Green Synthesis of Pyrazolo [3,4]-Pyrimidine-thiones by using Ionic liquid 2-methyl-Imidazolium-Oxalate as Potent EAC receptor antagonists

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Abstract:

Pyrazolo-Pyrimidines are Heterocycles having nitrogen based hetero atom which is the main composition to exhibit pharmacological efficacy. Since they resemble purine, have a great role in the field of medicinal chemistry. After thorough literature survey, we planned to synthesize the new compounds of Pyrazolo-Pyrimidines by different synthetic methodology followed the green chemistry approach. We are herein reported that one pot solvent-free syntheses of Pyrazolo-Pyrimidines met green chemistry via Ethyl acetoacetate, Hydrazine. hydrate, thiourea & different benzaldehydes by using Ionic liquid 2-methyl-imidazolium-Oxalate under Ultrasonicator. Both Conventional and Ultrasonic methods were employed & Comparisinal studies have been done. It was found that Ultrasonic method completed the reaction quicker than the Conventional method. The Ionic liquid 2-methyl-imidazolium-Oxalate is proved to be a good catalyst for the organic syntheses by attaining good yield. All the synthesized compounds were confirmed by ¹HNMR, IR & ¹³C-NMR spectra. The compounds were tested for in-vitro anticancer activity against Ehrlich Ascites Carcinoma (EAC) cell line. Most compounds revealed significant anticancer activity relative to doxorubicin as positive control with IC₅₀ values.

1. Introduction:

Pyrazolo [3,4-*d*]Pyrimidines have remarkable efficacy in the last decade for a consequence of their wide usage as a pharmaceutically important class of compounds [1]. As literature survey, Pyrazolo-pyrimidine derivatives have considerable potential in the field of chemotherapy, as they were found to exhibit their antitumor activity by inhibiting different types of

enzymes such as Cyclin-dependent kinase [2–4], Src and Abl tyrosine kinase [5], glycogen synthase kinase-3 [6–8], adenosine deaminase [9], and epidermal growth factor receptor protein tyrosine kinase. We revealed the synthesis of Pyrazolo [3,4-d] Pyrimidine-Thiones via Ethyl acetoacetate, Hydrazine. Hydrate, thiourea & different benzaldehydes by the ultra-sonication under solvent free condition. This methodology attained green chemistry. Reactions carried under ultrasonication were more effective for the reaction time and also for the high yield of the products than the conventional method [10]. 2-Mim⁺ Oxalate⁻ was used as a catalyst & found more efficient due to its ionic nature [11]. All the synthesized compounds were analysed by FT-IR, ¹HNMR, ¹³CNMR & Elemental analyses. The potency of these compounds was screened for In vitro test of anticancer activity against Ehrlich Ascites Carcinoma cell lines.

Key Words: Pyrazolo [3,4-*d*]Pyrimidines, Ultrasonicator method, 2-methy-imidazolium-hydroxide, Solvent-free, Green chemistry, Ehrlich Ascites Carcinoma cell lines.

Özet: Pirazolo-Pirimidinler farmakolojik etkinlik göstermesi ana bileşim azot bazlı hetero atom içeren heterosikllerdir. onlar pürin benzerler beri tıbbi kimya alanında büyük bir role sahiptir. Ayrıntılı bir literatür araştırmasına sonra, farklı sentetik metodoloji ile Pirazolo-pirimidinlerin yeni bileşiklerini sentezlemek için planlanan yeşil kimya yaklaşımını takip etti. Bu yazıda, pirazolo pirimidinlerdir bir kap solventsiz sentezleri etil asetoasetat, Hidrazin ile yeşil kimya araya rapor edilmiştir. hidrat, tioure ve ultrasonikatör altında iyonik sıvı 2-metil-imidazolyum-oksalat kullanılarak farklı benzaldehitler. Hem Konvansiyonel ve Ultrasonik yöntemleri kullanıldı & Comparisonal çalışmalar yapılmıştır. Bu ultrasonik yöntem daha hızlı geleneksel yöntemle göre reaksiyonun tamamlandığı tespit edildi. İyonik sıvı 2-metil-imidazolyum-Oksalat iyi bir verim elde organik sentezler için iyi bir katalizör olduğu kanıtlanmıştır. Tüm sentezlenen bileşikler ¹H-NMR, IR ve ¹³C-NMR spektrumları ile doğrulanmıştır. bileşikler, Ehrlich ascites carcinoma (EAC) hücre hattına karşı in vitro anti-kanser etkinlik için test edilmiştir. En bileşikler IC50 değerleri ile pozitif kontrol olarak doksorubisin önemli anti-kanser aktivitesine saptandı.

