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Ionic Liquid Catalyzed Microwave Assisted Synthesis, Characterization and Anticancer Activity of Novel Pyrazolo[3,4-*d*]pyrimidine Derivatives

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A rapid and efficient method has been developed for the synthesis of pyrazolo[3,4-*d*]pyrimidines (**5a-j**) *via* microwave assisted method as well as conventional method using four components system in the presence of ionic liquid 2-methyl-imidazolium thiocyanate as a green catalyst. It was found that ionic liquid showed better reactivity than *p*-toluene-sulfonic acid (*p*-TSA) and L-proline. All the synthesized pyrazolo-pyrimidine derivatives were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR spectral and elemental analysis. Further, the *in vitro* anti-breast cancer activity was evaluated by performing cytotoxicity studies against MCF-7 cell lines at different concentrations of synthesized compounds ranging from 100 to 500 µg/mL. Among the series of compounds, compounds **5a** and **5d** showed significant cytotoxic effect at the lowest concentration. However, all the synthesized compounds proved to be efficient against inhibiting breast cancer cells.

Keywords: Pyrazolo[3,4-*d*]-pyrimidines, Microwave method, Ionic liquid, *p*-TSA, L-Proline, MCF-7 Breast cancer cell lines.

INTRODUCTION

Nitrogen based heterocyclic compounds are known to possess biological activities, literature survey reveals that pyrazolo-pyrimidine derivatives show various pharmacological activities such as anti-inflammatory, antimicrobial, antiviral, anticonvulsant, anticancer, *etc.* [1-3]. Pyrazolopyrimidines are classified into five types [I, II, III, IV, V] according to their mechanism of action on specific targets, leading to a broad range of research that has heightened the interest of researchers in exploring their biological profiles [4]. Some literatures revealed the synthesis of pyrazolo-pyrimidines under microwave irradiation by ionic liquids [5], which have extremely low vapour pressure and can be recycled. Several literature also reported four component reactions under solvent-free conditions [6,7] and such multi-components afforded quick reaction and good yields [8,9].

The hybrid technique, particularly in the synthesis of novel heterocyclic molecules, is exployed to optimize specific biological features such as high efficacy and selectivity, as well as to achieve novel biological activities that are separate from the components [10,11]. The extensive use of pyrazolopyrimidine and its derivatives in a variety of therapeutic applications led to the development of an interest in these compounds [12-14].

In the present investigation, an attempt has been made to synthesize substituted derivatives of pyrazolo-pyrimidine *via* four component reactions by microwave method as well as conventional method under solvent free conditions. The synthesized compounds were confirmed by FT-IR, ^{1}H NMR and ^{13}C

NMR spectral analysis. All the synthesized compounds were also screened for anticancer activity.

EXPERIMENTAL

All chemicals and reagents were procured from SDFCL Company, India. Melting points were determined in open capillary tubes in Buchi B-540 melting point apparatus and are uncorrected. The progress of the reactions were monitored by performing thin layer chromatography using silica gel glass plates. FT-IR spectra were recorded on Vertex version from Bruker, USA. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 using DMSO-d_6 as solvent and tetramethylsilane as an internal standard.

General procedure for the synthesis of pyrazolo[3,4-*d*]-pyrimidinones (5a-j): To a solution of ethyl acetoacetate (0.02 mmol), hydrazine hydrate (0.02 mmol), urea (0.02 mmol) and substituted aromatic aldehydes (0.02 mmol)/L-ascorbic acid/furfural/aliphatic dialdehydes were mixed while stirring. An ionic liquid 2-methyl-imidazolium thiocyanate (0.01 mmol) was added gently to the mixture and placed in a microwave oven until the completion of the reaction. On the other hand, the same reaction was conducted under room temperature by conventional stirring with magnetic stirrer. The progress of both the reactions was monitored by performing TLC using petroleum ether and ethyl acetate (8:2) as an eluent. After the completion of the reaction, the solid product was precipitated, filtered, washed with hot water (3 × 20 mL) thoroughly, dried and finally recrystallized with hot ethanol (3 × 20 mL) to get pure product (**Scheme-I**).

