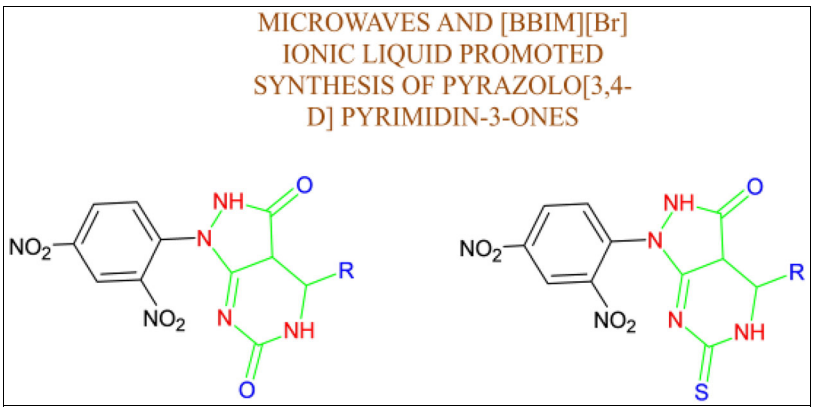
Synthesis of Some Substituted Pyrazolopyrimidine Derivatives: An Environmentally Benign Approach

Arpit Kumar Pathak, Sharoni Gupta, Pinki B. Punjabi, Garima Ameta, and Chetna Ameta*

Microwave Synthesis Laboratory, Department of Chemistry, University College of Science, M. L. Sukhadia University, Udaipur 313001, Rajasthan, India
*E-mail: chetna.ameta@yahoo.com

Received September 26, 2018
DOI 10.1002/jhet.3587
Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com).



Pyrazolopyrimidines constitute a medicinally important class of heterocyclic compounds. Herein, we report an efficient and environmentally benign method for the synthesis of pyrazolo[3,4-*d*]pyrimidin-3-ones from 2,4-dinitrophenylhydrazine and diethyl malonate under microwave irradiation in 1,3-dibutylimidazolium bromide ionic liquid. The synthesized compounds were analyzed by elemental analysis and standard spectroscopic techniques. The compounds were screened for their antibacterial and antifungal activities.

J. Heterocyclic Chem., **00,** 00 (2019).

INTRODUCTION

Synthesis of N-substituted heterocycles using microwave irradiation has attracted the interest of chemists worldwide because it is a highly efficient and time-saving process. Environmentally benign synthesis of N-heterocycles under microwave irradiation has been reported in literature [1–6]. The use of ionic liquids in organic synthesis has further enhanced the efficiency of the entire process of synthesis, simply owing to their several advantages such as low melting point, high thermal stability [7,8], negligible vapor pressure [9], and high ionic conductivity [10]. Conventionally, the ionic liquids have been synthesized in stirred tanks using batch or semibatch processes. But these methods were time and energy consuming [11]; hence, in recent years, the chemists have shifted their interest towards clean and efficient techniques such as microwave irradiation [12,13] and sonochemical processes [14] for synthesis of ionic liquid.

Amongst N-substituted heterocycles, pyrimidine is one of the most important molecules. Pyrimidine skeleton is involved in biologically important molecules such as DNA and RNA [15]. Hetero-fused and substituted pyrimidines are well known as antiviral, antibacterial, anti-AIDS, anti-inflammatory, antimalarial, antifungal, and anticancer agents [16]. Furthermore pyrazoles fused or substituted with other heterocycles constitute the core of several pharmacological products [17]. Pyrazole derivatives exhibit remarkable antimicrobial, analgesic, antipyretic, and anti-inflammatory properties [18–20].

A number of methods for pyrazole-fused pyrimidines have been reported earlier, but these methods have their drawbacks such as excessive use of solvents, time-consuming processes, poor yields, and expensive experimental set-up. To overcome these drawbacks, an efficient and eco-friendly method for the synthesis of pyrazole-fused pyrimidine derivatives under microwave irradiation in the presence of 1,3-dibutylimidazolium bromide ionic liquid is reported here.

EXPERIMENTAL SECTION

The reactions were carried out in a microwave synthesizer (Ragatech, Pune). Thin-layer chromatography (TLC) was carried out on silica plates precoated with silica (0.2 mm), and visualization was accomplished by iodine vapors. NMR spectra were taken on a Bruker Avance II 400 NMR Spectrometer (Bruker Corp., Billerica, MA) in DMSO solvent using tetramethylsilane

as internal standard, and chemical shifts were expressed in δ ppm. The Fourier transform infrared spectra were recorded on Perkin Elmer Spectrum RX-IFTIR spectrometer. Melting points were measured using Gallenkamp's melting point apparatus and are uncorrected. Solvents and reagents were purchased from commercial sources and were used without further purification.

Preparation of ionic liquid 1,3-dibutylimidazolium bromide [bbim][Br] (1.2*).* The synthesis of ionic liquid was carried out in two steps. The first step involved synthesis of butyl imidazole (**1.1**) from the imidazole (**1**) (0.68 g, 0.01 mol) using *n*-butyl lithium as a base and *n*-butyl bromide (1.37 g, 0.01 mol) as an alkylating agent under microwave exposure at 240 W for 10 min. An equimolar ratio of *n*-butyl imidazole and butyl bromide mixture was taken in the second step and heated intermittently in a microwave synthesizer at 300 W until a clear monophase was obtained. The resulting ionic liquid was cooled and washed several times with diethyl ether to remove the unspent reagent. The product was dried under vacuum to obtain a viscous 1,3-dibutylimidazolium bromide ionic liquid (Figure 1).

