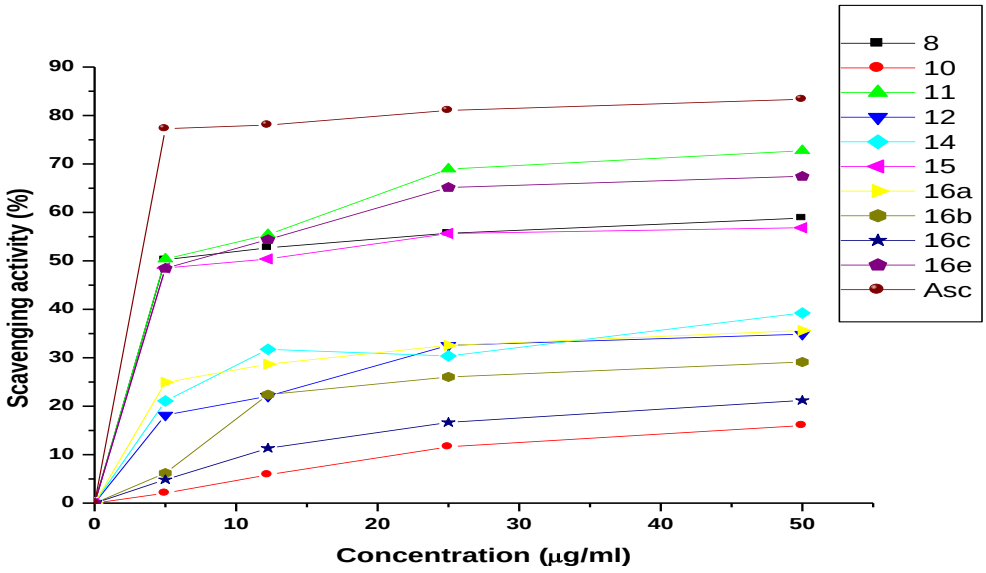


Fig. 2: Scavenging antioxidant percentage of the tested compounds.

DPPH radical scavenging assay: The antioxidant activities of the tested compounds were measured by using the DPPH radical scavenging assay according to Ardestani and Yazdanparast with ascorbic acid as standard [27]. Each tested sample and ascorbic acid (50 mg) was dissolved in 1 ml DMSO, the dissolved sample (250 ml) was added to 1 ml DPPH/DMSO solution (6 mg 50 mL^{-1}) and the total volume was adjusted to 3 ml with DMSO. An equal volume of DMSO was used as a control. After vortexing the mixture was incubated for 30 min in dark at room temperature. Absorbance was measured using a spectrophotometer at 517 nm. DPPH radical scavenging % = 1 - (A sample / A control) x 100. Serial dilutions (5–50 mg/ml) of each compound were measured by the same assay to obtain the EC50 according to Brand-Williams et al [28].

Among the tested compounds, compound **11** has the most potent antioxidant activity and was found to be close to the value obtained for standard ascorbic acid. This very promising result could be due to the presence of two sulphur atoms. This result was in agreement with that reported by Ali et al. [29]. Also, compound **16e** showed a good result, while compounds **8** and **15** had relatively good antioxidant activities. The other tested compounds were considerably less effective as radical scavenging and can be arranged in the order that **14**> **16a**> **12**> **16b**> **16c** >**10**. While, compounds **9** and **16d** showed a negative effect.

CONCLUSIONS

A new simple and efficient route for the synthesis of pyrazolo[3,4-*d*]pyrimidines was reported. The process is much greener compared with the traditional one in terms of the reaction time, yield and the energy required. Some of the synthesized products showed promising antimicrobial and antioxidant activities against some microorganisms.

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