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1. **Giriş:** Pirazolo [3,4-*d*] primidin bileşimlerinin farmasötik olarak önemli bir sınıf olarak geniş bir kullanım sonucunda son on yıl içinde dikkate değer bir etkiye sahip [1]. bu tür Sikline bağımlı kinaz [2-4], Src ve Abl tirozin kinaz [enzimlerin farklı inhibe etmek suretiyle bir antitümör aktivitesi arzettiği bulunmuştur literatür araştırması olarak, pirazolo-pirimidin-türevleri, kemoterapi alanında önemli bir potansiyele sahiptir [5], glikojen sentaz kinaz-3 [6-8], adenosin deaminaz [9] ve epidermal büyüme faktörü reseptörü protein tirozin kinaz

. Biz etil asetoasetat, Hidrazin ile pirazolo [3,4-d] pirimidin-tion sentezini ortaya koymuştur. Hidrat, tioüre ve çözücüsüz şartlar altında ultrasonikasyonla farklı benzaldehitler. Bu metodoloji yeşil kimya kazanmıştır. ultrasonikasyon altında gerçekleştirilen tepkimeler, geleneksel yöntemle göre daha yüksek ürün verimi için reaksiyon süresi ve aynı zamanda daha etkili olmuştur. 2-Mim + Oxalate- nedeniyle iyonik doğaya bir katalizör olarak kullanılan ve daha etkili bulunmuştur. Tüm sentezlenen bileşikler, FT-IR, ¹H-NMR, ¹³C-NMR ve eleman analizi ile analiz edilmiştir. Bu bileşiklerin gücü Ehrlich ascites carcinoma hücre çizgilerine karşı anti-kanser aktivitesi in vitro deney için taranmıştır.

Anahtar kelimeler: Pirazolo [3,4-d] primidin, ultrasonikatör yöntem olup, 2-metil-imidazolyum-hidroksit, solvent içermeyen, Yeşil kimya, Ehrlich ascites carcinoma hücre çizgileri.

2. Materials used

All Chemicals were purchased by SDFCL Company. Reactions were managed by Ultrasonic bath & magnetic stirrer (REMI). Melting point was determined by Open Capillary tubes in Buchi B-540 melting point apparatus. The reaction was monitored by thin layer chromatography using silica gel glass plates. FT-IR (Vertex version from Bruker), ¹HNMR (Bruker, 400MHz), ¹³C-NMR & Elemental analyser were used. Biological activity (EAC cell lines) has been screened by **Cytixon Biosolutions Pvt. Ltd.**, Hubballi – 580031, Karnataka, India.

2.1 Materials Used for Biological Study

CELL CULTURE LAB

PROTOCOL- P4

Date: 30-10-2018

MTT Assay Protocol and Results

Principle:

The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-

diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

Procedure:

1. The cells were trypsinized and aspirated into a 5ml centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM HG medium, such that 200µl of suspension contained approximately 10,000 cells.
2. To each well of the 96 well microtitre plate, 200µl of the cell suspension was added and the plate was incubated at 37°C and 5% CO₂ atmosphere for 24 h.
3. After 24 h, the spent medium was aspirated. 200µl of different test concentrations (**100, 200 and 300µg/ml from stock**) of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% CO₂ atmosphere for **24 h**.
4. The plate was removed from the incubator and the drug containing media was aspirated. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37°C and 5% CO₂ atmosphere for 3 h.
5. The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan.
6. 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% (IC₅₀) was generated from the dose-response curve for the cell line.

3. Experimental Section

General Procedure for the Synthesis of Pyrazolo [3,4-d]Pyrimidine-Thiones:

To a Solution of Ethyl acetoacetate (10mmol), add hydrazine.hydrate (10mmol), thiourea (10mmol) & different benzaldehydes (10mmol) in Round Bottom flask. The reaction mixture along with 2-methy-imidazolium-Oxalate was kept in an Ultrasonic bath to proceed & monitored

by TLC. The solid precipitate out in the solution & filtered off. The crude solid was recrystallized by hot water, ethanol & finally dried.

Preparation of 2-methyl-imidiazolium-hydroxide:

2-methyl-imidazole (10mmol) was treated with sodium Oxalate (10mmol) & dissolved in an acetone (30ml) solvent. The mixture was kept on magnetic stirrer for 30 mins. The reaction was monitored by TLC. The cleaned transparent solution was obtained.

