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Research Article

GREEN SYNTHESIS OF PYRAZOLO [3,4]-PYRIMIDINE-THIONES USING IONIC LIQUID 2-METHYL-IMIDAZOLIUM-OXALATE AS POTENT EHRLICH ASCITES CARCINOMA RECEPTOR ANTAGONISTS

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ABSTRACT

Objective: Pyrazolopyrimidines are heterocyclic molecules containing nitrogen as the main composition, and hence, they exhibit pharmacological efficacy. They are analogs of purines so that possessing wide applications in the field of medicinal chemistry. The main objective of this study is to synthesize different derivatives of pyrazole-pyrimidine classes by adopting simple methodology as well as by employing green chemistry. The purpose of the synthesis of these molecules is to study the antitumor activity against Ehrlich ascites carcinoma (EAC) cell lines.

Methods: After literature studies, it makes us to involve in the research of synthetic organic chemistry, especially to synthesize new compounds of pyrazolopyrimidines. We are reported solvent-free synthesis of pyrazolo [3,4-*d*]-pyrimidine-thiones through ethyl acetoacetate, hydrazine hydrate, thiourea, and different benzaldehydes. An ionic liquid 2-methyl-imidazolium-oxalate catalyzed the reactions under ultrasonication bath. Both conventional and ultrasonic methods were employed and comparison studies have been made. It was found that ultrasonic method completed the reaction quicker than the conventional method. All the synthesized compounds were confirmed their structures by ^{1}HNMR, Fourier transform infrared, ^{13}C-NMR, and elemental analysis spectra. The compounds were tested for *in vitro* anticancer activity against EAC cell lines. Most compounds revealed significant anticancer activity relative to doxorubicin as a positive control with inhibitory concentration (IC_{50}) values.

Results: Ultrasonication method is a simple method under which all the reactions were completed at faster time (<7 min) compared to the convention method. Among eight molecules, **8a** and **8d** completed the reactions at a faster rate. We reported IC_{50} values of all the molecules, in which **8e** and **8g** were exhibited excellent potency against EAC cell lines at different concentrations.

Conclusions: Ultrasonication method is an excellent method for the organic synthesis. We are herein reported that under this method, all the reactions are completed within 7 min. Hence, it is superior method than the conventional method. All synthesized molecules have shown good inhibitor potency against EAC cell lines. Among them, two molecules **8e** and **8g** have shown excellent inhibitor potency.

Keywords: Ehrlich ascites carcinoma cell lines, Green chemistry, 2-methyl-imidazolium-hydroxide, Pyrazolo [3,4-*d*]-pyrimidine-thiones, Solvent free, Ultrasonicator method.

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INTRODUCTION

Pyrazolo [3,4-*d*]-pyrimidines are analogs of most purine-based drugs, having huge scope in the past decade for a consequence of their wide usage in medicinal field [1]. On literature studies, pyrazolopyrimidine 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 synthesized pyrazolo [3,4-*d*]- pyrimidine-thiones through ethyl acetoacetate, hydrazine hydrate, thiourea, and different benzaldehydes by the ultrasonication 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 and found more efficient due to its ionic nature [11]. All the synthesized compounds were analyzed by Fourier transform infrared (FT-IR), ^{1}HNMR, ^{13}CNMR, and elemental analysis. The potency of these compounds was screened for *in vitro* test of anticancer activity against Ehrlich ascites carcinoma (EAC) cell lines. Most of the compounds showed excellent potency (inhibitory concentration [IC_{50}] values) against EAC cell lines.