IR (KBr pellet): ν (cm^{-1}), 3089 (C–H), 2960 (C–H), 2871, 2826 (C–H), 1630 (C═C), 1600 (C═N), and 1085 (C–N). ^{1}H-NMR: (δ, ppm, 400 MHz, DMSO-d_6), 0.50 (t, 6H, J = 6.9 Hz, CH_3), 1.20–1.30 (m, 4H, CH_2), 1.75 (m, 4H, CH_2), 2.15 (t, 2H, J = 7.4 Hz, CH_2), 3.70 (t, 2H, J = 7.4 Hz, CH_2), 6.90 (d, 2H, J = 7.6 Hz, CH), and 9.41 (s, 1H, CH). ^{13}C-NMR: (δ, ppm), 12.5–30.1 (C-2 imidazole), 57.0 (C-1′-N butyl), 55.0 (C-1″-N^+ butyl), and 124–133.0 (aromatic imidazole ring carbon).

Microwave-induced synthesis of 1-(2,4-dinitrophenyl) pyrazolidine-3,5-dione (4). A mixture of 2,4-dinitrophenyl hydrazine (1.98 g, 0.01 mol) (**2**) and diethyl malonate (1.6 g, 0.01 mol) (**3**) was dissolved in 1,3-dibutylimidazolium bromide ionic liquid (10 mL) in the presence of acidic medium (acetic acid, 2 mL). The reaction mixture was irradiated for 6 to 8 min in MW at 300 W. The viscous mass obtained after the completion of reaction was poured into ice-cold water with vigorous stirring and left for complete precipitation. The solid product (**4**) was filtered, washed, and dried. The purity of compound was ascertained by TLC using ethyl acetate: benzene (7:3) as mobile phase (Figure 2).

1-(2,4-Dinitrophenyl)pyrazolidine-3,5-dione (4). This was obtained in 85% yield; mp 160–162°C; IR: (ν, cm^{-1}), 3317 (N–H), 3000 (Ar C–H), 2926 and 2850 (CH_2), 1644 (>N–C═O), 1612 (NH–C═O), 1406 (N–N), 1500, 1350 (–NO_2), 1573, 1488, and 1406 (aromatic ring), and 1060 (C–N). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 2.55 (s, 2H, CH_2), 8.70 (s, 1H, N–H), and 7.20–7.80 (m, 3H, Ar–H). *Anal*. Calcd for $C_9H_6N_4O_6$: C 40.61; H 2.27; N 21.05%. Found: C 40.21; H 2.05; N 20.90%.

General procedure for microwave-induced synthesis of 4-(substituted phenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5a–e). In an Erlenmeyer flask, 1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (**4**) (2.6 g, 0.01 mol), different aromatic aldehydes (0.01 mol), and NaOH (two to three drops) in 10 mL of ionic liquid ([bbim][Br]) were taken and mixed thoroughly to prepare chalcones (**5a**–**e**). The well-stirred reaction mixture was irradiated by microwaves for 7 to 8 min at 720 W. The course of the reaction was monitored by TLC, and the resultant mass was poured into ice-cold water with vigorous stirring. The solid product was filtered, washed, and dried. The purity of compounds was ascertained by TLC using ethyl acetate:benzene (7:3) as mobile phase.

4-(4-Chlorophenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5a). This was obtained in 70% yield; mp 265–268°C; IR: (ν, cm^{-1}), 3425 (N–H), 3263 (═C–H), 3050 (Ar C–H), 1635 (>N–C═O), 1609 (NH–C═O), 1550, 1480, and 1450 (aromatic ring), 1500 and 1350 (–NO_2), 1416 (N–N), 1073 (C–N), 831 (C–Cl), and 740 and 717 (*m*-substituent dinitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.70 (s, 1H, N–H), 7.28–7.80 (m, 7H, Ar–H), and 8.25 (s, 1H, ═C–H). *Anal*. Calcd for $C_{16}H_9ClN_4O_6$: C 49.44; H 2.33; N 14.41%. Found: C 49.10; H 2.10; N 14.10%.

4-(3-Nitrophenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5b). This was obtained in 72% yield; mp 272–275°C; IR: (ν, cm^{-1}), 3422 (N–H), 3260 (═C–H), 3000 (Ar C–H), 1650 (>N–C═O), 1620 (NH–C═O), 1550, 1440, and 1460 (aromatic ring), 1500 and 1350 (–NO_2), 1416 (N–N), 1073 (C–N), and 740 and 717 (*m*-substituted dinitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.70 (s, 1H, N–H), 7.25–7.78 (m, 7H, Ar–H), and 8.00 (s, 1H, ═C–H). *Anal*. Calcd for $C_{16}H_9N_5O_8$: C 48.13; H 2.27; N 17.54%. Found: C 48; H 2.20; N 17.40%.

4-(4-Hydroxyphenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5c). This was obtained in 65% yield; mp 250–252°C; IR: (ν, cm^{-1}), 3430 (N–H), 3400 (O–H), 3258 (═C–H), 3000 (Ar C–H), 1652 (>N–C═O), 1625 (NH–C═O), 1573, 1488, and 1406 (aromatic ring), 1500 and 1340 (–NO_2), 1410 (N–N), 1060 (C–N), 1200 (C–O),

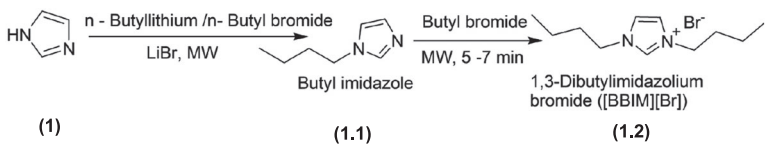


Figure 1. Preparation of ionic liquid 1,3-dibutylimidazolium bromide [bbim][Br].