3-Methyl-4-phenyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]-pyrimidin-6-one (5a): Orange solid, m.p.: 233 ºC, yield: 2.28 g, 87%; Anal. calcd. (found) % for $C_{12}H_{10}N_4O$ (*m.w.* 226): C, 63.71 (63.70); H, 4.42 (4.41); N, 24.78 (24.69); O, 7.07 (7.04). IR (KBr, ν_{max}, cm^{-1}): 3459 (-NH, *str.*), 3050 (=C-H, *str.*), 2948 (-C-H, *str.*), 1670 (C=O), 1624 (C=C, *str.*), 1446 (-CH_3, bend.); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.13 (3H, s), 4.59 (1H, s), 7.53 (2H, dddd, *J* = 8.0, 7.4, 1.2, 0.4 Hz), 7.78 (1H, tt, *J* = 7.4, 1.6 Hz), 8.03 (2H, dddd, *J* = 8.0, 1.6, 1.5, 0.4 Hz); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.76 (C_3-N, pyrazole), 77.02 (C_7-N, pyrimidine), 77.29 (C_{14}-N, pyrimidine), 128.56 (C_3-pyrazole), 128.79 (C_7-pyrimidine), 131.21 (C_8-C_{12}, aromatic), 134.06 (C_{12}, aromatic), 162.11 (C_{15}=O).

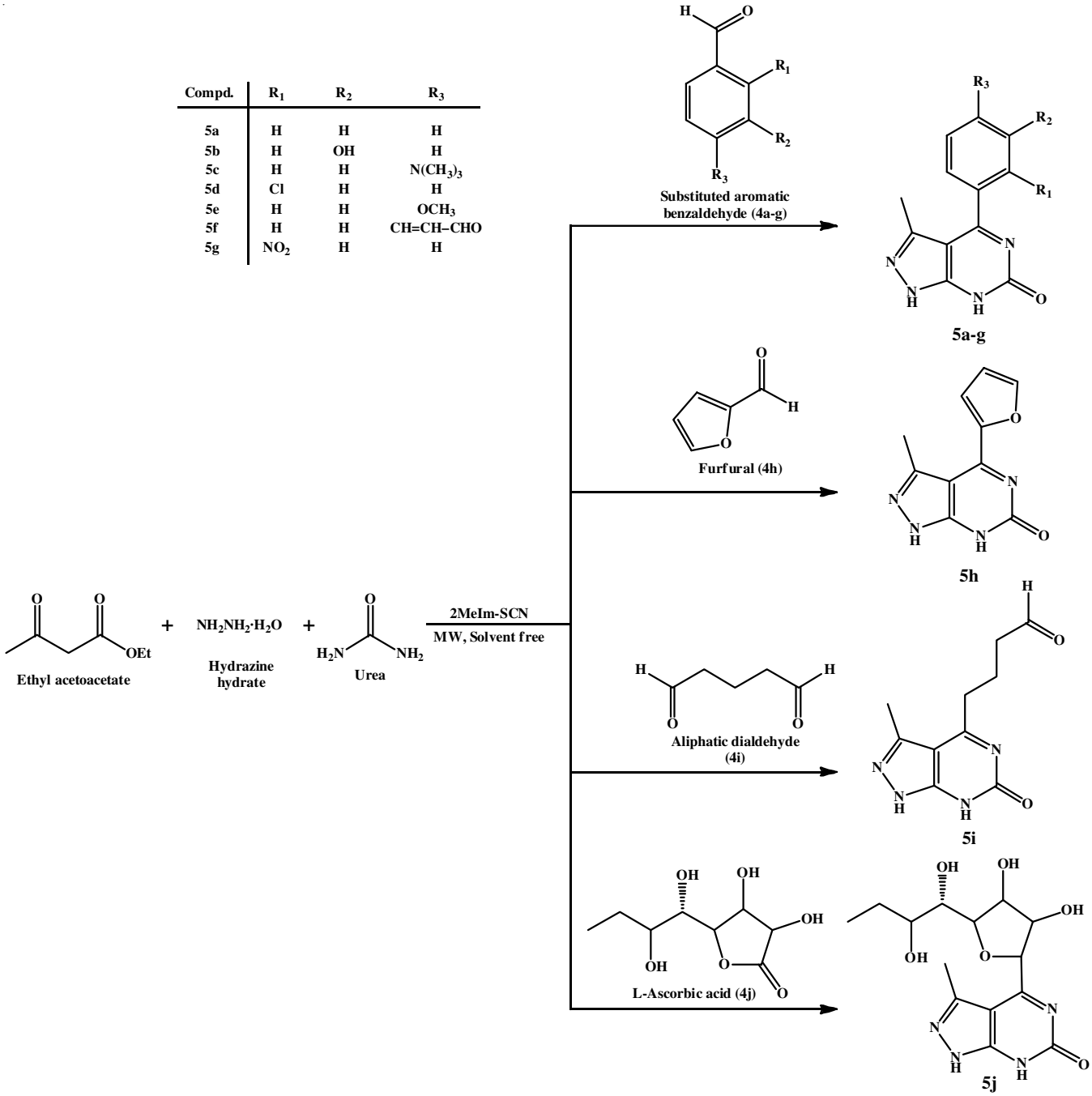
4-(3-Hydroxyphenyl)-3-methyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (5b): Brown solid, m.p.: 186 ºC, yield: 2.38 g, 91.00%; Anal. calcd. (found) % for $C_{12}H_{10}N_4O_2$ (*m.w.* 242): C, 59.50 (59.46); H, 4.13 (4.08); N, 23.14 (23.11); O, 13.22 (13.20). IR (KBr, ν_{max}, cm^{-1}): 3354 (OH *str.*, Ar), 3282 (NH *str.*), 3096 (=C-H), 1584 (C=C arom.), 1487 (CH_3, *bend.*); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.957-2.523 (CH_3, s), 4.813 (1H, s), 6.535-6.606 (1H, ddd, *J* = 8.7, 7.8, 1.6 Hz), 7.524 (1H, ddd, *J* = 8.1, 7.8, 1.5 Hz), 8.60 (CH=NH, s); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 138.749, 130.120, 122.067, 118.57, 114.15, 29.72 (=C_3-CH_3, pyrazole), 76.77 (C_3-N, pyrazole), 77.03 (C_7-N, pyrimidine), 77.30 (C_{16}-N, pyrimidine), 114.15 (C_1, pyrazole), 118.57 (C_2- pyrazole), 122.07 (C_3-pyrazole), 130.12 (C_7-pyrimidine), 138.75 (C_{10}-aromatic), 161.88 (C_{16}=O).