METHODS

All chemicals were purchased by SDFCL Company. Reactions were managed by ultrasonic bath and 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 (TLC) using silica gel glass plates. FT-IR (Vertex version from Bruker), ^{1}HNMR (Bruker, 400 MHz), ^{13}C-NMR, and elemental analyzer were used. Biological activity (EAC cell lines) has been screened by Cytxon Biosolutions Pvt. Ltd., Hubballi – 580031, Karnataka, India.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay protocol

The cells were trypsinized and aspirated into a 5 ml centrifuge tube. Cell pellet was obtained by centrifugation at 300×g. The cell counted was adjusted, using Dulbecco's Modified Eagle's Medium HG medium, such that 200 µl of suspension contained approximately 10,000 cells. To each well of the 96-well microtiter plates, 200 µl of the cell suspension was added and the plate was incubated at 37°C and 5% CO_2 atmosphere for 24 h. After 24 h, the spent medium was aspirated. 200 µl of different test concentrations (100, 200, and 300 µg/ml from stock) of test drugs were added to the respective wells. The plate was then incubated at 37°C and 5% CO_2 atmosphere for 24 h. The plate was removed from the incubator and the drug-containing media were aspirated. 200 µl of

media containing 10% MTT reagent was then added to each well to get a final concentration of 0.5 mg/ml and the plate was incubated at 37°C and 5% CO_2 atmosphere for 3 h.

General procedure for the synthesis of pyrazolo [3,4-*d*]-pyrimidine-thiones

To a solution of ethyl acetoacetate (10 mmol), add hydrazine hydrate (10 mmol), thiourea (10 mmol), and different benzaldehydes (10 mmol) in a round bottom flask. The reaction mixture along with 2-methyl-imidazolium-oxalate was kept in an ultrasonic bath to proceed and monitored by TLC. The solid precipitate out in the solution and filtered off. The crude solid was recrystallized by hot water, ethanol, and finally dried.

Preparation of 2-methyl-imidiazolium-oxalate

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

3-methyl-4-phenyl-1,3a-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidine-6-thione: (8a)

Yellow crystals, m.p. 218–220°C, yield (81.00%). IR (KBr) *ν*max/ cm^{-1} 3343 (NH), 1652 (C=S). ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*ppm): 3.80 (s, 3H, OCH_3), 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). ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ*ppm):55.5 (C,OCH_3), 87.6 (C,C_3–Pyrazolopyrimidine), 106.0 (C,C_6–Pyrazolopyrimidine), 114.7, 118.2, 120.8, 123.7, 126.7, 129.6, 129.8, 129.9(14C,Ar), 134.8 (C,C3a–pyrazolopyrimidine), 137.8, 142.8, 145.7 (3C,Ar), 147.7 (C,C_7–pyrazolopyrimidine), 163.2 (C=S).

Anal. calculated (%) for $C_{12}H_{10}N_4S$ (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-6*H*-Pyrazolo[3,4-*d*] pyrimidine-6-thione: (8b)

Yellow crystals, m.p. 219–221°C, yield(74.00%). IR (KBr) *ν*max/ cm^{-1} 3334 (NH), 1648 (C=S). ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*ppm): 2.45 (s, 3H, CH_3), 3.6 (s, 3H, OCH_3), 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=4.7Hz), 9.33 (s, 1H, NH), 10.01 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ* ppm): 21.5 (C,CH_3), 55.6 (C,OCH_3), 86.9 (C,C3–pyrazolopyrimidine), 107.1 (C,C_6–pyrazolopyrimidine), 113.9, 119.1, 120.1, 123.5, 127.5, 129.0, 129.4, 129.6 (14C,Ar), 133.8 (C,C_{3a}–pyrazolopyrimidine), 138.8, 142.2, 146.4 (3C, Ar), 163.1 (C=S).

Anal. calculated (%) for $C_{13}H_8N_4OSCl$ (303.00): C, 51.48; H, 2.64; N, 18.48; O, 5.28; S, 10.56; Cl, 11.55. Found: C, 51.45H, 2.56; N, 18.56; O, 5.27; S, 10.55; Cl, 11.60%.

3-methyl-4-(2-methoxy-phenyl)-1,3a-dihydro-6*H*-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). ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ* ppm): 3.80 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 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). ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ* ppm): 55.5 (C,OCH_3), 55.6 (C,OCH_3), 87.4 (C,C_3–pyrazolopyrimidine), 106.4 (C,C_6–pyrazolopyrimidine), 113.9, 114.2, 119.0, 120.0, 122.4, 123.5, 128.9, 131.3 (14C,Ar), 134.0 (C,C_{3a}–pyrazolopyrimidine), 162.2 (C,Ar), 163.2 (C=O).