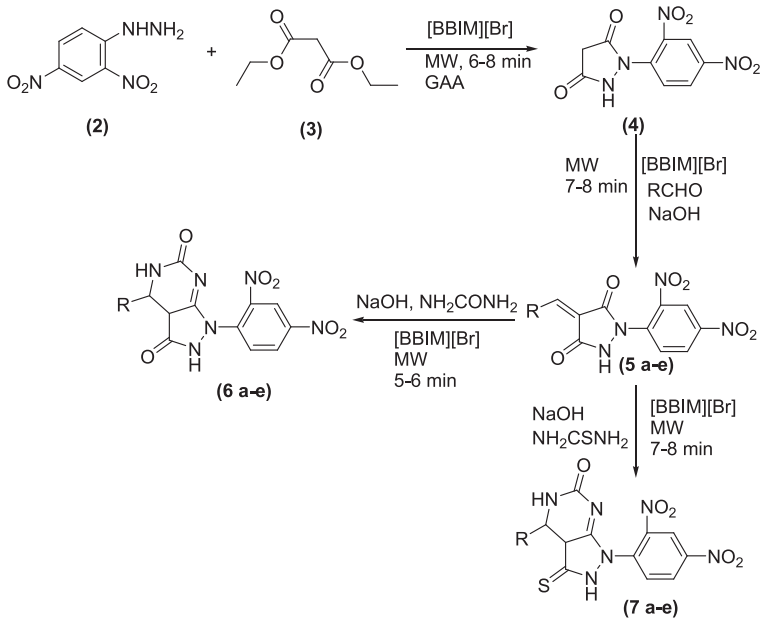


Figure 2. Microwave assisted synthesis of pyrazolo[3,4-*d*]pyrimidin-3-ones.

840 (*p*-substituent), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.70 (s, 1H, –N–H), 7.25–7.80 (m, 7H, Ar–H), 8.2 (s, 1H, ═C–H), and 10.50 (s, 1H, OH). *Anal*. Calcd for $C_{16}H_{10}N_4O_7$: C 51.90; H 2.72; N 15.13%. Found: C 51.50; H 2.40; N 15.00%.

4-(2-Chlorophenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5d). This was obtained in 75% yield; mp 285–288°C; IR: (ν, cm^{-1}), 3415 (N–H), 3050 (Ar C–H), 3270 (═C–H), 1650 (>N–C═O), 1625 (NH–C═O), 1570, 1480, and 1450 (aromatic ring C–H bending), 1490 and 1360 (–NO_2 coupled vibration), 1073 (C–N), 1410 (N–N), 830 (C–Cl), and 740 and 717 (*m*-disubstituted dinitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.70 (s, 1H, –N–H), 7.20–7.80 (m, 7H, Ar–H), and 8.20 (s, 1H, ═C–H). *Anal*. Calcd for $C_{16}H_9ClN_4O_6$: C 49.44; H 2.33; N 14.41%. Found: C 49.10; H 2.15; N 14.10%.

4-(4-Nitrophenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione (5e). This was obtained in 80% yield; mp 300–302°C; IR: (ν, cm^{-1}), 3425 (N–H), 3275 (═C–H), 3000 (Ar C–H), 1642 (>N–C═O), 1615 (NH–C═O), 1550, 1450, and 1470 (aromatic ring), 1520 and 1360 (–NO_2), 1073 (C–N), 1416 (N–N), 835 (*p*-substituent NO_2 group), and 740 and 717 (*m*-substituted dinitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.70 (s, 1H, N–H), 7.20–7.78 (m, 7H, Ar–H), and 8.05 (s, 1H, ═C–H). *Anal*. Calcd for $C_{16}H_9N_5O_8$: C 48.13; H 2.27; N 17.54%. Found: C 47.90; H 2.05; N 17.20%.

The physical data of compounds **4** and **5** (**a**–**e**) are given in Table 1.

General procedure for microwave-induced synthesis of 4-(substituted phenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3a*H*-pyrazolo[3,4-*d*]pyrimidine-3,6-dione (6a–e). The chalcone (**5a**) (3.88 g, 0.01) was dissolved in 10 mL of ionic liquid in an Erlenmeyer flask. Then, urea (0.6 g, 0.01 mol) and NaOH (two to three drops) were added and mixed thoroughly. The well-stirred mixture was exposed to microwaves for 5 to 6 min at 720 W. The completion of the reaction was monitored by TLC. After

Table 1

Physical data of synthesized compounds **4** and **5a**–**e**.

Compound	R	Molecular formula	Molecular weight	mp, °C	Yield, %	Time, min
4	—	$C_9H_6N_4O_6$	266	160–162	85	7
5a	4-Cl-C_6H_4	$C_{16}H_9ClN_4O_6$	388	265–268	70	7
5b	3-NO_2-C_6H_4	$C_{16}H_9N_5O_8$	399	272–275	72	6
5c	4-OH-C_6H_4	$C_{16}H_{10}N_4O_7$	370	250–252	65	9
5d	2-Cl-C_6H_4	$C_{16}H_9ClN_4O_6$	388	285–288	75	8
5e	4-NO_2-C_6H_4	$C_{16}H_9N_5O_8$	399	300–302	80	8

the reaction mixture was cooled, it was poured into crushed ice. The obtained solid was filtered, washed with water, and recrystallized from ethanol. The purity of compounds was analyzed by TLC using ethyl acetate: benzene (7:3) as mobile phase. The recovery of ionic liquid was carried out by evaporation of water and reused in the next step. The process was repeated with other chalcones (**5b**–**e**).