4-[4-(Dimethylamino)phenyl]-3-methyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (5c): Orange solid, m.p.: 164 ºC, yield: 2.41 g, 94%; Anal. calcd. (found) % for $C_{14}H_{15}N_5O$ (*m.w.* 269): C, 62.45 (62.44); H, 5.57 (5.52); N, 26.02 (26.00); O, 5.94 (5.93). IR (KBr, ν_{max}, cm^{-1}): 3441 (-NH, *str.*), 3413 (NH_2, Ar), 2910 (-CH, *str.*), 1666 (C=O *str.*, pyrimidine), 1603 (C=C, Ar), 1523 (CH_3, *bend.*); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.286 (3H, s), 2.827 (6H, s), 6.770 (2H, ddd, *J* = 8.4, 1.1, 0.5 Hz), 7.713 (2H, ddd, *J* = 8.4, 1.9, 0.5 Hz), 9.687 (CH=NH, s); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 40.18 (=C_3-CH_3, pyrazole), 76.77 (C_3-N, pyrazole), 77.03 (C_7-N, pyrimidine), 77.29 (C_{20}-N, pyrimidine), 110.98 (C_1, pyrazole), 111.71 (C_2-pyrazole), 122.18 (C_3-pyrazole), 129.85 (C_7-pyrimidine), 152.08 (C_8-C_{11}, arom.), 160.788 (C_9, C_{10}, C_{15}, C_{16}, arom.), 190.31 (C_{18}-C=O, pyrimidine).

