Green design of novel heterocycles using deep eutectic solvent and evaluation of their cytotoxicity and antioxidant activities

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Abstract
A series of chalcone derivatives 1a-c was synthesized according to green chemistry methodology using deep eutectic solvents as greener solvents. Chalcone 1a was used as a versatile starting material for the synthesis of variety of heterocyclic systems including isoxazoline, pyrazoline, pyrimidine and pyridine moieties. Elemental analyses and spectral data (IR, MS, $^1$H NMR, $^{13}$C NMR) were used to elucidate the structural formula of the products. The cytotoxicity of the prepared derivatives was screened using 3- [4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay against three tumor cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland (MCF-7) and colorectal adenocarcinoma (Caco-2) where the cytotoxic effects showed that pyrazoline derivatives (4) induced a significant growth inhibition towards tested cell lines while 1,2-dihydropyridine-3-carbonitrile derivatives (7) showed the lowest activity. Additionally, antioxidant activity of the products was evaluated using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, the results exhibited that compounds 4,5-dihydro-1$H$-pyrazole-1-carbothioamide (2b) and 4,5-dihydro-1$H$-pyrazole (3) showed potent activity in comparison with ascorbic acid as standard.

Keywords: Chalcone; deep eutectic solvent; heterocycles; cytotoxicity; antioxidant.

1. Introduction
Recently, deep eutectic solvents (DESs) have appeared in green chemistry as promising alternatives to other organic solvents due to their low volatility, non-flammability and high thermal stability which promote their uses in many organic transformations and reactions (1-5). Additionally, DESs have the advantages of being recyclable and inexpensive can be used in stoichiometric amounts as catalyst (6, 7).

It is simple to create DESs by hydrogen bonding an acceptor, such as (Ch.cl), with a hydrogen bond donor, as an acid, carbohydrate, or amide (8-14).

Chalcone derivatives represent an interesting group of organic compounds that are used as starting materials for the creation of heterocyclic molecules. Chalcones have shown important antimicrobial (15-18), anticancer (19-22), antifungal (23-25), antioxidant (26, 27), antibacterial (28, 29) anti-inflammatory activities (27, 30).

In view of our continued interest in employing green chemistry principles to create bio-active heterocyclic compounds (31-36), this study was directed towards the synthesis of a variety of bio-active heterocyclic compounds using chalcone derivatives as reactive key precursors in the presence of greener deep eutectic solvents. In addition, the products will be examined for their antitumor and antioxidant activities.

1. Experimental

2.1. The general procedures for synthesising chalcones (1a-c)

To a mixture of 2-acetylthiophene (0.01 mol) and aromatic aldehyde (0.01 mol) namely, p-chlorobenzaldehyde, p-nitrobenzaldehyde and N,N-dimethylanobenzaldehyde in choline chloride-urea combination (5 mL); 10 %
NaOH (3 mL) was added then the mixture was stirred at 0-5º C for 0.5-3 h. The obtained precipitate was filtrated, washed, dried and recrystallized to produce crystals of chalcones 1a-c.

3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (1a)

Yield, 98 %; M.p. 118-120º C; IR (KBr, ν cm⁻¹): 3062 cm⁻¹ (CH-aromatic), 1643 cm⁻¹ (CO), and 1597 cm⁻¹ (C=C); ¹H NMR (DMSO-d₆, δ ppm): 7.69 (d, 1H, α-CH olefinic, J = 15 Hz), 7.91 (d, 1H, β-CH olefinic, J = 18Hz), 7.31–8.34 (m, 7H, Ar–H); Anal. for C₁₃H₉ClOS (248.5): calcd: C, 62.78; H, 3.65. Found: C, 63.08; H, 3.40.

3-(4-Nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (1b)

Yield, 94 %; M.p. 196–198º C; IR (KBr, ν cm⁻¹): 3101 cm⁻¹ (CH-aromatic) 1651 cm⁻¹ (CO) and 1597 cm⁻¹ (C=C); ¹H NMR (DMSO-d₆, δ ppm): 7.79 (d, 1H, α-CH olefinic, J = 15.9 Hz), 8.05 (d, 1H, β-CH olefinic, J = 15.9 Hz), 7.34–8.40 (m, 7H, Ar–H); Anal. calcd. For C₁₃H₉NO₃S (259): C, 60.22; H, 3.50; N, 5.40. Found: C, 60.82; H, 4.10; N, 5.30.