General procedure of microwave-induced synthesis of 4-(substituted phenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3a*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (7 a-e). Chalcone (**5a**) (3.88 g, 0.01 mol), thiourea (0.76 g, 0.01 mol), and NaOH (two to three drops) in 10 mL of ionic liquid were taken in an Erlenmeyer flask and mixed thoroughly. The well-stirred mixture was irradiated in a microwave oven for 7 to 8 min at 720 W. The completion of the reaction was monitored by TLC. The reaction mixture was then cooled at room temperature and poured into crushed ice. The obtained solid was filtered, washed with water, and recrystallized from ethanol. The purity of compounds was analyzed by TLC using ethyl acetate:benzene (7:3) as mobile phase. The ionic liquid was recovered by evaporation of water and reused. The same process was repeated with other chalcones (**5b**–**e**).

4-(4-Chlorophenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3aH-pyrazolo[3,4-d]pyrimidine-3,6-dione (6a). This was obtained in 75% yield; mp 80–82°C; IR: (ν, cm^{-1}), 3285 (N–H), 3010 (C–H, Ar–H), 1677 (C═O), 1650 (C═N), 1409 (N–N), 1510 and 1340 (NO_2 coupled vibration), 1550, 1480, and 1450 (aromatic ring), 1155 (C–N), 840 (C–Cl *p*-substituent), and 700 and 742 (*m*-substituent). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.50 (s, 1H, N–H), 8.10 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 2.90 (d, 1H, J = 4.4 Hz, CH), and 3.50 (d, 1H, J = 3.6 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 44.7 (C-4 pyrazolopyrimidine), 115–130.5 (CH aromatic), 132.5 (C4″-Cl aromatic), 133.0 (C-2′-NO_2 aromatic), 139.0 (C-4′-NO_2 aromatic), 142.0 (C-1″ aroma tic), 144.4 (C-1′ aromatic), 160.9 (C-7 pyrazolopyrimidine), 164.0 (C-7a pyrazolopyrimidine), and 174.5 (C-7 pyrazolopyrimidine). MS: (*m*/*z*, %), 430 $[M]^{+\cdot}$, 432 $[M + 2]^{+\cdot}$, 395 $[M\text{-}Cl]^{+\cdot}$, 263 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$, 319 $[M\text{-}C_6H_4Cl]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}ClN_6O_6$: C 47.40; H 2.57; N 19.51%. Found: C 47.26; H 2.40; N 19.35%.

4-(3-Nitrophenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3aH-pyrazolo[3,4-d]pyrimidine-3,6-dione (6b). This was obtained in 70% yield; mp 50–52°C; IR: (ν, cm^{-1}), 3280 (N–H), 3055 (C–H, Ar–H), 1673 (C═O), 1650 (C═N), 1557, 1440, and 1460 (aromatic ring), 1530 and 1361 (NO_2 coupled vibration), 1147 (C–N), and 717 and 730 (*m*-substituent NO_2 bending). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO), 8.50 (s, 1H, N–H), 8.10 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 3.00 (d, 1H, J = 4.6 Hz, CH), and 3.65 (d, 1H, J = 3.8 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 44.7 (C-4 pyrazolopyrimidine), 115.0–132.5 (CH aromatic), 133.0 (C-2′-NO_2 aromatic), 140.5 (C-4′-NO_2 aromatic), 144.5 (C-1′ aromatic), 142.5 (C-3 pyrazolopyrimidine), 147.9 (C-3″-NO_2 aromatic), 162.5 (C-7a pyrazolopyrimidine), 160.9 (C-7 pyrazolopyrimidine ring), and 174.0 (C-3 pyrazolopyrimidine). MS: (*m*/*z*, %), 441 $[M]^{+\cdot}$, 395 $[M\text{–}NO_2]^{+\cdot}$, 274 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$, 319 $[M\text{-}C_6H_4NO_2]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}N_7O_8$: C 46.27; H 2.51; N 22.22%. Found: C 46.15; H 2.35; N 22.05%.

4-(4-Hydroxyphenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3aH-pyrazolo[3,4-d]pyrimidine-3,6-dione (6c). This was obtained in 65% yield; mp 90–93°C, IR: (ν, cm^{-1}), 3405 (OH), 3280 (N–H), 3010 (Ar C–H), 1678 (C═O), 1650 (C═N), 1573, 1480 and 1410 (aromatic ring), 1560 and 1360 (–NO_2 coupled vibration), 1410 (N–N), 1060 (C–N), 835 (*p*-substituent), and 740 and 717 (*m*-substituted dinitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO-d_6), 8.50 (s, 1H, N–H), 8.15 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 11.44 (s, 1H, OH), 2.90 (d, 1H, J = 4.4 Hz, CH), and 3.65 (d, 1H, J = 3.8 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 44.7 (C-4 pyrazolopyrimidine), 113–130.6 (CH aromatic), 133.0(C-2′-NO_2 aromatic), 140.0 (C-4′-NO_2 aromatic), 142.5 (C-1″ aromatic), 144.4 (C-1′ aromatic), 154.9 (C-4″-OH aromatic), 160.5 (C-6 pyrazolopyrimidine), 162.9 (C-7a pyrazolopyrimidine), and 174.5 (C-3 pyrazolopyrimidine). MS: (*m*/*z*, %), 412 $[M]^{+\cdot}$, 394 $[M\text{-}H_2O]$, 319 $[M\text{-}C_6H_4OH]^{+\cdot}$, 245 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{12}N_6O_7$: C 49.52; H 2.93; N 20.38%. Found: C 49.25; H 2.78; N 20.20%.