Anal. calculated (%) for $C_{13}H_{12}N_4OS$ (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-6*H*-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 ($CDCl_3$, 400 MHz, *δ*ppm):3.71 (s, 3H, OCH_3), 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). ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ*ppm): 55.6 (C,OCH_3), 88.0 (C,C_3–pyrazolopyrimidine), 107.0 (C,C_6-pyrazolopyrimidine), 114.0, 119.2, 120.1, 123.7, 129.1, 129.1, 130.6, 131.8 (14C,Ar), 163.2 (C=S).

Anal. calculated (%) for $C_{12}H_{10}N_4OS$ (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-6*H*-pyrazolo[3,4-*d*] pyrimidine-6-thione: (8e)

Yellow crystals, m.p. 278–280°C, yield (84.00%); IR (KBr) *ν*max/ cm^{-1} 3363 (NH), 1656 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz, *δ*ppm): 3.71 (s, 3H, OCH_3), 6.79 (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). ^{13}C-NMR (DMSO-d_6, 100 MHz, *δ*ppm): 55.7 (C,OCH_3), 87.6 (C,C_3–pyrazolopyrimidine), 106.9 (C,C_6–pyrazolopyrimidine), 114.4, 119.1, 120.5, 123.3, 129.4, 129.8, 131.0, 131.6 (14C,Ar), 133.7 (C,C_{3a}–pyrazolopyrimidine), 163.7 (C=S).

Anal. calculated ($C_{12}H_9N_5O_2S$, %) 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-6*H*-pyrazolo[3,4-*d*] pyrimidine-6-thione: (8f)

Yellow crystals, m.p. 237–239°C, yield (67.00%); IR (KBr) *ν*max/ cm^{-1} 3341 (NH), 1649 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz, *δ*ppm): 3.71 (s, 3H, OCH_3), 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). ^{13}C-NMR (DMSO-d_6, 100 MHz, *δ* ppm): 55.4 (C,OCH_3), 86.8 (C,C_3–pyrazolopyrimidine), 108.5 (C,C_6-pyrazolopyrimidine), 114.5, 115.8, 115.9, 118.7, 119.5, 123.6, 126.5, 129.1 (14C, Ar), 132.3 (C,C_{3a}–pyrazolopyrimidine), 162.2 (C=S).

Anal. calculated (%) for ($C_{12}H_8N_6O_4S$, %) 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-6*H*-pyrazolo[3,4-*d*] pyrimidine-6-thione: (8g)

Yellow crystals, m.p. 233–235°C, yield(76.00%); IR (KBr) *ν*max/ cm^{-1} 3351 (NH), 1658 (C=S). ^{1}H-NMR (DMSO-d_6, 400 MHz, *δ*ppm): 3.76 (s, 3H, OCH_3), 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). ^{13}C-NMR (DMSO-d_6, 100 MHz, *δ*ppm): 55.7 (C,OCH_3), 86.8 (C,C_3–pyrazolopyrimidine), 107.3 (C,C_6–pyrazolopyrimidine), 114.8, 119.2, 120.8, 126.3(7C, Ar), 128.1, 129.8(2C), 130.1(2C,Ar), 162.9 (C=S).

Anal. calculated (%) for $C_{12}H_{10}N_4O_2S$ (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-6*H*-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 ($CDCl_3$, 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). ^{13}C-NMR ($CDCl_3$, 100 MHz, δppm): 21.1 (C,CH3), 55.5 (C,OCH3), 87.5 (C,C_3–pyrazolopyrimidine), 106.8 (C,C_6–pyrazolopyrimidine), 114.4, 119.2, 120.1, 127.3, 129.3, 129.7, 133.7 (14C, Ar), 134.2 (C,C_{3a}–pyrazolopyrimidine), 163.2 (C=O).