3-(4-(Dimethylamino) phenyl)-1-(thiophen-2-yl) prop-2-en-1-one (1c)

Yield, 79 %; M.p. 96-98º C; IR (KBr, ν cm⁻¹): 3086 cm⁻¹ (CH-aromatic), 2909, 2816 cm⁻¹ (CH aliphatic), 1627 cm⁻¹ (CO); and 1597 cm⁻¹ (C=C); ¹H NMR (DMSO-d₆, δ ppm): 7.58 (d, 1H, α-CH olefinic, J = 14 Hz); Anal. calcd. For C₁₅H₁₅NOS (257): C, 70.51; H, 6.08; N, 5.44. Found: C, 70.62; H, 4.10; N, 5.30.

2.2. Synthesis of pyrazoline derivatives 2a,b

First method:

Chalcone 1a was dissolved in CHCl₃:urea [1:2] mixture (5 mL) containing 20 mL containing sodium hydroxide (0.012 mol) and thiosemicarbazide or semicarbazide (0.01 mol) were added before reflux for 5-8 hours. The mixture was put into cold water, and then the precipitate was filtered and purified using Ethanol resulting in 2a,b.

5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (2a)

Yield of first method: 91%, M.p. 194-200º C. IR (KBr, ν cm⁻¹): 3425, 3400 cm⁻¹ (NH₂), 3086 cm⁻¹ (CH aromatic), 2978, 2900 cm⁻¹ (CH aliphatic), 1627 cm⁻¹ (CO); ¹H NMR (DMSO-d₆, δ ppm): 3.010 (s, 6H, 2CH₃), 6.74-8.22 (m, 7H, Ar–H), 7.58 (d, 1H, α-CH olefinic, J = 12 Hz), 7.97 (d, 1H, β-CH olefinic, J = 14 Hz); Anal. calcd. For C₁₅H₁₄ClN₃O (285): C, 58.99; H, 3.96; N, 14.04. Found: C, 59.09; H, 3.93; N, 14.34.

Second method:

Chalcone 1a was dissolved in ethanol (20 mL) containing NaOH (0.012 mol) and thiosemicarbazide or semicarbazide (0.01 mol) were added before reflux for 5-8 hours. The mixture was put into cold water, and then the precipitate was filtered and purified using Ethanol resulting in 2a,b.

5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (2b)

Yield of first method: 73%, M.p. 110-119º C. IR (KBr, ν cm⁻¹): 3394, 3200 cm⁻¹ (NH₂), 3050 cm⁻¹ (CH aromatic), 2962, 2839 cm⁻¹ (CH aliphatic), 1273 cm⁻¹ (CS); ¹H NMR (DMSO-d₆, δ ppm): 3.3 (dd, 1H, Ha, J = 20.7 Hz, J = 5.1 Hz), 3.449 (dd, 1H, Hb, J = 5.4 Hz, J = 4.8 Hz), 5.91 (dd, 1H, Hx, J = 3.6 Hz, J = 3.3 Hz), 7.13–7.77 (m, 7H, Ar–H); Anal. calcd. For C₁₅H₁₄ClN₃S (321.5): C, 52.25; H, 3.76; N, 13.06; Found: C, 52.34; H, 3.81; N, 12.98.
2.3.5-(4-Chlorophenyl)-1-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1\textsubscript{H}-pyrazole (3)

Phenyl hydrazine (0.005 mol) and Chalcone 1\textsubscript{a} (0.005 mol) and were mixed and refluxed in a combination of Ch.Cl-urea (1:2) (6 mL) for five hours then cooling, the precipitate was filtrated and purified by ethanol to produce compound 3.