4-(2-Chlorophenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3aH-pyrazolo[3,4-d]pyrimidine-3,6-dione (6d). This was obtained in 80% yield; mp 110–112°C; IR: (ν, cm^{-1}), 3290 (N–H), 3020 (C–H, Ar–H), 1660 (C═O), 1630 (C═N), 1570, 1480, and 1450 (aromatic ring), 1500 and 1340 (–NO_2 coupled vibration), 1410 (N–N), 1073 (C–N), 840 (C–Cl), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO-d_6), 8.55 (s, 1H, N–H), 8.10 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 3.00 (d, 1H, J = 4.8 Hz, CH), and 3.50 (d, 1H, J = 3.6 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 44.7 (C-4 pyrazolopyrimidine), 113.5–129.0 (CH aromatic), 132.5 (C-2″-Cl aromatic), 132.9 (C-2′-NO_2 aromatic), 140.0 (C-4′-NO_2 aromatic), 142.4 (C-1″ aromatic), 143.7 (C-1′ aromatic), 160.0 (C-6 pyrazolopyrimidine), 162.5 (C-7a pyrazolopyrimidine), and 174.6 (C-3 pyrazolopyrimidine). MS: (*m*/*z*, %), 430 $[M]^{+\cdot}$, 432 $[M + 2]^{+\cdot}$, 395 $[M\text{-}Cl]^{+\cdot}$, 263 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$, 319 $[M\text{-}C_6H_4Cl]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}ClN_6O_6$: C 47.40; H 2.57; N 19.51%. Found: C 47.20; H 2.52; N 19.34%.

4-(4-Nitrophenyl)-1-(2,4-dinitrophenyl)-1,2,4,5-tetrahydro-3aH-pyrazolo[3,4-d]pyrimidine-3,6-dione (6e). This was obtained in 64% yield; mp 115–118°C; IR: (ν, cm^{-1}), 3290 (N–H), 3022 (C–H, Ar–H), 1690 (C═O), 1630 (C═N), 1500 and 1350 (NO_2 coupled vibration), 1570, 1450, and 1470 (aromatic ring), 1400 (N–N), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO-d_6), 8.55 (s, 1H, N–H), 8.15 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), and 3.00 (d, 1H, *J* = 4.6 Hz, CH), and 3.90 (d, 1H, *J* = 4.0 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.0 (C-3a pyrazolopyrimidine), 44.5(C-4 pyrazolopyrimidine), 115.0–130.6 (CH aromatic), 132.5 (C-2′-NO_2 aromatic), 139.5 (C-4′-NO_2 aromatic), 142.0 (C-1″ aromatic), 144.4 (C-1′ aromatic), 146.5 (C-4″-NO_2 aromatic), 160.5 (C-6 pyrazolopyrimidine), 165.0 (C-7a pyrazolopyrimidine), and 174.0 (C-3 pyrazolopyrimidine). MS: (*m*/*z*, %), 441 $[M]^{\cdot+}$, 395 $[M–NO_2]^{+\cdot}$, 274 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$, 319 $[M\text{-}C_6H_4NO_2]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}N_7O_8$: C 46.27; H 2.51; N 22.22%. Found: C 46.15; H 2.35; N 22.05%.

4-(4-Chlorophenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3aH-pyrazolo[3,4-d]pyrimidin-3-one (7a). This was obtained in 77% yield; mp 170–172°C; IR: (ν, cm^{-1}), 3260 (N–H), 3000 (C–H, Ar–H), 1690 (C═O), 1630 (C═N), 1570, 1480, and 1450 (aromatic ring), 1500 and 1350 (NO_2 coupled vibration), 1410 (N–N), 1250 (C═S), 830 (C–Cl *p*-substituent), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO-d_6), 8.55 (s, 1H, N–H), 8.20 (s, 1H, N–H), 7.20–7.78 (m, 7H aromatic), 3.25 (d, 1H, *J* = 5.8 Hz, CH), and 3.90 (d, 1H, *J* = 4.6 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 52.7 (C-4 pyrazolopyrimidine), 115.0–128.9 (CH aromatic), 131.0 (C-4″-Cl aromatic), 132.9 (C-2′-NO_2 aromatic), 139.0 (C-4′-NO_2 aromatic), 141.5 (C-1″ aromatic), 144.4 (C-1′ aromatic), 160.9 (C-7a pyrazolopyrimidine), 174.8 (C-3 pyrazolopyrimidine), 182.0 (C-6 pyrazolopyrimidine). MS: (*m*/*z*, %), 446 $[M]^{\cdot+}$, 448 [M + 2], 411 $[M\text{-}Cl]^{+\cdot}$,335 $[M\text{-}C_6H_4Cl]^{+\cdot}$, 279 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}ClN_6O_5S$: C 45.70; H 2.48; N 18.81%. Found: C 45.55; H 2.20; N 18.60%.