Anal. calculated (%) for $C_{14}H_{14}N_4S$ (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%.

RESULTS AND DISCUSSION

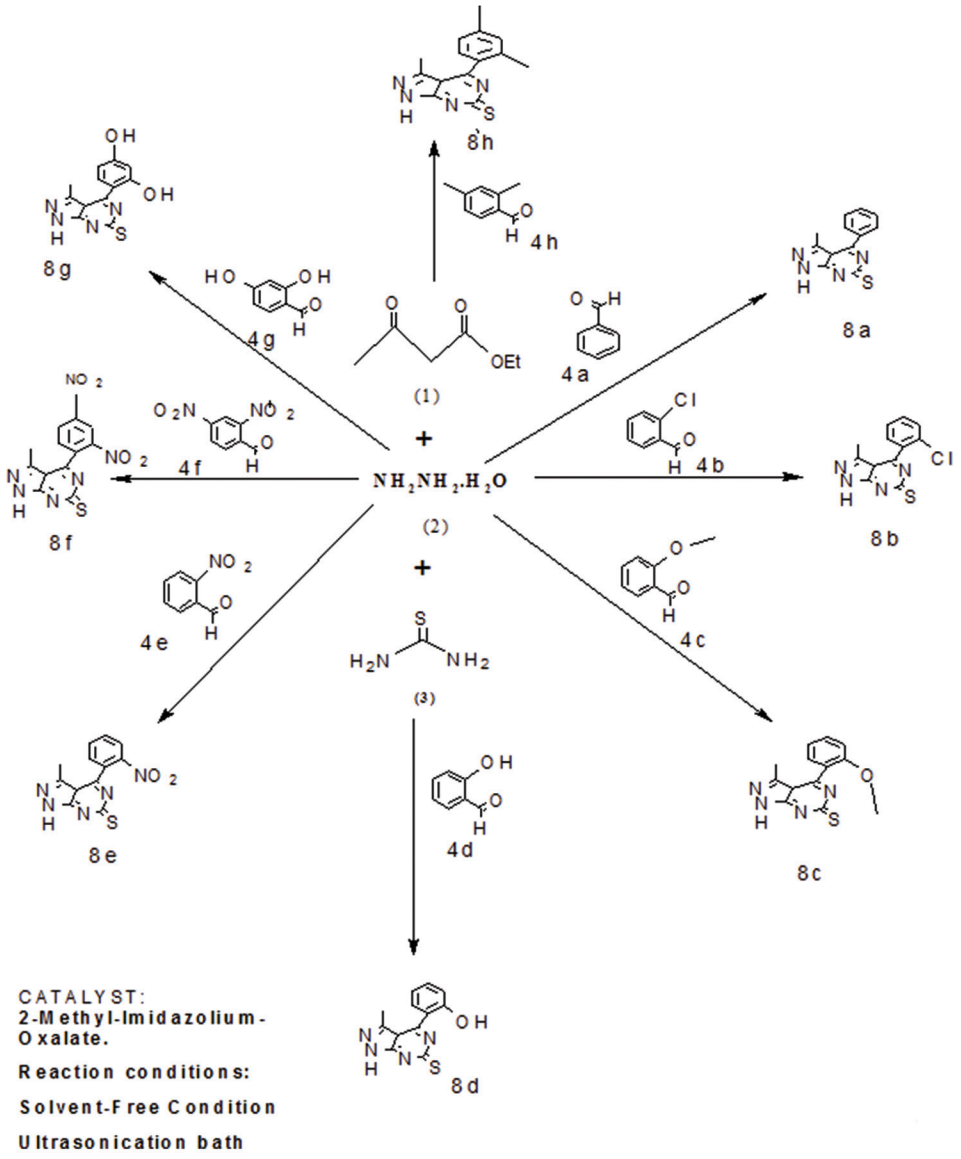
All synthesized molecules are confirmed by FT-IR, 3300–3400 cm^{-1} (NH group of pyrazole) and 1630–1660 cm^{-1} (C=S), ^{1}HNMR by 6.7 ppm–8.5 ppm confirms aromatic hydrogen, and 8.5–10.1 indicated the presence of -NH protons. To follow the green chemistry, solvent-free reactions were maintained under ultrasonication **Reaction Scheme-1** [12]. Ionic liquids found to be better catalysts for organic synthesis. Bronsted acidic ionic liquid 2-methyl-imidazolium-oxalate catalyzed 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 (Table 1) [13].