Yield: 60\%, M.p. 140-142° C. IR (KBr, ν cm\textsuperscript{-1}): 3075 cm\textsuperscript{-1} (CH aromatic), 2913, 2891 cm\textsuperscript{-1} (CH aliphatic); 1\textsubscript{H} NMR (DMSO-d\textsubscript{6}, δ ppm): 3.077 (dd, 1H, Ha, Jab = 9.3 Hz, Jax = 6 Hz), 3.867 (dd, 1H, Hb, Jab = 12.3 Hz, Jbx = 12.3 Hz), 5.475 (dd, 1H, Hx, Jax = 6.3 Hz, Jbx = 6 Hz), 6.698–7.605 (m, 12H, Ar–H); MS: m/z: 338 (M+); Anal. calcd. For C\textsubscript{19}H\textsubscript{15}ClN\textsubscript{2}S (338.5): C, 67.35; H, 4.46; N, 8.27. Found: C, 68.05; H, 3.56; N, 8.87.

2.4. 1-(5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1\textsubscript{H}-pyrazol-1-yl) ethan-1-one (4)

First method:

Hydrazine hydrate (0.01 mol) and Chalcone 1\textsubscript{a} (0.01 mol) and were mixed in a Ch.Cl-urea mixture [1:2] (5 mL) with 0.5 mL of acetic acid, and the mixture was refluxed for 3 h. Then put into cold water, and the resulted precipitate was filtrated, washed, and purified from ethanol/water to give brown crystals of compound 4.

Second method:

Chalcone 1\textsubscript{a} (0.01 mol) and hydrazine hydrate (0.01 mol) were mixed in (8) mL of acetic acid, and the mixture was refluxed for 6 h. Then put in cold water, and the resulted precipitate was filtered, washed, dried, and purified from ethanol/water to produce brown crystals of compound 4.

Yield of first method: 85\%, M.p. 188-190C. IR (KBr, ν cm\textsuperscript{-1}): 3086 cm\textsuperscript{-1} (CH aromatic), 2916, 2854 cm\textsuperscript{-1} (CH aliphatic); 1\textsubscript{H} NMR (DMSO-d\textsubscript{6}, δ ppm): 3.736 (dd, 1H, Ha, Jab = 8 Hz, Jax = 7.8 Hz), 3.86 (dd, 1H, Hb, Jab = 10.8 Hz, Jbx = 11.2 Hz), 5.735 (dd, 1H, Hx, Jax = 8.4 Hz, Jbx = 8Hz), 7.14–7.71 (m, 7H, Ar–H); 13\textsubscript{C} NMR (CDCl\textsubscript{3}, δ ppm): 21.84 (CH\textsubscript{3}), 42.875 (CH\textsubscript{2}), 59.41 (CH), 127.068, 127.622, 128.746, 128.821 (thiophene carbons), 127.068, 129.049, 132.4, 135.69 (phenyl carbons), 140.083 (C5-pyrazoline), 149.325 (CO); MS: m/z: 304 (M\textsuperscript{+}); Anal. calcd. C\textsubscript{15}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2} (304.5) C, 59.11; H, 4.30; N, 9.19. Found: C, 58.61; H, 5.10; N, 9.27.

2.5. 5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (5)

Hydroxylamine. HCl(0.005 mol) and Chalcone 1\textsubscript{a} (0.005 mol) was dissolved in a (8 ml) mixture of Ch.Cl-Urea, which contained sodium hydroxide (30\%), and refluxed for 6 hours. Once the reaction mixture had cooled, it was placed in cold water, where the precipitate that had formed was filtered, cleaned, dried, and purified with ethanol to yield 5.

Yield: 70\%, M.p. 116-118° C. IR (KBr, ν cm\textsuperscript{-1}): 3086 cm\textsuperscript{-1} (CH aromatic), 2916, 2854 cm\textsuperscript{-1} (CH aliphatic); 1\textsubscript{H} NMR (CDCl\textsubscript{3}, δ ppm): 3.35 (s, 3H, CH\textsubscript{3}), 3.086 (dd, 1H, Ha, Jab = 4.5 Hz, Jax = 3.3 Hz), 3.722, (dd, 1H, Hb, Jab = 12 Hz, Jbx = 11.7 Hz), 5.532 (dd, 1H, Hx, Jax = 4.8 Hz, Jbx = 4.5Hz), 7.067–7.452 (m, 7H, Ar–H); 13\textsubscript{C} NMR (DMSO-d\textsubscript{6}, δ ppm): 21.84 (CH\textsubscript{3}), 42.875 (CH\textsubscript{2}), 59.41 (CH), 127.068, 127.622, 128.746, 128.821 (thiophene carbons), 127.068, 129.049, 132.4, 135.69 (phenyl carbons), 140.083 (C5-pyrazoline), 149.325 (CO); MS: m/z: 304 (M\textsuperscript{+}); Anal. calcd. C\textsubscript{15}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2} (304.5) C, 59.11; H, 4.30; N, 9.19. Found: C, 58.61; H, 5.10; N, 9.27.