4-(2-Chlorophenyl)-3-methyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (5d): Pale yellow, m.p.: 135 ºC, yield: 2.17 g, 81%); Anal. calcd. (found) % for $C_{12}H_8N_4OCl$ (*m.w.* 259): C, 55.59 (55.54); H, 3.08 (3.02); N, 21.62 (21.59); O, 6.17 (6.15); Cl, 13.51 (13.50). IR (KBr, ν_{max}, cm^{-1}): 3449 (NH *str.*), 3068 (=C-H, *str.*), 1615 (C=C, Ar), 1697 (C=O), 1465 (CH_3, *bend.*), 750 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.509 (3H, s), 3.330 (1H, s), 7.486 (1H, ddd, *J* = 8.7, 7.8, 1.6 Hz), 7.508 (1H, ddd, *J* = 8.1, 7.8, 1.5 Hz), 8.169 (2H, 8.15 (ddd, *J* = 8.7, 1.5, 0.5 Hz), 8.19 (=CH-NH, s); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.76 (C_3-N, pyrazole), 77.02 (C_7-N, pyrimidine), 77.29 (C_{20}-N, pyrimidine), 124.52 (C_1, pyrazole), 124.80 (C_2-pyrazole), 128.69 (C_3-pyrazole), 129.61 (C_7-pyrimidine), 131.57 (C_8-C_{12}, arom.), 133.60 (C_9, arom.), 133.71 (C_{11}, arom.), 134.09 (C_{12}, arom.), 149.10 (C_{13}, arom.), 158.47 (C_{14}, arom.), 188.16 (C_{16}=O).

4-(4-Methoxyphenyl)-3-methyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one:(5e): Pale yellow, m.p.: 140 ºC, yield: 2.0 g, 76%); Anal. calcd. (found) % for $C_{13}H_{12}N_4O_2$ (*m.w.* 256): C, 60.93 (60.91); H, 4.68 (4.64); N, 21.85 (21.79); O, 12.50 (12.49). IR (KBr, ν_{max}, cm^{-1}): 3483 (-NH, *str.*), 2966 (=C-H, *str.*), 1602 (C=C, *str.*), 1461 (CH_3, *bend.*), 1250 (C-O-C); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.503 (3H, s), 3.317 (1H, s) 7.128 2H, ddd, *J* = 8.3, 1.3, 0.4 Hz) 8.386 (2H, ddd, *J* = 8.3, 1.5, 0.4 Hz), 9.707(=CH-NH, s); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.761 (C_3-N, pyrazole), 77.020 (C_7-N, pyrimidine), 77.293 (C_{14}-N, pyrimidine), 128.565 (C_3-pyrazole), 128.786 (C_7-pyrimidine), 131.214 (C_8-C_{12}, arom.), 134.055 (C_{12}, arom.), 162.112 (C_{15}=O).

3-Methyl-4-{4-[(*Z*)-2-phenylethenyl]-phenyl}-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (5f): Yellow solid, m.p.: 197 ºC, yield: 2.3 g, 98%); Anal. calcd. (found) % for $C_{14}H_{11}N_4O$ (*m.w.* 251): C, 66.93 (66.91); H, 4.38 (4.34); N, 22.31 (22.29); O, 6.37 (6.33). IR (KBr, ν_{max}, cm^{-1}): 3481 (NH *str.*, pyrazole), 3038 (=CH *str.*, alkene), 3020 (C-H *str.*, arom.), 1629 (C=C *str.*, alkene), 1586 (C=C *str.*, arom.), 1683 (C=O, pyrimidine), 975.5 (=C-H bending, alkene), 1595 (N-H, bending); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.500 (3H, s), 4.690 (1H, s), 7.128 (1H, d, *J* = 10.5 Hz), 7.295-7.380 (4H, 7.300 (tt, *J* = 7.7, 1.3 Hz), 7.330 (dddd, *J* = 7.9, 7.7, 1.8, 0.4 Hz), 7.350 (d, *J* = 10.5 Hz)), 7.450 (2H, dddd, *J* = 7.9, 1.6, 1.3, 0.4 Hz), 7.620 (2H, ddd, *J* = 8.3, 1.9, 0.5 Hz), 8.380 (2H, ddd, *J* = 8.3, 1.7, 0.4 Hz), 9.700 (1H, s –C-NH, pyrazole); ^{13}C NMR



Scheme-I: Synthetic route of pyrazolo[3,4-*d*]pyrimidine-one derivatives (**5a-j**) using four components system

(DMSO-d_6, 400 MHz) δ ppm: 76.770 (C_3-N, pyrazole), 77.030 (C_7-N, pyrimidine), 77.293 (C_{12}-N, pyrimidine), 119.254 (C_1, pyrazole), 125.321 (C_2, pyrazole), 127.451 (C_3, pyrazole), 127.864 (C_7, pyrimidine), 135.706 (C_8, alkene), 163.725 (C_1-N), 193.75 (C_{12}=O, pyrimidine).