The conventional method was managed at room temperature by stirring. Under this method, the reaction times for all the molecules are higher and yields were lower than ultrasonication method.

From Table 1, under ultrasonication, we found that **8a** and **8d** molecules completed the reaction at shortest time and yielded the highest quantity (%). Due to electron releasing group present at the 2-position of phenyl substituent, both **8a** and **8d** are found to be stable molecules. Hence, these molecules yield excellent. Similarly, **8e**, **8f,** and **8g** molecules possess electron-withdrawing group at phenyl substituent and, therefore, yield lower than the molecules possessing electron releasing group.

Under the convention method, **8c** and **8d** compounds ended the reactions at shortest time. **8e** and **8f** molecules yield lower than the remaining molecules due to the presence of electron-withdrawing group at phenyl substituent.

The preliminary investigation of the anticancer activity of the newly synthesized pyrazolopyrimidine derivatives was screened *in vitro* against EAC. The IC_{50} values of these molecules were determined (Tables 2-4) [14-17]. From the data, we concluded that at 100 µg/ml,



Reaction Scheme-1: Schematic representation for the synthesis of pyrazolo-pyrimidine-thiones (8a-8h)

the higher anticancer activity of molecules **8e** and **8g** (Table 1 and Fig. 1) is 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.

From Fig. 2, at 200 µg/ml, **8e** and **8g** molecules have excellent IC_{50} values.

Table 1: Comparison data for the compounds b/w conventional and ultrasonicator methods

Compounds	Conventional method at RT time (min)	Yield (%)	Ultrasonic method at 80°C time (min)	Yield (%)
8a	10	79.00	2	81.00
8b	12	76.00	3	74.00
8c	8	68.00	4.5	76.00
8d	10	78.00	2	88.00
8e	14	63.00	4	84.00
8f	11	69.00	6	67.00
8g	14	74.00	3	76.00
8h	12	73.00	7	75.00

Table 2: Binding energy and IC_{50} values of compounds (100 µg/ml) against EAC

Tested compounds	IC_{50} values in µg/ml	Binding energy
8a	84	51.21
8b	151	54.92
8c	123	51.77
8d	108	52.11
8e	49	48.39
8f	185	59.90
8g	79	54.33
8h	94	57.89
Doxorubicin	59	46.73

EAC: Ehrlich ascites carcinoma, IC_{50}: Inhibitory concentration

Table 3: Binding energy and IC_{50} values of compounds (200 µg/ml) against EAC

Tested compounds	IC_{50} values in µg/ml	Binding energy
8a	86	54.33
8b	123	51.12
8c	78	48.72
8d	185	57.53
8e	66	42.43
8f	211	51.81
8g	35	54.11
8h	144	56.99
Doxorubicin	76	47.88

EAC: Ehrlich ascites carcinoma, IC_{50}: Inhibitory concentration

Table 4: Binding energy and IC_{50} values of compounds (300 µg/ml) against EAC

Tested compounds	IC_{50} (µg/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

EAC: Ehrlich ascites carcinoma, IC_{50}: Inhibitory concentration

From Fig. 3, at 300 µg/ml, **8e** and **8g** also possess excellent IC_{50} values.