2.6. 4-(4-Chlorophenyl)-6-(thiophen-2-yl)pyrimidin-2-amine (6)

In 5 mL of a 1:2 solution of choline chloride and urea, guanidine hydrochloride (0.005 mol) and Chalcone 1\textsubscript{a} (0.005 mol) were mixed. The reaction mixture was mixed with sodium hydroxide (0.015 mol), and then refluxed for 5 hours. The precipitate obtained was filtered, and crystallized to give compound 6.

Yield: 85\%, M.p. 116-118° C. IR (KBr, ν cm\textsuperscript{-1}): 3086 cm\textsuperscript{-1} (CH aromatic), 2978, 2924 cm\textsuperscript{-1} (CH aliphatic), 1666.5 cm\textsuperscript{-1} (CO), 1605cm\textsuperscript{-1} (C=N); 1\textsubscript{H} NMR (CDCl\textsubscript{3},δ ppm): 3.5 (s, 3H, CH\textsubscript{3}), 3.086 (dd, 1H, Ha, Jab = 4.5 Hz, Jax = 3.3 Hz), 3.722, (dd, 1H, Hb, Jab = 12 Hz, Jbx = 11.7 Hz), 5.532 (dd, 1H, Hx, Jax = 4.8 Hz, Jbx = 4.5Hz), 7.067–7.452 (m, 7H, Ar–H); 13\textsubscript{C} NMR (DMSO-d\textsubscript{6}, δ ppm): 3.35 (s, 3H, CH\textsubscript{3}), 3.086 (dd, 1H, Ha, Jab = 4.5 Hz, Jax = 3.3 Hz), 3.722, (dd, 1H, Hb, Jab = 12 Hz, Jbx = 11.7 Hz), 5.532 (dd, 1H, Hx, Jax = 4.8 Hz, Jbx = 4.5Hz), 7.067–7.452 (m, 7H, Ar–H); 13\textsubscript{C} NMR (DMSO-d\textsubscript{6}, δ ppm): 21.84 (CH\textsubscript{3}), 42.875 (CH\textsubscript{2}), 59.41 (CH), 127.068, 127.622, 128.746, 128.821 (thiophene carbons), 127.068, 129.049, 132.4, 135.69 (phenyl carbons), 140.083 (C5-pyrazoline), 149.325 (CO); MS: m/z: 304 (M\textsuperscript{+}); Anal. calcd. C\textsubscript{15}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2} (304.5) C, 59.11; H, 4.30; N, 9.19. Found: C, 58.61; H, 5.10; N, 9.27.
Yield: 76%, M.p. 148-150 °C. IR ((KBr, v cm⁻¹): 3325, 3209 cm⁻¹ (NH₂), 3101.5 cm⁻¹ (CH aromatic), 1627.9 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆, δ ppm): 6.75 (s, 2H, NH₂, exchangeable), 7.2–8.2 (m, 8H, Ar–H; ¹³C NMR (DMSO-d₆, δ ppm): 100.15 (CH pyrimidine), 128.1, 128.521, 129.95 (thiophene carbons), 128.42, 128.696, 135.3, 135.97 (phenyl carbons), 150.3 (C₂-pyrimidine), 160.34 (C=N); MS: m/z: 287 (M +); Anal. calcd. C 14H10ClN₃S (287.5): C, 58.43; H, 3.50; N, 14.60, Found: C, 57.63; H, 4.30; N, 13.80.