3-methyl-4-phenyl-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8a). Yellow crystals, m.p. 218–220 ° C, yield (81.00%). IR (KBr) $\nu_{\max}$ /cm⁻¹ 3343 (NH), 1652 (C=S). ¹H-NMR (CDCl₃, 400MHz, δ ppm): 3.80 (s, 3H, OCH₃), 6.82 (d, 2H, J = 9.0 Hz, ArH), 7.00 (d, 1H, J = 4.8 Hz, pyrimidine), 7.12 (t, 1H, ArH), 7.33–7.46 (m, 5H, ArH), 7.62 (d, 2H, J = 9.0 Hz, ArH), 7.54 (d, 2H, J = 8.4 Hz, ArH), 8.11 (d, 2H, J = 8.3 Hz, ArH), 8.48 (d, 1H, J = 4.8 Hz, pyrimidine), 9.39 (s, 1H, NH), 10.05 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.5 (C, OCH₃), 87.6 (C, C₃–pyrazolo-pyrimidine), 106.0 (C, C₆–pyrazolo-pyrimidine), 114.7, 118.2, 120.8, 123.7, 126.7, 129.6, 129.8, 129.9 (14C, Ar), 134.8 (C, C_{3a}–pyrazolo-pyrimidine), 137.8, 142.8, 145.7 (3C, Ar), 147.7 (C, C₇–pyrazolo-pyrimidine), 148.6 (C, Ar), 154.8 (C, C₂–pyrazolo-pyrimidine), 156.8 (C, C₅–pyrazolo-pyrimidine), 163.2 (C=S). Anal. Calcd. (%) for C₁₂H₁₀N₄S (242.00): C, 59.78; H, 4.26; N, 23.28, S, 12.68. Found: C, 59.69; H, 4.11; N, 23.00, S, 13.2%.

4-(2-chlorophenyl)-3-methyl-1,3a-dihydro-6H-Pyrazolo[3,4-d]pyrimidine-6-thione:

(8b). Yellow crystals, m.p. 219–221 ° C, yield (74.00%). IR (KBr) $\nu_{\max}$ /cm⁻¹ 3334 (NH), 1648 (C=S). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.45 (s, 3H, CH₃), 3.6 (s, 3H, OCH₃), 6.77 (d, 2H, J = 8.9 Hz, ArH), 6.66 (d, 1H, J = 4.7 Hz), 7.06 (t, 1H, ArH), 7.26 (d, 2H, J = 8.3 Hz, ArH), 7.28 (t, 2H, ArH), 7.56 (d, 2H, J = 8.9 Hz, ArH), 7.72 (d, 2H, J = 7.6 Hz, ArH), 8.10 (d, 2H, J = 8.1 Hz, ArH), 8.33 (d, 1H, J = 4.7 Hz), 9.33 (s, 1H, NH), 10.01 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.5 (C, CH₃), 55.6 (C, OCH₃), 86.9 (C, C₃–pyrazolo-pyrimidine), 107.1 (C, C₆–pyrazolo-pyrimidine), 113.9, 119.1, 120.1, 123.5, 127.5, 129.0, 129.4, 129.6 (14C, Ar), 133.8 (C, C_{3a}–pyrazolo-pyrimidine), 138.8, 142.2, 146.4 (3C, Ar), 147.8 (C, C₇–pyrazolo-pyrimidine), 149.4 (C, Ar), 154.6 (C, C₂–pyrazolo-pyrimidine),

157.9 (C, C5 –pyrazolo-pyrimidine), 163.1 (C=S). Anal. Calcd. (%) for C₁₃ H₈ N₄OSCl (303.00): C, 51.48; H, 2.64; N, 18.48; O, 5.28; S, 10.56; Cl, 11.55 Found: C, 51.45 H, 2.56; N, 18.56; O, 5.27; S, 10.55; Cl, 11.60%.

3-methyl-4-(2-methoxy-phenyl)-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8c). Yellow crystals, m.p. 206–208 °C, yield (76.00%). IR (KBr) ν_{max} /cm⁻¹ 3340 (NH), 1646 (C=O). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 3.80 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 6.87 (d, 2H, J = 8.9 Hz, ArH), 6.89 (d, 1H, J = 4.8 Hz), 7.05 (d, 2H, J = 8.8 Hz, ArH), 7.11 (t, 1H, ArH), 7.37 (t, 2H, ArH), 7.60 (d, 2H, J = 8.9 Hz, ArH), 7.72 (d, 2H, J = 7.6 Hz, ArH), 8.18 (d, 2H, J = 8.8 Hz, ArH), 8.40 (d, 1H, J = 4.8 Hz), 9.36 (s, 1H, NH), 10.02 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.5 (C, OCH₃), 55.6 (C, OCH₃), 87.4 (C, C3 –pyrazolo-pyrimidine), 106.4 (C, C6 –pyrazolo-pyrimidine), 113.9, 114.2, 119.0, 120.0, 122.4, 123.5, 128.9, 131.3 (14C, Ar), 134.0 (C, C3a –pyrazolo-pyrimidine), 138.6, 146.0 (2C, Ar), 147.7 (C, C7 –pyrazolo-pyrimidine), 149.3 (C, Ar), 154.3 (C, C2 –pyrazolo-pyrimidine), 157.5 (C, C5 –pyrazolo-pyrimidine), 162.2 (C, Ar), 163.2 (C=O). Anal. Calcd. (%) for C₁₃ H₁₂ N₄ OS (272.00): C, 57.35; H, 4.41; N, 20.58; O, 5.88; S, 11.76. Found: C, 57.41; H, 4.45; N, 20.54; O, 5.79; S, 11.97 %.