3-Methyl-4-(2-nitrophenyl)-1,3a-dihydro-6*H*-pyrazolo-[3,4-*d*]pyrimidin-6-one (5g): Pale yellow, m.p.: 190 ºC, yield: 2.13 g, 81%); Anal. calcd. (found) % for $C_{12}H_9N_5O_2$ (*m.w.* 255): C, 56.47; H, 3.53 (56.42); N, 27.45 (27.42); O, 12.55 (12.51). IR (KBr, ν_{max}, cm^{-1}): 3449 (NH *str.*), 3068 (=C-H, str.), 1615 (C=C, Ar), 1697 (C=O), 1465 (CH_3, *bend.*); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.12 (3H, s), 6.56 (1H, s), 7.11 (1H, ddd, *J* = 8.7, 7.8, 1.6 Hz), 7.69 (1H, ddd, *J* = 8.1, 7.8, 1.5 Hz), 8.16-8.17 (2H, 8.15 (ddd, *J* = 8.7, 1.5, 0.5 Hz), 8.97 (ddd, *J* = 8.1, 1.6, 0.5 Hz), 10.25 (CH=NH, pyrazole); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.761 (C_3-N, pyrazole), 77.020 (C_7-N, pyrimidine), 77.293 (C_{20}-N, pyrimidine), 124.524 (C_1, pyrazole), 124.802 (C_2- pyrazole), 128.690 (C_3-pyrazole), 129.611 (C_7-pyrimidine), 131.569 (C_8-C_{12}, arom.), 133.595 (C_9, arom.), 133.710 (C_{11}, arom.), 134.094 (C_{12}, arom.), 149.097 (C_{13}, arom.), 158.474 (C_{14}, arom.), 188.163 (C_{16}=O).

3-Methyl-4-(tetrahydrofuran-2-yl)-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (5h): Brown solid, m.p.: 202 ºC, yield: 2.1 g, 90.05%); Anal. calcd. (found) % for $C_{10}H_8N_4O_2$ (*m.w.* 216): C, 55.55 (55.52); H, 3.70 (3.68); N, 25.92 (25.90); O, 14.81 (14.77). IR (KBr, ν_{max}, cm^{-1}): 3442 (-NH, *str.*), 2063

(C=C, furan), 1634 (C=O, pyrimidine); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.51 (3H, s), 7.52-7.54 (1H, dd, *J* = 3.5, 1.8 Hz), 7.896 (1H, dd, *J* = 1.8, 0.9 Hz), 8.733 (=CH-NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.770 (C_3-N, pyrazole), 77.030 (C_7-N, pyrimidine), 77.293 (C_{14}-N, pyrimidine), 112.334 (C_1, pyrazole), 116.941 (C_2-pyrazole), 145.881 (C_8-furan), 149.442 (C_{10}-furan), 151.026 (C_{14}=O).

4-(3-Methyl-6-oxo-3a,6-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-yl)butanal (5i): White solid, m.p.: 175 ºC, yield: 1.85 g, 79%); Anal. calcd. (%) for $C_{10}H_{12}N_4O_2$ (*m.w.* 220): C, 54.54 (54.52); H, 5.54 (5.48); N, 25.45 (25.41); O, 14.54 (14.50). IR (KBr, ν_{max}, cm^{-1}): 3374 (-NH, *str.*), 3149 (=C-H, arom.), 2956 (-CH, *str.*), 1704 (H-C=O), 1610 (N-H *bend.*, pyrazole), 1436 (C=C arom. *str.*), 1355 (C-N *str.*); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.20-1.26 (2H, 1.90 (quint, *J* = 7.5 Hz), 1.529 (quint, *J* = 7.5 Hz), 1.84 (3H, s), 2.35-2.52 (2H, 2.52 (td, *J* = 7.5, 6.9 Hz), 2.52 (td, *J* = 7.5, 6.9 Hz), 2.63-2.72 (2H, 2.67 (td, *J* = 7.5 Hz), 2.76 (t, *J* = 7.5 Hz), 3.33 (1H, s), 10.5 (1H, t, *J* = 6.9 Hz); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 29.71 (C_5-pyrimidine, alkane), 76.76 (C_3-N, pyrazole), 77.01 (C_4-N, pyrimidine).