4-(3-Nitrophenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3aH-pyrazolo[3,4-d]pyrimidin-3-one (7b). This was obtained in 64% yield; mp 160–162°C; IR: (ν, cm^{-1}), 3250 (N–H), 3010 (C–H, Ar–H), 1670 (C═O), 1650 (C═N), 1550, 1440 and 1460 (aromatic ring), 1515 and 1360 (NO_2 coupled vibration), 1230 (C═S), 1115 (C–N), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR (δ, ppm, 400 MHz, DMSO-d_6), 8.50 (s, 1H, N–H), 8.25 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 3.00 (d, 1H, *J* = 5.0 Hz, CH), and 3.50 (d, 1H, *J* = 4.2 Hz, CH). ^{13}C-NMR: (δ, ppm), 41.5 (C-3a pyrazolopyrimidine), 52.7 (C-4 pyrazolopyrimidine), 112.5–141.5 (CH aromatic), 132.6 (C-2′-NO_2 aromatic), 139.6 (C-4′-NO_2 aromatic), 142.1 (C-1″ aromatic), 143.4 (C-1′ aromatic), 148.5 (C-3″-NO_2 aromatic), 160.5 (C-7a pyrazolopyrimidine), 174.5 (C-3 pyrazolopyrimidine), and 182.0 (C-6 pyrazolopyrimidine). MS: (*m*/*z*, %), 457 $[M]^{\cdot+}$, 411 $[M–NO_2]^{+\cdot}$, 335 $[M\text{-}C_6H_4NO_2]^{+\cdot}$, 290 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$. *Anal*. Calcd for $C_{17}H_{11}N_7O_7S$: C 44.64; H 2.42; N 21.44%. Found: C 44.50; H 2.25; N 21.25%.

4-(4-Hydroxyphenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3aH-pyrazolo[3,4-d]pyrimidin-3-one (7c). This was obtained in 70% yield; mp 252–255°C; IR: (ν, cm^{-1}), 3290 (N–H), 3400 (OH), 1625 (C═O), 1580 (C═C), 1510 and 1350 (–NO_2 coupled vibration), 1060 (C–N), 1410 (N–N), 1270 (C═S), 1573, 1488, and 1406 (aromatic ring), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.50 (s, 1H, N–H), 8.20 (s, 1H, N–H), 7.20–7.70 (m, 7H, Ar–H), 11.38 (s, 1H, OH), 3.05 (d, 1H, *J* = 5.4 Hz, CH), and 3.95 (d, 1H, *J* = 4.8 Hz, CH). ^{13}C-NMR spectrum, δ, ppm: 41.0 (C-3a pyrazolopyrimidine), 52.0 (C-4 pyrazolopyrimidine), 113.0–130.6 (CH aromatic), 132.5 (C-2′-NO_2 aromatic), 140.5 (C-4′-NO_2 aromatic), 142.5 (C-1″ aromatic), 143.4 (C-1′ aromatic), 154.5 (C-4″-OH aromatic), 160.9 (C-7a pyrazolopyrimidine), 174.0 (C-3 pyrazolopyrimidine), and 182.5 (C-6 pyrazolopyrimidine). Mass spectrum, *m*/*z* (I_{rel}, %): 428 $[M]^{\cdot+}$, 410 $[M\text{-}H_2O]^{+\cdot}$, 261 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$, 335 $[M\text{-}C_6H_4OH]^{+\cdot}$. Found, %: C 47.48; H 2.65; N 19.40. $C_{17}H_{12}N_6O_6S$. Calculated, %: C 47.66; H 2.82; N 19.62.

4-(2-Chlorophenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3aH-pyrazolo[3,4-d]pyrimidin-3-one (7d). This was obtained in 80% yield; mp 210–212°C; IR: (ν, cm^{-1}), 3290 (N–H), 3010 (Ar C–H), 1670 (C═O), 1650 (C═N), 1570, 1480, and 1450 (aromatic ring), 1500 and 1350 (NO_2 coupled vibration), 1250 (C═S), 1115 (C–N), 832 (C–Cl), and 740 and 717 (*m*-disubstituted nitro group). ^{1}H-NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.50 (s, 1H, N–H), 8.20 (s, 1H, N–H), 7.20–7.75 (m, 7H, Ar–H), 3.10 (s, 1H, *J* = 5.2 Hz, CH), and 3.60 (d, 1H, *J* = 4.4 Hz, CH). ^{13}C-NMR spectrum, δ, ppm: 41.0 (C-3a pyrazolopyrimidine), 52.2 (C-4 pyrazolopyrimidine), 113.5–129.0 (CH aromatic), 132.5 (C-2″-Cl aromatic), 133.0 (C-2′-NO_2 aromatic), 139.0 (C-4′-NO_2 aromatic), 142.8 (C-1″ aromatic), 143.4 (C-1′ aromatic), 160.2 (C-7a pyrazolopyrimidine), 174.5 (C-3 pyrazolopyrimidine), and 182.0 (C-6 pyrazolopyrimidine). Mass spectrum, *m*/*z* (I_{rel}, %): 446 $[M]^{\cdot+}$, 448 [M + 2], 411 $[M\text{-}Cl]^{+\cdot}$, 335 $[M\text{-}C_6H_4Cl]^{+\cdot}$, 279 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$. Found, %: C 45.55; H 2.55; N 18.60. $C_{17}H_{11}ClN_6O_5S$. Calculated, %: C 45.70; H 2.48; N 18.81.

