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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 tumour cell lines namely; hepatocellular carcinoma (HePG-2), mammary gland (MCF-7) and colorectala denocarcinoma (Caco-2) where the cytotoxic effects showed that pyrazoline derivatives(**4)** induced a significant growth inhibition towards tested cell lines while1,2-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*-dimethylaminobenzaldehyde 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. 118-120° C; IR (KBr, ν cm^{-1}): 3062 cm^{-1} (CH-aromatic), 1643 cm^{-1} (CO), and 1597 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 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 $C_{13}H_9ClOS$ (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. 196–198°C; IR (KBr, ν cm^{-1}): 3101 cm^{-1} (CH-aromatic) 1651 cm^{-1} (CO) and 1597 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6, 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 $C_{13}H_9NO_3S$ (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. 96-98° C; IR (KBr, ν cm^{-1}): 3086 cm^{-1} (CH-aromatic), 2909, 2816 cm^{-1} (CH aliphatic), 1627cm^{-1} (CO) and 1612 cm^{-1} (C=C); 1H NMR (DMSO-d_6, δ ppm): 3.010 (s, 6H, $2CH_3$), 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 $C_{15}H_{15}NOS$ (257): C, 70.01; H, 5.88; N, 5.44. Found: C, 70.51; H, 6.08; N, 4.54.

2.2. Synthesis of pyrazoline derivatives 2a,b

First method:

Chalcone **1a** was dissolved in ChCl.urea [1:2] mixture (5 mL) containing (20 mL) containing sodium hydroxide (0.012 mol) and thiosemicarbazide or semicarbazide (0.01 mol) was added before reflux for 3-4 hours. The reaction mixture was put into cold water, and then the precipitate that formed was filtered and purified using Ethanol resulting in **2a,b**.

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 in 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-1*H*-pyrazole-1-carboxamide (2a)

Yield of first method: 91%, M.p. 194-200° C. IR (KBr, ν cm^{-1}): 3425, 3400 cm^{-1} (NH_2), 3086 cm^{-1} (CH aromatic), 2978, 2900 cm^{-1} (CH aliphatic), 1627 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6, δ ppm): 3.026 (dd, 1H, Ha, Jab = 5.4 Hz, *J*ax = 5.2 Hz), 3.785 (dd, 1H, Hb, Jab = 11.7 Hz, Jbx = 12 Hz), 5.391 (dd, 1H, Hx, Jax = 5.1 Hz, Jbx = 5.4 Hz), 6.369 (s, 2H, NH_2, exchangeable with D_2O) 7.096–7.686 (m, 7H, Ar–H); MS: m/z: 305 (M^+); Anal. calcd. For $C_{14}H_{12}ClN_3OS$ (305.5): C, 54.99; H, 3.96; N, 13.74. Found: C, 55.09; H, 2.93; N, 14.34.

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

Yield of of first method: 73%, M.p. 110-119° C. IR (KBr, ν cm^{-1}): 3394, 3200 cm^{-1} (NH_2), 3050 cm^{-1} (CH aromatic), 2962, 2839 cm^{-1} (CH aliphatic), 1273 cm^{-1} (CS); ^{1}H NMR (DMSO-d_6, δ ppm): 3.3 (dd, 1H, Ha, Jab = 20.7 Hz, *J*ax = 5.1 Hz), 3.449 (dd, 1H, Hb, Jab = 5.4 Hz, Jbx = 4.8 Hz), 5.91 (dd, 1H, Hx, Jax = 3.6 Hz, Jbx = 3.3 Hz), 7.13–7.77 (m, 7H, Ar–H), 8.1 (s, 2H, NH_2, exchangeable); MS: m/z: 321 (M^+); Anal. calcd. For $C_{14}H_{12}ClN_3S_2$ (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*H*-pyrazole (3)

Phenyl hydrazine (0.005 mol) and Chalcone **1a** (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^{-1}): 3075 cm^{-1} (CH aromatic), 2913, 2891 cm^{-1} (CH aliphatic); ^{1}H NMR (DMSO-d_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 $C_{19}H_{15}ClN_2S$ (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*H*-pyrazol-1-yl) ethan-1-one (4)

First method:

Hydrazine hydrate (0.01 mol) and Chalcone **1a** (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 **1a** (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 filtrated, washed, dried, and purified from ethanol/water to produce brown crystalss of compound **4.**

Yield of first method: 85 %, M.p. 116-118° C. IR (KBr, ν cm^{-1}): 3086 cm^{-1} (CH aromatic), 2978, 2924 cm^{-1} (CH aliphatic), 1666.5 cm^{-1} (CO), 1605cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$,δ ppm): 3.35 (s, 3H, CH_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}C NMR (DMSO-*d6*, δ ppm): 21.84 (CH_3), 42.875 (CH_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^+); Anal. calcd. $C_{15}H_{13}ClN_2OS$ (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**1a** (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. 188-190C. IR (KBr, ν cm^{-1}): 3086 cm^{-1} (CH aromatic), 2916, 2854 cm^{-1} (CH aliphatic); ^{1}H NMR (DMSO-d_6,δ ppm): 3.376 (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); Anal. calcd. For $C_{13}H_{10}ClNOS$ (263.5) C, 59.20; H, 3.82; N, 5.31, Found: C, 58.20; H, 4.12; N, 4.91.