4-{(5*R*)-5-[(1*R*)-1,2-Dihydroxyethyl]-3,4-dihydroxy-2,5-dihydrofuran-2-yl}-3-methyl-1,3a-dihydro-6*H*-pyrazolo-[3,4-*d*]pyrimidin-6-one (5j): Creamy solid, m.p.: 205 ºC, yield: 2.50 g, 95%); Anal. calcd. (found) % for $C_{14}H_{20}N_4O_6$ (*m.w.* 340): C, 49.41 (49.40); H, 5.88 (5.88); N, 16.47 (16.44); O, 28.23 (28.20). IR (KBr, ν_{max}, cm^{-1}): 3297 (N-H, *str.*), 3185 (O-H, *str.*), 2981 (C-H *str.*, CH_3CH_2), 1678 (C=O, pyrimidine), 1612 (N-H, *bend.*), 1569 (C=C str., arom.), 1194 (C-O *str.*, alcoholic); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.23 (3H, s), 4.04 (2H, 3.51 (d, *J* = 5.5 Hz), 4.06 (d, *J* = 5.5 Hz), 4.08 (1H, td, *J* = 5.5, 4.7 Hz), 4.09 (1H, d, *J* = 4.7 Hz), 4.01 (1H, s), 6.08 (1H, s); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 76.77 (C_3-N, pyrazole), 77.03 (C_7-N, pyrimidine), 77.29 (C_{12}-N, pyrimidine), 112.334 (C_1, pyrazole), 116.941 (C_2-pyrazole), 145.881 (C_3-pyrazole), 149.442 (C_7-pyrimidine).

Anticancer activity: The human breast adenocarcinoma cell lines MCF-7 (ER Positive) were procured from National Centre for Cell Sciences (NCCS), Pune, India and were grown in DMEM HG medium containing fetal bovine serum (10%) and L-glutamine (2 mM). The MCF-7 cells were trypsinized and aspirated into a 5 mL centrifuge tube. The cell pellet was obtained by centrifugation at 300 × g. The cell count was adjusted, using DMEM HG medium, such that 200 µL of suspension contained approximately 10,000 cells. To each well of the 96 well micro-titre plates, 200 µL of cell suspension was added and the plate was incubated at 37 ºC and 5% CO_2 atmosphere for 24 h. After 24 h, the spent medium was aspirated. A 200 µL of different test concentrations (100, 200, 300, 400 and 500 µg/mL from stock) of test drugs were added to the respective wells. The plate was then incubated at 37 ºC and 5% CO_2 atmosphere for 24 h. The plate was removed from the incubator and the drug containing media was aspirated. The 200 µL of medium containing 10% MTT reagent was added to each well to get a final concentration of 0.5 mg/mL and the plate was incubated again at 37 ºC and 5% CO_2 atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Finally, 100 µL of solubilization solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line [15-17].

RESULTS AND DISCUSSION

This study focuses on the solvent-free microwave assisted synthesis of novel pyrazolo[3,4-d]pyrimidines (**5a-j**) using four component systems involving ethyl acetoacetate (0.2 mmol), hydrazine hydrate (0.2 mmol), malononitrile (0.2 mmol) and substituted aromatic aldehydes/L-ascorbic acid/furfural/aliphatic dialdehydes (0.2 mmol) in the presence of different catalysts. The reaction time of microwave mediated synthesis was found to be much less than conventional method in all the three catalytic reactions. The yield of the final product using ionic liquid, 2-methyl-imidazolium thiocyanate catalyzed reactions are excellently than *p*-toluene sulfonic acid (*p*-TSA) and L-proline (Table-1). In some cases, few compounds provided better yield with L-proline in comparison to *p*-TSA; nonetheless, the presence of L-proline as a catalyst affected the reactions.