4-(4-Nitrophenyl)-1-(2,4-dinitrophenyl)-6-thioxo-1,2,3a,4,5,6-hexahydro-3aH-pyrazolo[3,4-d]pyrimidin-3-one (7e). This was obtained in 65% yield; mp 190–192°C; IR: (ν, cm^{-1}), 3290 (N–H), 1672 (C═O), 1620 (C═N), 1580, 1440, and 1470 (aromatic ring), 1520 and 1361 (–NO_2

coupled vibration), 1409 (N–N), 1270 (C═S), and 1065 (C–N). ^{1}H-NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 8.55 (s, 1H, N–H), 8.20 (s, 1H, N–H), 7.20–7.80 (m, 7H, Ar–H), 3.00 (s, 1H, J = 5.0 Hz, CH), and 3.90 (d, 1H, J = 4.8 Hz, CH). ^{13}C-NMR spectrum, δ, ppm: 42.0 (C-3a pyrazolopyrimidine), 52.8 (C-4 pyrazolopyrimidine), 115.5–130.5 (CH aromatic), 132.5 (C-2′-NO_2 aromatic), 139.5 (C-4′-NO_2 aromatic), 142.5 (C-1″ aromatic), 143.4 (C-1′ aromatic), 146.0 (C-4″-NO_2 aromatic), 160.9 (C-7a pyrazolopyrimidine), 174.8 (C-3 pyrazolopyrimidine), and 182.0 (C-6 pyrazolopyrimidine). Mass spectrum, m/z (I_{rel}, %): 457 $[M]^{\cdot+}$, 411 $[M–NO_2]^{+\cdot}$, 335 $[M\text{-}C_6H_4NO_2]^{+\cdot}$, 290 $[M\text{-}C_6H_3N_2O_4]^{+\cdot}$. Found, %: C 44.45; H 2.30; N 21.20. $C_{17}H_{11}N_7O_7S$. Calculated, %: C 44.64; H 2.42; N 21.44.

RESULTS AND DISCUSSION

Condensation of diethyl malonate (**3**) with 2,4-dinitrophenyl hydrazine (**2**) in the presence of glacial acetic acid in 1,3-dibutylimidazolium bromide as solvent afforded compound **4**. The presence of IR band at 1644 cm^{-1} for cyclic C═O and disappearance of band at 3400 cm^{-1} due to NH_2 group confirm the structure of compound **4**. The ^{1}H-NMR signal at 2.55 δ ppm due active methylene group further characterizes compound **4** as 1-(2,4-dinitrophenyl)pyrazolidine-3,5-dione. Compound **4** on reaction with various substituted aromatic aldehydes in the presence of NaOH gave 4-(4-substituted phenyl)-1-(2,4-dinitrophenyl)pyrazolidine-3.5-diones (**5a**–**e**). The disappearance of signal at 2.55 ppm due to CH_2 group and appearance of multiplet for five aromatic protons at 6.4–9.5 ppm and ═C–H stretching frequency at 3200 cm^{-1} in IR spectra confirm compounds **5a**–**e**.

Compounds **5a**–**e** serve as intermediate for the synthesis of compounds **6a**–**e** and **7a**–**e**. In the first reaction pathway, treatment of compounds **5a**–**e** with urea in the presence of NaOH synthesized pyrazolopyrimidine derivatives (**6a**–**e**). The products were confirmed by the appearance of IR bands at 1650 cm^{-1} for C═N stretching, whereas disappearance of NMR signal at 8.2 ppm due to ═C–H proton. In the second reaction pathway, pyrazolopyrimidine derivatives (**7a**–**e**) were synthesized by treating compounds **5a**–**e** with thiourea in alkaline medium. The appearance of IR band at 1660 cm^{-1} for C═N stretching and IR band at 1270 cm^{-1} due to replacement of C═O band by C═S bond have confirmed compounds **7a**–**e**. Compounds **7a**–**e** were further confirmed by disappearance of NMR signal for ═C–H at 8.2 ppm.

The synthesized compounds were screened *in vitro* for their antimicrobial activities against Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and three strains of fungi *Aspergillus niger*, *Candida albicans*, and *Aspergillus clavatus* (Table 2). Micro broth dilution method was used to determine minimum growth inhibition concentration. Ampicillin was used as standard drug for antibacterial activity, and griseofulvin was used as standard antifungal drug.

The synthesized compounds **6a**, **6e**, **7b**, and **7d** showed minimum inhibitory concentration (MIC) values similar to those of the standard drug ampicillin. Further, it was found that replacing the hydrogen of phenyl ring with hydroxyl group at *p*-position in compounds **6c** and **7c** enhanced their activity, and they displayed excellent activity against *E. coli*. Compounds **6b** (3-NO_2) and **6d** (2-Cl) also exhibit good activity against *E. coli*.

Table 2

Microbial activity of synthesized compound (minimum inhibition concentration) (μg mL^{-1}).

Compound R		Bacteria (MIC, μg mL^{-1})				Fungi (MIC, μg mL^{-1})		
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus pyogenes*	*Aspergillus niger*	*Candida albicans*	*Aspergillus clavatus*
6a	4-Cl-C_6H_4	100	125	**125**	100	250	500	200
6b	3-NO_2-C_6H_4	**50**	150	250	125	500	500	500
6c	4-OH-C_6H_4	**25**	100	**62.5**	100	125	**250**	200
6d	2-Cl-C_6H_4	**62.5**	200	**100**	200	200	500	500
6e	4-NO_2-C_6H_4	100	125	**200**	125	500	1000	500
7a	4-Cl-C_6H_4	**50**	250	**200**	125	500	500	1000
7b	3-NO_2-C_6H_4	100	200	**200**	100	500	500	1000
7c	4-OH-C_6H_4	**50**	100	**125**	**62.5**	100	**200**	100
7d	2-Cl-C_6H_4	100	125	**200**	200	125	500	500
7e	4-NO_2-C_6H_4	125	200	250	100	1000	1000	1000
Standard: Ampicillin		100	Not active	250	100			
Griseofulvin						100	500	100

Bold values shows excellent activity as compared to standard drug.