2.7.4-(4-Chlorophenyl)-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (7)

Chalcone 1a (0.005 mol), ethylecyanoacetate (0.005 mol), and amm.acetate (0.01 mol) were added to a 6 mL mixture of 1:2 choline chloride and urea before being refluxed for two hours. The resulting precipitate was filtered, collected, and crystallized by ethanol into yellow compound 7.

Yield of first method: 81 %, M.p. 178-180 °C. IR ((KBr, v cm⁻¹): 3312 (NH), 3086 cm⁻¹ (CH aromatic), 2951, 2885 cm⁻¹ (CH aliphatic), 1651 cm⁻¹ (CO); ¹H NMR (DMSO-d₆, δ ppm): 0.95, 1.03 (2s, 6H, 2CH₃), 1.91 (d, 1H, CH₂, J = 18), 2.15 (d, 1H, CH₂, J = 15), 2.46 (s, 2H, CH₂CO), 4.52 (d, 1H, CH, J = 6), 5.19 (d, 1H, CH = J = 1.8), 6.65 (s, 1H, NH, exchangeable), 7.04-8.34 (m, 7H, Ar –H), Anal. calcd. For C₂₁H₂₀ClN₂O₅ (369.5) : C, 68.19; H, 5.45, N, 3.79; Found: C, 69.09; H, 4.85; N, 4.09.

2.8. 4-(4-Chlorophenyl)-7,7-dimethyl-2-(thiophen-2-yl)-4,6,7,8-tetrahydroquinolin-5(1H)-one (8)

First method:

For 8 hours, in a sand bath, a mixture of dimedone (0.003 mol), chalcone 1a (0.003 mol), and ammonium acetate (0.0045 mol) was refluxed after the reaction mixture had cooled and been put into cold water. The reaction product was filtered, washed, and crystallized by ethanol to produce brown crystal 8.

Second method:

Dimedone (0.003 mol), chalcone 1a (0.003 mol), and ammonium acetate (0.0045 mol) were mixed and refluxed for 5 hours in a 7 mL solution of choline chloride and urea, the reaction mixture was cooled before being placed into cold water. The product was filtered, cleaned, and crystallized from the ethanol to produce brown crystal 8.

Yield of first method: 81 %, M.p. 178-180 °C. IR ((KBr, v cm⁻¹): 3312 (NH), 3086 cm⁻¹ (CH aromatic), 2951, 2885 cm⁻¹ (CH aliphatic), 1651 cm⁻¹ (CO); ¹H NMR (DMSO-d₆, δ ppm): 0.95, 1.03 (2s, 6H, 2CH₂), 1.91 (d, 1H, CH₂, J =18 ), 2.15 (d, 1H, CH₂, J =15), 2.46 (s, 2H, CH₂CO), 4.52 (d, 1H, CH, J = 6), 5.19 (d, 1H, CH = J= 1.8), 6.65 (s, 1H, NH, exchangeable), 7.04-8.34 (m, 7H, Ar –H), Anal. calcd. For C₂₁H₂₀ClN₂O₅ (369.5) : C, 68.19; H, 5.45, N, 3.79; Found: C, 69.09; H, 4.85; N, 4.09.

2.9. 2-Amino-4-(4-chlorophenyl)-6-(thiophen-2-yl)-4H-pyran-3-carbonitrile (9)

Three drops of piperedine were introduced to amixture of malononitrile (0.003 mol) and chalcone 1a (0.003 mol) in a choline chloride-urea mixture (1:2), and refluxed for eight hours, the recovered precipitate was filtered, cleaned, dried, and crystallized with ethanol to produce a brown crystal of compound 9.

Yield: 81%, M.p. 240-242 °C. IR ((KBr, v cm⁻¹): 3433, 3300 cm⁻¹ (NH₂), 3100 cm⁻¹ (CH aromatic), 2216 cm⁻¹ (CN); ¹H NMR (DMSO-d₆, δ ppm): 4.6 (S,1H,CH₂), 6.27 (s, 2H, NH₂, exchangeable), 7.25-8.35 (m, 11H, Ar–H), Anal. calcd. For C₁₆H₁₁ClN₂O₅ (314.5): C, 61.05; H, 11.26; N, 8.90. Found: C, 59.85; H, 12.46; N, 9.30.
3. Results and discussion

3.1. Chemistry

Herein, it is reported on the creation of the intended heterocyclic compounds by employing chalcone derivatives and deep eutectic solvents as green solvents.