From Table-1, it also reveals that compound **5g** has obtained in excellent yield both under conventional and under microwave method. It could be due to electron withdrawing Nitro group. On the other hand, compound **5e** has led to relatively lower yield due to electron releasing methoxy group both under microwave as well as conventional stirring conditions. Furthermore, it has been determined that the exceptional yields are attributable to the electron-withdrawing properties of phenyl and phenolic functional groups.

Anticancer activity: Pyrazolopyrimidines have been reported significant efficacy against different types of cancer cell lines. In present investigation, *in vitro* screening of the synthesized pyrazolo-pyrimidine derivatives against MCF-7 human breast cancer cell lines by MTT assay revealed that compound **5a** showed significant cytotoxic property with IC_{50} less than 100 mg/mL. Compound **5** also proved to potential among the tested series with IC_{50} of 108 mg/mL (Table-2). All compounds, expect **5i** and **5j** showed potential cytotoxic effect with IC_{50} less than 500 mg/mL.

Conclusion

Using ionic liquid, 2-methyl-imidazolium thiocyanate catalyzed reaction, a series of pyrazolo[3,4-*d*]pyrimidines (**5a-j**) was successfully synthesized in high yields and shorter reaction time as compared to other catalysts *viz*. *p*-TSA and L-proline in the microwave assisted method. All the synthesized compounds were also evaluated for their cytotoxic activity against MCF-7 breast cancer cells. Some structural features were found to be beneficial to the antitumor activity of the synthesized compounds. In particular, compounds **5a** and **5d** showed significant cytotoxic effect at the lowest concentration.

TABLE-1
TIME AND YIELD (%) DATA OF SYNTHESIZED PYRAZOLO[3,4-*d*]PYRIMIDINE-ONE DERIVATIVES (**5a-j**) CATALYZED BY DIFFERENT CATALYSTS

Compd.	Ionic liquid				*p*-TSA				L-Proline			
	Conv. method (min)	Yield (%)	MW method (sec)	Yield (%)	Conv. method (min)	Yield (%)	MW method (sec)	Yield (%)	Conv. method (min)	Yield (%)	MW method (sec)	Yield (%)
5a	70	79.00	30	91.00	80	78.00	45	81.00	100	68.00	60	78.00
5b	60	82.00	60	94.00	100	76.00	60	78.00	120	76.00	60	82.00
5c	100	76.00	45	86.00	120	89.00	30	91.00	70	89.00	45	95.00
5d	80	78.00	90	88.00	60	83.00	90	83.00	90	63.00	90	72.00
5e	50	66.00	30	74.00	75	82.00	45	92.00	80	84.00	45	89.00
5f	90	82.00	110	87.00	90	77.00	60	79.00	100	77.00	90	81.00
5g	100	87.00	90	96.00	100	81.00	120	86.00	120	81.00	100	88.00
5h	75	83.00	75	95.00	140	72.00	45	82.00	170	71.00	60	83.00
5i	120	77.00	100	90.00	120	71.00	90	76.00	140	71.00	100	79.00
5j	100	80.00	45	81.00	80	71.00	45	71.00	70	71.00	30	73.00

TABLE-2
In vitro CYTOTOXICITY DATA OF AGAINST MCF-7 CELL LINES AT DIFFERENT CONCENTRATIONS OF SYNTHESIZED COMPOUNDS (**5a-j**)

Compounds	% Viability at different concentrations					IC_{50} values (µg/mL)
	100	200	300	400	500	
5a	93.75	88.90	50.79	47.18	22.88	359.01
5b	85.38	72.71	37.41	0.350	0.170	256.19
5c	64.96	64.52	57.43	55.36	36.17	389.69
5d	36.79	37.76	35.56	31.60	26.23	< 100.00
5e	52.55	39.348	25.70	17.07	7.83	108.19
5f	69.71	42.51	38.20	28.96	15.93	> 500 µg/mL; Calculated: 4825
5g	62.76	57.13	41.10	36.70	15.14	237.13
5h	99.20	93.13	69.19	46.65	10.12	361.60
5i	66.98	65.66	66.19	65.31	64.96	209.90
5j	96.83	94.71	95.24	91.98	80.63	> 500 µg/mL; Calculated = 1497.14

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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