4-(2-hydroxyphenyl)-3-methyl-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8d), Yellow crystals, m.p. 252–253 °C, yield (88.00%); IR (KBr) ν_{max} /cm⁻¹ 3348 (NH), 1641 (C=S). ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 3.71 (s, 3H, OCH₃), 6.78 (d, 2H, J = 9.0 Hz, ArH), 6.54 (d, 1H, J = 4.7 Hz, pyrimidine), 7.33 (t, 1H, ArH), 7.35 (t, 2H, ArH), 7.57 (d, 4H, J = 8.8 Hz, ArH), 7.77 (d, 2H, J = 8.6 Hz, ArH), 8.45 (d, 2H, J = 8.7 Hz, ArH), 8.52 (d, 1H, J = 4.7 Hz, pyrimidine), 9.44 (s, 1H, NH), 9.99 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 55.6 (C, OCH₃), 88.0 (C, C3 –pyrazolo-pyrimidine), 107.0 (C, C6 –pyrazolo-pyrimidine), 114.0, 119.2, 120.1, 123.7, 129.1, 129.1, 130.6, 131.8 (14C, Ar), 133.9 (C, C3a –Pyrazolo-pyrimidine), 134.6, 138.0, 138.7 (3C, Ar), 145.3 (C, C7 –pyrazolo-pyrimidine), 149.7 (C, Ar), 154.6 (C, C2 –pyrazolo-pyrimidine), 157.9 (C, C5 –pyrazolo-pyrimidine), 163.2 (C=S); Anal. Calcd. (%) for C₁₂H₁₀N₄OS (258.00): C, 55.81; H, 3.87; N, 21.70; O, 6.20; S, 12.40 Found: C, 55.74; H, 3.90; N, 21.85; O, 6.22; S, 12.31%.

3-methyl-4-(2-nitrophenyl)-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8e), Yellow crystals, m.p. 278–280 ° C, yield (84.00%); IR (KBr) $\nu_{\max}$ /cm⁻¹ 3363 (NH), 1656 (C=S). ¹H-NMR (DMSO-d₆, 400 MHz, δ ppm): 3.71 (s, 3H, OCH₃), 6.97 (d, 2H, J = 9.0 Hz, ArH), 7.22 (t, 1H, ArH), 7.36 (t, 2H, ArH), 7.45 (d, 1H, J = 4.8 Hz), 7.56 (d, 2H, J = 9.0 Hz, ArH), 7.71 (d, 2H, J = 7.6 Hz, ArH), 7.90 (d, 2H, J = 8.7 Hz, ArH), 8.21 (d, 2H, J = 8.7 Hz, ArH), 8.72 (d, 1H, J = 4.8 Hz), 9.28 (s, 1H, NH), 10.04 (s, 1H, NH). ¹³C-NMR (DMSO-d₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 87.6 (C, C₃-pyrazolo-pyrimidine), 106.9 (C, C₆-pyrazolo-pyrimidine), 114.4, 119.1, 120.5, 123.3, 129.4, 129.8, 131.0, 131.6 (14C, Ar), 133.7 (C, C_{3a}-pyrazolo-pyrimidine), 133.4, 136.1, 138.7 (3C, Ar), 145.2 (C, C₇-pyrazolo-pyrimidine), 149.5 (C, Ar), 154.8 (C, C₂-pyrazolo-pyrimidine), 157.1 (C, C₅-pyrazolo-pyrimidine), 163.7 (C=S). Anal. calcd. (C₁₂H₉N₅O₂S, %) for (287.00): C, 50.17, H, 3.13; N, 24.39, O, 11.15, S, 11.15; Found: C, 50.45; H, 3.05; N, 24.34, O, 11.01, S, 11.21 %.