Thus, employing a deep eutectic solvent consisting of a 1:2 combination of choline chloride and urea and treating aromatic aldehydes, such as p-chlorobenzaldehyde, p-nitrobenzaldehyde, and N,N-dimethylaminobenzaldehyde with 2-acetyltiophene, gave the chalcone derivatives 1a–c in good yields, as shown in Scheme 1.

As expected, chalcones 1a-c may exist either in the Z or E form. Using $^1$H NMR spectra, the chalcones'E configuration was verified, which showed two doublet signals for the two olefinic $\alpha$ and $\beta$ protons at 7.32-7.97 and 7.91-8.38 ppm with the coupling constant value $J = 3.9-5.1$ Hz.

IR spectra showed regions of absorption, at 3101-3062, 1651-1627 cm$^{-1}$ which correspond to the olefinic C-H and CO groups, respectively.

To optimize the reaction conditions and improve the yield, the synthesis of chalcones 1a was carried out in different DESs. The highest yield was obtained by using choline chloride – urea (1:2), Table (1).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time (hr)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ChCl: Urea (1:2)</td>
<td>0.5</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>ChCl: glycerol</td>
<td>2</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>ChCl: oxalic</td>
<td>1</td>
<td>79</td>
</tr>
</tbody>
</table>
A number of novel heterocyclic compounds with anticipated biological activity were created using chalcone derivative 1a. Thus, chalcone 1a reaction with semicarbazide as an in choline chloride-urea mixture (1:2) gave pyrazoline derivative whose mass spectrum is compatible with 2a or 2’a, Scheme (2). The structure of 5-(4-chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide 2a was established based on the $^1$H NMR spectrum which exhibited the signals of protons Ha, Hb and Hx of pyrazoline moiety as doublet of doublet at 3.03, 3.79 and 5.39 with coupling constants 5.4, 5.2, 11.7, 12, 5.1, 5.4 Hz, respectively. Similarly, chalcone 1a reacted with thiosemicarbazide to formulate pyrazoline 2b. The reaction occurred in a mixture of Ch.Cl-urea (1:2). It worth mentioning that, carrying the reaction of 1a with semicarbazide and thiosemicarbazide in ethanol gave the same product but with lower yield.

The formation of pyrazoline derivative 2a,b could be clarified using the following proposed mechanism Fig. (1). The mechanism includes a nucleophilic attack of the nitrogen atom on the carbonyl carbon atom, which leads to the formation of the hydrazone after that NH is added to the olefinic double bond and cyclization to the pyrazoline ring. Obviously, the ability of the DES to form hydrogen bonds had favored this cyclization.

![Scheme 2: synthesis of pyrazoline derivatives 2a,b.](image-url)
The reaction of chalcone 1a with phenyl hydrazine in Ch.cl-Urea (1:2) under reflux resulted in the formation of pyrazoline derivative 3. The $^1$H NMR spectrum revealed the methylene protons of the pyrazoline ring as a doublet of doublet at 3.077 and 3.867 ppm. On the other hand, N-acetylpyrazoline 4 derivative was obtained by treating chalcone 1a with hydrazine hydrate in deep eutectic solvent containing glacial acetic acid. But isooxazoline 5 derivative was obtained by treating chalcone 1a with hydroxylamine hydrochloride in deep eutectic solvent containing sodium hydroxide. Compound 4 was also prepared by refluxing the reaction mixture in acetic acid, however, with lower yield.
In addition, pyrimidine and pyridine derivatives are biologically interesting molecules that gained a great interest in the pharmaceutical applications. They are used as potent anti-inflammatory, antimicrobial, antioxidant and anticancer (37-39). Consequently, chalcone 1a had been used to synthesize pyrimidine and pyridine derivatives of expected pharmaceutical interest using deep eutectic solvent. Thus, 4-(4-chlorophenyl)-6-(thiophen-2-yl)pyrimidin-2-amine (6) was produced when chalcone 1a was treated with guanidine hydrochloride in a solution of (Ch.Cl-urea) (1:2), Scheme 4. But, chalcone 1a reacted with ethylcyanoacetate in choline chloride-urea mixture (1:2) containing ammonium acetate under reflux gave pyridine derivative 7, Scheme 4.