All the synthesized compounds showed good activity when *P. aeruginosa* strain was used for conducting MIC test with the ampicillin standard. Ampicillin standard drug does not show any activity against *P. aeruginosa* bacteria. Compound **6c** (4-OH) showed excellent activity against *P. aeruginosa* strain. Compounds **6a**, **6c**, and **7c** displayed excellent MIC value than did other compounds against *S. aureus* strain. Compounds **7c** (4-OH), **7b** (3-NO_2), and **7e** (4-NO_2) showed good activity against *S. pyogenes* strain. Owing to high electronegativity and mesomeric effect of –OH group, the compounds with –OH group showed more polarity and permeability than did other compounds. Thus, these compounds easily enter the cell wall of bacteria and show good activity.

Compound **7c** exhibited activity equivalent to that of griseofulvin against *A. niger*. Compounds **6a**, **6b**, **6d**, **7a**, and **7d** showed moderate activities against *C. albicans*. Excellent activities were shown by compounds **6c** and **7c** against *C. albicans*. When *A. clavatus* was used as a strain, compound **7c** showed excellent activity. Thus, all the synthesized compounds showed good antibacterial activity and moderate to weak antifungal activity.

CONCLUSION

An efficient and environmentally benign method for the synthesis of pyrazolopyrimidine derivatives under microwave irradiation in the presence of 1,3-dibutylimidazolium bromide ionic liquid has been developed. In the present investigation, imidazolium-based ionic liquid was prepared under the microwave irradiation by the N-alkylation method. Thereafter, the 1,3-dibutylimidazolium bromide ionic liquid was used both as a catalyst and as solvent to synthesize the pyrazolopyrimidine derivatives. The method offers several advantages such as rapid synthesis with excellent yields of products, simple preparation, non-volatility, easy recovery, and reusability of ionic liquid. The biological screenings of prepared derivatives reveal good antibacterial activities and moderate antifungal activities.

REFERENCES AND NOTES

[1] Mady, M. F.; Saleh, T. S.; El-Kateb, A. A.; El-Rehman, N. M. A.; El-Moez, S. I. A. Res Chem Intermediat 2016, 42, 753.

[2] Faty, R. M.; Rashed, M. S.; Youssef, M. M. Molecules 2015, 20, 1842.

[3] Tiwari, U.; Ameta, C.; Rawal, M. K.; Ameta, R.; Punjabi, P. B Indian J Chem Sec B 2013, 52B, 432.

[4] Tiwari, U.; Ameta, C.; Sharma, S.; Sharma, M.; Pathak, A. K.; Punjabi, P. B. Eur Chem Bull 2013, 242.

[5] Vyas, R.; Swarnkar, N.; Sancheti, A.; Verdia, J.; Punjabi, P. B. J Indian Chem Soc 2008, 85, 1217.

[6] Sharulatha, V.; Indumathi, U.; Priyadarshini, S. Int J Res Engg Technol 2014, 3, 1.

[7] Rogers, R. D.; Seddon, K. R. Science 2003, 302, 792.

[8] Sheldon, R. Green Chem 2005, 7, 267.

[9] Earle, M. J.; Esperanc, J. M. S. S.; Gilea, M. A.; Lopes, J. N. C.; Rebelo, L. P. N.; Magee, J. W.; Seddon, K. R.; Widegren, J. A. Nature 2006, 439, 831.

[10] Sakaebe, H.; Matsumoto, H. Electrochem Commun 2003, 5, 594.

[11] Deetlefs, M.; Seddon, K. R. In Handbook of Green Chemistry, Green Solvents, Ionic LiquidsWasserscheid, P.; Stark, A. Eds.; Wiley: Weinheim, 2010; Vol 6, p 1.

[12] Namboodri, V. N.; Varma, R. S. Tetrahedron Lett 2002, 43, 5381.

[13] Messali, M.; Ahmed, S. A. Green Sustain Chem 2011, 1, 70.

[14] Ameta, G.; Pathak, A. K.; Ameta, C.; Ameta, R.; Punjabi, P. B. J Mol Liq 2015, 211, 934.

[15] Basavaiah, D.; Sathyanaryana, T. Tetrahedron Lett 2002, 43, 4301.

[16] Selvam, T. P.; James, C. R.; Dniandev, P. V.; Valzita, S. K. Res Pharm 2012, 2, 1.

[17] Dastan, A.; Kulkarni, A.; Torok, B. Green Chem 2012, 14, 17.

[18] Matsui, H.; Sakanashi, Y.; Oyama, T. M.; Oyama, Y.; Yokota, S.; Ishida, S.; Okano, Y.; Oyama, T. B.; Nishimura, Y. Toxicology 2008, 248, 142.

[19] Steel, H. C.; Tintinger, G. R.; Anderson, R. Chem Biol Drug Des 2008, 72, 225.

[20] Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y. N.; Al-aizari, F. A.; Ansar, M. Molecules 2018, 23, 134.