4-(2,4-dinitrophenyl)-3-methyl-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8f), Yellow crystals, m.p. 237–239 ° C, yield (67.00%); IR (KBr) $\nu_{\max}$ /cm⁻¹ 3341 (NH), 1649 (C=S). ¹H-NMR (DMSO-d₆, 400 MHz, δ ppm): 3.71 (s, 3H, OCH₃), 6.95 (d, 2H, J = 9.0 Hz, ArH), 7.16 (t, 1H, ArH), 7.38 (d, 1H, J = 4.9 Hz), 7.35 (d, 2H, J = 7.6 Hz, ArH), 7.52 (t, 2H, ArH), 7.54 (d, 2H, J = 9.0 Hz, ArH), 7.79 (d, 2H, J = 8.6 Hz, ArH), 8.35 (d, 2H, J = 8.9 Hz, ArH), 8.79 (d, 1H, J = 4.8 Hz), 9.33 (s, 1H, NH), 10.05 (s, 1H, NH). ¹³C-NMR (DMSO-d₆, 100 MHz, δ ppm): 55.4 (C, OCH₃), 86.8 (C, C₃-pyrazolo-pyrimidine), 108.5 (C, C₆-pyrazolo-pyrimidine), 114.5, 115.8, 115.9, 118.7, 119.5, 123.6, 126.5, 129.1 (14C, Ar), 132.3 (C, C_{3a}-pyrazolo-pyrimidine), 133.3, 138.4 (2C, Ar), 145.0 (C, C₇-pyrazolo-pyrimidine), 147.1 (C, Ar), 151.1 (C, C₂-pyrazolo-pyrimidine), 154.1 (C, C₅-pyrazolo-pyrimidine), 156.6 (C, Ar), 162.2 (C=S). Anal. Calcd. (%) for (C₁₂H₈N₆O₄S, %) for (332.00): C, 43.37, H, 2.40; N, 25.30, O, 19.27, S, 9.63; Found: C, 43.45; H, 2.57; N, 25.50, O, 19.58, S, 8.90 %.

4-(2,4-dihydroxyphenyl)-3-methyl-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8g), Yellow crystals, m.p. 233–235 ° C, yield (76.00%); IR (KBr) $\nu_{\max}$ /cm⁻¹ 3351 (NH), 1658 (C=S). ¹H-NMR (DMSO-d₆, 400 MHz, δ ppm): 3.76 (s, 3H, OCH₃), 7.05 (d, 2H, J = 8.4 Hz, ArH), 7.22 (t, 1H, ArH), 7.42 (t, 2H, ArH), 7.47 (t, 1H, J = 4.9 Hz), 7.74 (d, 2H, J

= 7.8 Hz, ArH), 7.82 (d, 2H, J = 8.5 Hz, ArH), 7.91 (d, 1H, J = 4.6 Hz,), 8.29 (d, 1H, J = 4.4 Hz), 8.58 (d, 1H, J = 2.8 Hz,), 8.71 (d, 1H, J = 4.4 Hz, pyrimidine), 9.44 (s, 1H, NH), 10.07 (s, 1H, NH). ¹³C-NMR (DMSO-d₆, 100 MHz, δ ppm): 55.7 (C, OCH₃), 86.8 (C, C₃-pyrazolo-pyrimidine), 107.3 (C, C₆-pyrazolo-pyrimidine), 114.8, 119.2, 120.8, 126.3 (7C, Ar), 128.1, 129.8 (2C), 130.1 (2C, Ar), 133.2 (C), 133.9 (C, C_{3a}-pyrazolo-pyrimidine), 134.5, 137.1 (2C, Ar), 139.4 (C), 147.3 (C, Ar), 150.8 (C, C₂-pyrazolo-pyrimidine), 154.4 (C, C₅-pyrazolo-pyrimidine), 156.3 (C, C₇-pyrazolo-pyrimidine), 162.9 (C=S). Anal. Calcd. (%) for C₁₂H₁₀N₄O₂S (274.00): C, 52.55; H, 3.64; N, 20.43, O, 11.67, S, 11.67. Found: C, 52.35; H, 3.70; N, 20.71; O, 11.51; S, 11.73 0%.

4-(2,4-dimethylphenyl)-3-methyl-1,3a-dihydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione:

(8h). Yellow crystals, m.p. 251–253 °C, yield (75.00%); IR (KBr) ν_{max}/cm⁻¹ 3372 (NH), 1662 (C=S); ¹H-NMR (CDCl₃, 400 MHz, δ ppm): 2.35 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 6.88 (d, 2H, J = 9.0 Hz, ArH), 6.95 (d, 1H, J = 4.8 Hz, pyrimidine), 7.18 (d, 2H, J = 8.2 Hz, ArH), 7.40 (d, 2H, J = 8.2 Hz, ArH), 7.60–7.64 (m, 5H, ArH), 8.10 (d, 2H, J = 8.2 Hz, ArH), 8.45 (d, 1H, J = 4.8 Hz,), 9.41 (s, 1H, NH), 9.95 (s, 1H, NH). ¹³C-NMR (CDCl₃, 100 MHz, δ ppm): 21.1 (C, CH₃), 55.5 (C, OCH₃), 87.5 (C, C₃-pyrazolo-pyrimidine), 106.8 (C, C₆-pyrazolo-pyrimidine), 114.4, 119.2, 120.1, 127.3, 129.3, 129.7, 133.7 (14C, Ar), 134.2 (C, C_{3a}-pyrazolo-pyrimidine), 136.2, 142.4, 146.6 (3C, Ar), 147.8 (C, C₇-pyrazolo-pyrimidine), 149.6 (C, Ar), 154.4 (C, C₂-pyrazolo-pyrimidine), 157.8 (C, C₅-pyrazolo-pyrimidine), 163.2 (C=O). Anal. Calcd. (%) for C₁₄H₁₄N₄S (270.00): C, 62.22; H, 5.16; N, 20.74; S, 11.85. Found: C, 62.20; H, 5.11; N, 20.79; S, 11.90%.