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. The experimental binding energies (ΔGexp) were calculated from the measured IC_{50} by Equation (1), using the gas constant (R) and the temperature (T) [18-20].

$$\Delta G_{exp} = -RT \ln IC_{50} \quad (1)$$

Where, "ΔGexp" is the binding energy of the molecule with cancer cell lines,

"R" is the universal gas constant (8.314 J $K^{-1}mol^{-1}$),

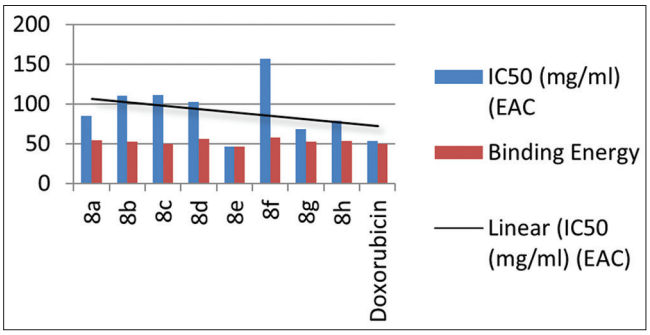


Fig. 1: Graphical representation of compounds (100 µg/ml) against inhibitory concentration 50 values of Ehrlich ascites carcinoma cell lines

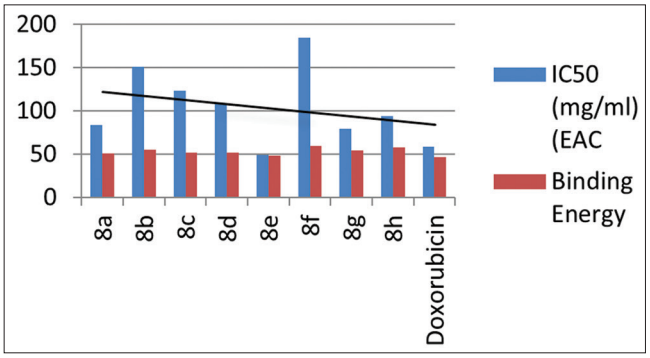


Fig. 2: Graphical representation of compounds (200 µg/ml) against inhibitory concentration 50 values of Ehrlich ascites carcinoma cell lines

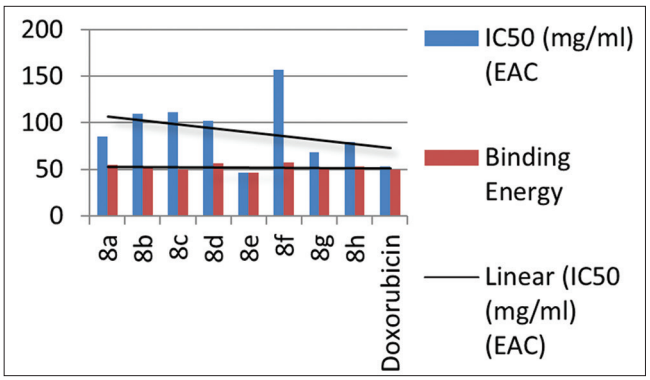


Fig. 3: Graphical representation of compounds (300 µg/ml) against inhibitory concentration 50 values of Ehrlich ascites carcinoma cell lines

"T" is the temperature at which a molecule binds (298°K) at room temperature,
"IC_{50}" is the inhibitory concentration of the molecule.

Results and interpretation

The IC_{50} values of the test compounds for EAC cell line for 24 h treatment were found to be

Sample name	Ehrlich ascites carcinoma cell line inhibitory concentration 50 (in µg/ml) 24 h
Doxorubicin	15
**Sample 8a	<100
Sample 8b	>100
**Sample 8c	>100.00
	Calculated value: 88.5
**Sample 8d	>100.00
	Calculated value: 149.71
Sample 8e	<100.00
Sample 8f	>150.00
Sample 8g	<100.00
Sample 8h	<100.00

CONCLUSIONS

We reported that organic reactions were brought very good yield by momentarily under ultrasonic waves compared to the conventional method. 2-methyl-imidazolium-oxalate, an ionic liquid was found to be a good catalyst for organic syntheses. Pyrazolopyrimidines proved as potent molecules for cancer treatment. All the synthesized molecules exhibited moderate inhibition against EAC cell lines. **8e** and **8g** have the most potent IC_{50} values.

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CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally in the design, development, review, and finalization of the contents of the manuscript.

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