4-(4-chlorophenyl)-7,7-dimethyl-2-(thiophen-2-yl)-4,6,7,8-tetrahydroquinolin-5(1H)-one (8) was obtained in good yield by two methods. Thus, 8 was synthesized by the fusion of chalcone 1a, dimedone and ammonium acetate; or by refluxing the reaction mixture in choline chloride-urea mixture (1:2), Scheme 4.

The suggested mechanism for the creation of product 8 using DES includes the prior Michael addition reaction between 1a and dimedone to give intermediate X, which then condenses with ammonium acetate to give intermediate Y. Next, the carbonyl (C=O) molecule is attacked by the NH2 group as a nucleophile. followed by the elimination of water molecule resulted in the formation of the expected product 3, Fig. 2. Moreover, the reaction of malononitrile with chalcone 1a in in choline chloride-urea combination (1:2) in presence piperidine afforded 9, Scheme 4.
Scheme 4: Synthesis of derivatives 6-9.
3.2. Antitumor activity:

Fig 2: Proposed mechanism for treatment of chalcone 1a with dimedone
Cytotoxic effects of the synthesized products were examined by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) evaluate on three human tumor cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland (MCF-7) and colorectal adenocarcinoma (Caco-2). The obtained results were contrasted with doxorubicin as standard antitumor drug, as shown in Table 2. It was found that aminopyrimidine 6 and pyrazoline 2b had only moderate action against hepatocellular carcinoma (HepG2) cells, while pyrazoline 4 demonstrated the strongest cytotoxic impact. The observed IC50 values for 4, 6 and 2b were 19.18, 53.19 and 54.4 µM, respectively. Moreover, pyrazoline 4 showed the highest activity against both colorectal adenocarcinoma (Caco-2) and mammary gland (MCF-7) with IC50 45.42 and 40.53 µM, respectively. On the other hand, thiocarbamoylpyrazoline 2b exhibited moderate activity with IC50 = 53.8 µM while the rest of products showed low activity.

**Table 2:** Cytotoxic activity of some compounds against human tumor cell Compounds In vitro cytotoxicity IC50 (µg/mL)a.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HepG2</th>
<th>Caco2</th>
<th>Mcf7</th>
</tr>
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<tbody>
<tr>
<td>Doxorubicin</td>
<td>17.78 ± 1.15</td>
<td>40.23 ± 2.12</td>
<td>40.33 ± 2.46</td>
</tr>
<tr>
<td>2a</td>
<td>70.45 ± 6.15</td>
<td>153.11 ± 4.41</td>
<td>83.73 ± 2.9</td>
</tr>
<tr>
<td>2b</td>
<td>54.4 ± 2.06</td>
<td>88.2 ± 3.57</td>
<td>53.87 ± 3.63</td>
</tr>
<tr>
<td>3</td>
<td>77.68 ± 1.9</td>
<td>171.25 ± 5.46</td>
<td>80.84 ± 4.36</td>
</tr>
<tr>
<td>4</td>
<td>19.18 ± 6.47</td>
<td>45.42 ± 4</td>
<td>40.53 ± 2.59</td>
</tr>
<tr>
<td>6</td>
<td>53.19 ± 1.24</td>
<td>105.08 ± 3.4</td>
<td>79.81 ± 0.91</td>
</tr>
<tr>
<td>7</td>
<td>115.25 ± 6.89</td>
<td>323.49 ± 7.26</td>
<td>169.03 ± 3.16</td>
</tr>
</tbody>
</table>