Reaction Scheme:

Schematic representation for the synthesis of Pyrazolo-Pyrimidines (8a-8h)

General Reaction Scheme:

Different substituted Pyrazolo[3,4-d]-Pyrimidinones (8a-8h)

R=H, R'=H (8a)

R= 2-Cl, R'=H (8b)

R=2-OCH₃, R'=H (8c)

R=2-OH, R'=H (8d)

R= 2-NO₂, R'=H (8e)

R,R'= 2-NO₂,4-NO₂ (8f)

R,R'=2-OH,4-OH (8g)

R,R'=2-CH₃,4-CH₃ (8h)

4. Results & Discussions:

All synthesized compounds are confirmed by FT-IR, 3300-3400 cm⁻¹ (NH group of Pyrazole), 1630-1660 cm⁻¹ (C=S), ¹HNMR by 6.7ppm to 8.5 ppm confirms Aromatic hydrogen & 8.5 to 10.1 indicated the presence of NH protons. In order to follow the green chemistry, solvent-free reactions were maintained under Ultra-sonication [reaction scheme 12]. Ionic liquids found to

be better catalysts for organic synthesis. Bronsted acid ionic liquid 2-methyl-imidazolium-Oxalate catalysed the reactions for good yield as well as instant completion. Experimental data revealed that Ultrasonic method is a more superior method than Conventional. Reaction times and yield of the products were studied for both methods (See Table-4.1) [13-14].

Table-4.1: Comparisomal data for the compounds b/w Conventional & Ultrasonicator methods

Compounds	Catalyst	Conventional Method stirring at RT Time (min)	Yield (%)	Ultrasonicator Method Time (min)	Yield (%)
8a	2-Methyl-Imidazolium-Oxalate			2	
8b		10	79.00	4.5	81.00
8c		12	76.00	3	74.00
8d		8	68.00	2	76.00
8e		10	78.00	4	88.00
8f		14	63.00	6	84.00
8g		11	69.00	3	67.00
8h		14	74.00	7	76.00
		12	73.00		75.00

The preliminary investigation of the anticancer activity of the newly synthesized Pyrazolo-Pyrimidine derivatives was screened against EAC. The IC₅₀ values of these compounds were determined (see Table-4.2, 4.3 & 4.4) [11-14].

[15-17] From the data, we concluded that at 100µg/ml of compounds, the higher anticancer activity of molecules **8e** & **8g** (see Table-4.1) are attributed to the presence of nitro group(2-position) and two OH groups in addition to C=S group that facilitates the C-S-Bonding with the active sites which increase its reactivity respectively. Both **8e** and **8g** showed excellent inhibitory activity than Doxorubicin (see Table-4.2). At 200µg/ml, the molecule **8e** showed excellent than Doxorubicin (see Table-4.3). At 300µg/ml, **8e** molecule exhibited excellent

activity than Doxorubicin (see Table-4.4). The compound (100 µg/ml, 200 µg/ml & 300 µg/ml) 8f exhibited poor IC₅₀ value than all other molecules and Doxorubicin.

The difference between IC₅₀ and binding energy results from the fact, in an enzymatic binding assay of a competitive enzyme inhibitor, the inhibitor is not the only molecule trying to bind the enzyme's active site. It is competing with the substrate, so the concentration of ligand needed to reduce the enzymatic activity (EAC) by 50% depends on the concentration of substrate and how tightly it binds the enzyme [18]. The experimental binding energies (ΔG_{exp}) were calculated from the measured inhibition constants (IC₅₀) by equation 12, using the gas constant (R) and the temperature (T). $\Delta G_{\text{exp}} = -RT \ln \text{IC}_{50}$

Table-4.2: Binding Energy & IC₅₀ values of compounds (100 µg/ml) against EAC

Tested compounds	IC ₅₀ (mg/ml) (EAC)	Binding Energy
8a	86	54.323
8b	123	51.121
8c	78	48.723
8d	185	57.533
8e	66	42.439
8f	211	51.811
8g	35	54.111
8h	144	56.991
Doxorubicin	76	47.881

Table-4.3: Binding Energy & IC₅₀ values of compounds (200 µg/ml) against EAC

Tested compounds	IC ₅₀ (mg/ml) (EAC)	Binding Energy
8a	84	51.21
8b	151	54.92
8c	123	51.77
8d	108	52.11
8e	49	48.39
8f	185	59.9
8g	79	54.33
8h	94	57.89
Doxorubicin	59	46.73

Table-4.4: Binding Energy & IC₅₀ values of compounds (300 µg/ml) against EAC

Tested compounds	IC50 (mg/ml) (EAC	Binding Energy
8a	85	54.62
8b	110	52.51
8c	111	49.78
8d	102	56.34
8e	46	46.77
8f	157	57.74
8g	68	52.35
8h	79	53.31
Doxorubicin	53	49.99

Fig-4.1: Graphical representation of Compounds (100µg/ml) against IC₅₀ values of EAC cell lines

Fig-4.2: Graphical representation of Compounds (200µg/ml) against IC₅₀ values of EAC cell lines

Fig-4.3: Graphical representation of Compounds (300µg/ml) against IC₅₀ values of EAC cell lines

Results and Interpretation:

The IC₅₀ values of the test compounds for **MCF-7** cell-line for 24 hour treatment were found to be [19, 20, 21 & 22]:

Sample name	MCF-7 cell line IC ₅₀ (in µg/ml) 24hr
Doxorubicin	15 µg/ml
**Sample 8a	<100µg/ml
Sample 8b	222.19µg/ml
**Sample 8c	>300µg/ml Calculated Value: 4285µg/ml
**Sample 8d	>300µg/ml Calculated Value: 1497.14µg/ml
Sample 8e	378.69µg/ml
Sample 8f	217.13µg/ml
Sample 8g	329.01 µg/ml
Sample 8h	101.19µg/ml

**For IC₅₀ values outside of the experimental concentration range taken, the values provided above are only expected values, re-testing may be required at the higher/lower concentration range for confirmation.

Note: Calculations and images are attached in the mail. The images at higher drug concentrations may not be clear due to particles from drug precipitation in the media.

3. Conclusions

We reported that Organic reactions were brought very good yield by momentarily under Ultrasonic waves compared to Conventional method. 2-methyl-Imidazolium-Oxalate, an ionic liquid was found to be a good catalyst for Organic syntheses.

Pyrazolo-Pyrimidines proved as potent molecules for Cancer treatment. All the synthesized molecules exhibited moderate inhibition against EAC cell lines. **8e** and **8g** were the most potent molecules having IC₅₀ value 35 mg/ml.

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References

- [1]. Abd El Razik, H.A.; Abdel Wahab, A.E. Synthesis and Biological Evaluation of Some Novel Fused Pyrazolo-pyrimidines as Potential Anticancer and Antimicrobial Agents. Arch. Pharm. Chem. Life Sci. 2011, 11, 184–196. [[CrossRef](#)] [[PubMed](#)]
 - [2]. Aggarwal, R.; Masan, E.; Kaushik, P.; Kaushik, D.; Sharma, C.; Aneja, K.R. Synthesis and biological evaluation of 7-trifluoromethylpyrazolo[1,5-a]pyrimidines as anti-inflammatory and antimicrobial agents. J. Fluor. Chem. 2014, 168, 16–24. [[CrossRef](#)]
 - [3] Kaping, S.; Kalita, U.; Sunn, M.; Singha, L.I.; Vishwakarma, J.N. A facile, regioselective synthesis of pyrazolo[1,5-a]pyrimidine analogs in the presence of KHSO₄ in aqueous media assisted by ultrasound and their anti-inflammatory and anti-cancer activities. Monatsh. Chem. 2016, 147, 1257–1276. [[CrossRef](#)]
 - [4] Deshmukh, S.; Dingore, K.; Gaikwad, V.; Jachak, M. An efficient synthesis of Pyrazolo [1,5-a]pyrimidines and evaluation of their antimicrobial activity. J. Chem. Sci. 2016, 128, 1459–1468. [[CrossRef](#)]
 - [5] El-Mekabaty, A.; Habib, O.M.O.; Moawad, E.B.; Hasel, A.M. Synthesis and Antioxidant Activity of New Pyrazolo[1,5-a]Pyrimidine Derivatives Incorporating a Thiazol-2-ylidiazanyl Moiety. J. Heterocycl. Chem. **2016**, 53, 1820–1826. [[CrossRef](#)]
 - [6] Hassan, A.S.; Masoud, D.M.; Sroor, F.M.; Askar, A.A.; Synthesis and biological evaluation of pyrazolo[1,5-a]pyrimidine-3-carboxamide as antimicrobial agents. Med. Chem. Res. **2017**, 26, 2909–2919. [[CrossRef](#)]
-