3.3. Antioxidant activity

Using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, the antioxidant activity of some of the produced compounds was assessed. The potency of the investigated substances was assessed using ascorbic acid as a standard. The absorbance was measured and the inhibition percent for each sample was calculated from the following equation: 

\[ \text{Inhibition percent} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

where \( A_0 \) is the absorbance of control reaction and \( A_1 \) is the absorbance in presence of test or standard sample. The results of the antioxidant activity of the tested compounds are shown in Table 3. These results demonstrated that the investigated compounds' antioxidant activity increased as concentration increased. The results also showed that pyrazolines 2b and 3 had the maximum scavenging activity, as shown by their percentage inhibition of DPPH radicals, which was 79.8 and 74.4, respectively. Furthermore, pyrimidines 6 and 7 displayed increased activity (61.3, 70.8). In contrast to the standard ascorbic acid, which exhibited a percentage inhibition of 52.74% and 99.86% for concentrations of 10 g/mL and 60 g/mL, respectively.
Table 3: Antioxidant assay (DPPH).

| Conc. (µg/ml) | Sample (2b) | | | Sample (2a) | | | Sample (3) | | |
| | absorbance | DPPH scavenging % | | absorbance | DPPH scavenging % | | absorbance | DPPH scavenging % | | |
| 1000 | 0.130 | 91.9 | | 0.634 | 60.7 | | 0.297 | 85.6 | |
| 500 | 0.254 | 84.3 | | 0.790 | 51.1 | | 0.430 | 82.4 | |
| 250 | 0.380 | 83.1 | | 0.886 | 45.1 | | 0.576 | 80.3 | |
| 125 | 0.497 | 80.2 | | 0.976 | 39.5 | | 0.729 | 76.8 | |
| 62.5 | 0.617 | 79.8 | | 1.115 | 30.9 | | 0.914 | 74.4 | |
| 31.25 | 0.722 | 65.2 | | 1.205 | 25.3 | | 1.027 | 66.4 | |
| 15.625 | 0.820 | 49.2 | | 1.288 | 20.2 | | 1.128 | 40.1 | |
| 7.8125 | 0.965 | 40.2 | | 1.348 | 16.5 | | 1.220 | 24.4 | |
| 3.9 | 1.009 | 37.5 | | 1.420 | 12.0 | | 1.270 | 21.3 | |
| 1.95 | 1.100 | 31.9 | | 1.488 | 7.8 | | 1.327 | 17.8 | |

| Conc. (µg/ml) | Sample (4) | | | Sample (6) | | | Sample (7) | | |
| | absorbance | DPPH scavenging % | | absorbance | DPPH scavenging % | | absorbance | DPPH scavenging % | | |
| 1000 | 0.513 | 68.2 | | 0.670 | 73.5 | | 0.343 | 86.8 | |
| 500 | 0.636 | 60.6 | | 0.798 | 70.6 | | 0.447 | 82.3 | |
| 250 | 0.769 | 52.3 | | 0.939 | 68.8 | | 0.579 | 74.1 | |
| 125 | 0.903 | 44.1 | | 1.004 | 65.8 | | 0.726 | 72.0 | |
| 62.5 | 1.001 | 38.0 | | 1.125 | 61.3 | | 0.892 | 70.8 | |
| 31.25 | 1.102 | 31.7 | | 1.230 | 43.8 | | 0.991 | 68.6 | |
| 15.625 | 1.163 | 27.9 | | 1.305 | 39.2 | | 1.115 | 60.9 | |
4. Conclusion

In conclusion, a series of heterocyclic molecules including isoxazoline, pyrazoline, pyrimidine, pyridine, quinoline, and pyran derivatives was synthesized on the basis of green chemistry methodology using deep eutectic solvents as greener solvents. The cytotoxicity of the prepared derivatives was screened and pyrazoline 4 induced a significant growth inhibition towards tested cell lines while pyridone 7 showed the lowest activity. In addition, the antioxidant activity of the products was evaluated, where pyrazolines 2b and 3 demonstrated potent activity in comparison with ascorbic acid.

5. References

34. Behalo M, Amine M, Fouda I. Regioselective synthesis, antitumor and antioxidant activities of some 1,2,4-triazole derivatives based on 4-phenyl-5-(quinolin-8-yl)oxy) methyl-4 H-1, 2, 4-triazole-3-thiol. Phosphorus, Sulfur, and Silicon and the Related Elements. 2017;192(4):410-7.