[7] Kumar, A.K.A.; Bodke, Y.D.; Lakra, P.S.; Sambasivam, G.; Bhat, K.G. Design, synthesis and anti-cancer evaluation of a novel series of pyrazolo[1,5-a]pyrimidine substituted diamide derivatives. *Med. Chem. Res.*

2017, 26, 714–744. [[CrossRef](#)]

[8] Rahmouni, A.; Souiei, S.; Belkacem, M.A.; Romdhane, A.; Bouajila, J.; Ben Jannet, H. Synthesis and biological evaluation of novel pyrazolo-pyrimidines derivatives as anticancer and anti-5-lipoxygenase agents. *Bioorg. Chem.* **2016**, 66, 160–168. [[CrossRef](#)] [[PubMed](#)]

[9] Zhao, M.; Ren, H.; Chang, J.; Zhang, D.; Yang, Y.; He, Y.; Qi, C.; Zhang, H. Design and Synthesis of pyrazolo[1,5-a]pyrimidine derivatives bearing nitrogen mustard moiety and evaluation of their antitumor activity in vitro and in vivo. *Eur. J. Med. Chem.* **2016**, 119, 183–196. [[CrossRef](#)] [[PubMed](#)]

[10] Li, J.; Zhao, Y.F.; Zhao, X.L.; Yuan, X.Y.; Gong, P. Synthesis and Anti-tumor Activities of Novel Pyrazolo[1,5-a]pyrimidines. *Arch. Pharm. Chem. Life Sci.* **2006**, 339, 593–597. [[CrossRef](#)] [[PubMed](#)]

[11] Ahmed, O.M.; Mohamed, M.A.; Ahmed, R.R.; Ahmed, S.A. Synthesis and anti-tumor activities of some new pyridines and pyrazolo[1,5-a]pyrimidines. *Eur. J. Med. Chem.* **2009**, 44, 3519–3523. [[CrossRef](#)] [[PubMed](#)]

[12] Abdel-Aziz, H.A.; Saleh, T.S.; El-Zahabi, H.S.A. Facile Synthesis and In-Vitro Antitumor Activity of Some Pyrazolo[3,4-b]pyridines and Pyrazolo[1,5-a]pyrimidines Linked to a Thiazolo[3,2-a]benzimidazole Moiety. *Arch. Pharm. Chem. Life Sci.* **2010**, 343, 24–30. [[CrossRef](#)]

[13] Hassan, A.S.; Mady, M.F.; Awad, H.M.; Hafez, T.S. Synthesis and antitumor activity of some new pyrazolo[1,5-a]pyrimidines. *Chin. Chem. Lett.* **2017**, 28, 388–393. [[CrossRef](#)]

[14] Hassan, A.S.; Hafez, T.S.; Osman, S.A.M.; Ali, M.M. Synthesis and In Vitro cytotoxic activity of novel pyrazolo[1,5-a]Pyrimidines and related Schiff bases. *Turk. J. Chem.* **2015**, 39, 1102–1113. [[CrossRef](#)].

[15] Ribeiro DA, Marques ME, Salvadori BDM. In vitro cytotoxic and non-genotoxic effects of gutta-percha solvents on mouse lymphoma cells by single cell gel (comet) assay. *Braz Dent J* 2006;17:228–32.

[16] Elgazwy A-SSH, Nassar IF, Jones PG. Ethyl -5-amino-1-[(4-methylphenyl)sulfonyl]-1H-pyrazole-4-carboxylate., *Acta Cryst.*, 2013;E69:o1376.,

[17] An information portal to biological macromolecular structures., Available from: <http://www.rcsb.org/pdb>.

[18] Humphrey, W., Dalke, A. & Schulten, K. VMD -Visual Molecular Dynamics. *J. Molec. Graph.* 14, 33-38 (1996).

[19] MTT Cell Proliferation Assay Instruction Guide – ATCC, VA, USA www.atcc.org

[20] Gerlier D., and N. Thomasset. *J. Immunol. Methods* 94: 57-63, 1986. Alley, M.C., et al.

[21] *Cancer Res.* 48: 589-601, 1988. Mosmann, T. *J. Immunol. Methods* 65: 55-63, 1983.

[22] Alley, M. C., Scudiere, D. A., Monks, A., Czerwinski, M., Shoemaker, R. II., and Boyd, M. R. Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. *Proc. Am. Assoc. Cancer Res.*, 27: 389, 1986

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