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 **1a** (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: 76%, M.p. 148-150° C. IR ((KBr, ν cm^{-1}): 3325, 3209 cm^{-1} (NH_2), 3101.5 cm^{-1} (CH aromatic), 2924, 2862 cm^{-1} (CH aliphatic), 1627.9 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6,δ ppm): 6.75 (s, 2H, NH_2, exchangeable), 7.2–8.2 (m, 8H, Ar–H; ^{13}C NMR (DMSO-d_6,δ ppm): 100.15 (CH pyrimidine), 128.1, 128.521, 129.95 (thiophene carbons), 128.42, 128.696, 135.3, 135.97 (phenyl carbons), 160.34 (C2-pyrimidine), 163.23 (C4 pyrimidine), 163.722 (C-NH_2);MS: m/z: 287 (M^+); Anal. calcd. $C_{14}H_{10}ClN_3S$ (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), ethylcyanoacetate (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: 56%, M.p.283-285° C. IR ((KBr, ν cm^{-1}): 3286, 3093 cm^{-1} (NH_2,OH), 3039 cm^{-1} (CH aromatic), 2214 cm^{-1} (CN), 1643 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6, δ ppm): 7.23-8.07 (m, 8H, Ar–H,12.83 (s, 2H, NH_2, exchangeable); MS: m/z: 312 (M^+); Anal. calcd. For $C_{16}H_9ClN_2OS$ (312.5): C, 61.44; H, 2.90; N, 8.96. Found: C, 62.74; H, 2.40; N, 9.16.

2.8. 4-(4-Chlorophenyl)-7,7-dimethyl-2-(thiophen-2-yl)-4,6,7,8-tetrahydroquinolin-5(1*H*)-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 crystallised from the ethanol to produce brown crystal **8**.

Yield of first method: 81 %, M.p. 178-180° C. IR ((KBr, ν cm^{-1}): 3312 (NH), 3086 cm^{-1} (CH aromatic), 2951, 2885 cm^{-1} (CH aliphatic), 1651 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6,δ ppm): 0.95, 1.03 (2s, 6H, $2CH_3$), 1.91 (d, 1H, CH_2, j =18), 2.15 (d, 1H, CH_2, J= 15), 2.46 (s, 2H, CH_2CO), 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 $C_{21}H_{20}ClNOS$ (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)-4*H*-pyran-3-carbonitrile (9)

Three drops of pipredine 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 crystallised with ethanol to produce a brown crystal of compound **9**.

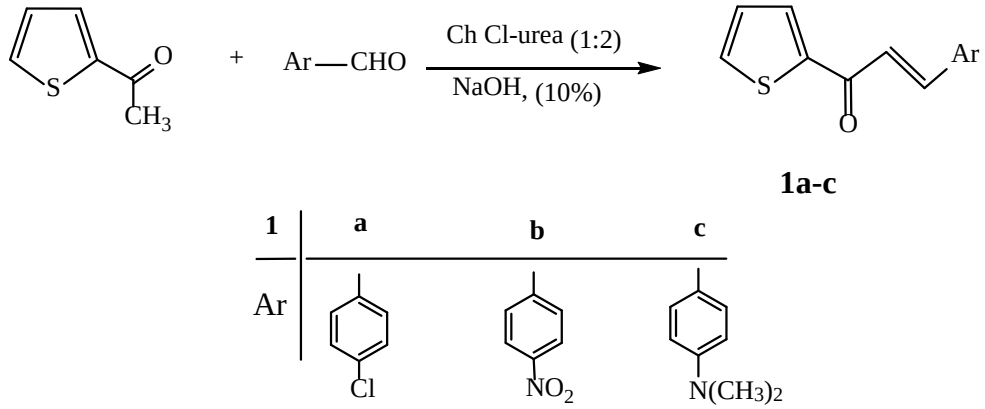
Yield: 81%, M.p. 240-242° C. IR ((KBr, ν cm^{-1}): 3433, 3300 cm^{-1} (NH_2), 3100 cm^{-1} (CH aromatic), 2216 cm^{-1} (CN); ^{1}HNMR (DMSO-d_6,δ ppm): 4.6 (S,1H,CH_2), 6.27 (s, 2H, NH_2, exchangeable), 7.25-8.35 (m, 11H, Ar–H), Anal. calcd. For $C_{16}H_{11}ClN_2OS$ (314.5): C, 61.05; H, 11.26; N, 8.90. Found: C, 59.85; H, 12.46; N, 9.30.

3. Results and discussion

3.1.Chemistry

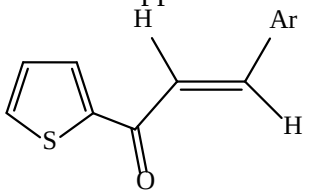
Herin, it is reported on the creation of the intended heterocyclic compounds by employing chalcone derivatives and deep eutectic solvents as green solvents.

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-acetylthiophene, gave the chalcone derivatives **1a–c** in good yields, as shown in Scheme 1.

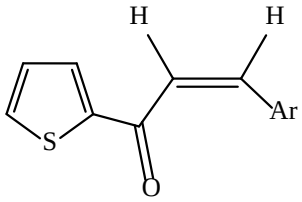


Scheme 1: Using DES (ChCl-Urea, 1:2) in the syntheses of chalcone derivatives **1a–c**.

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 α and *β* 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.



E



Z

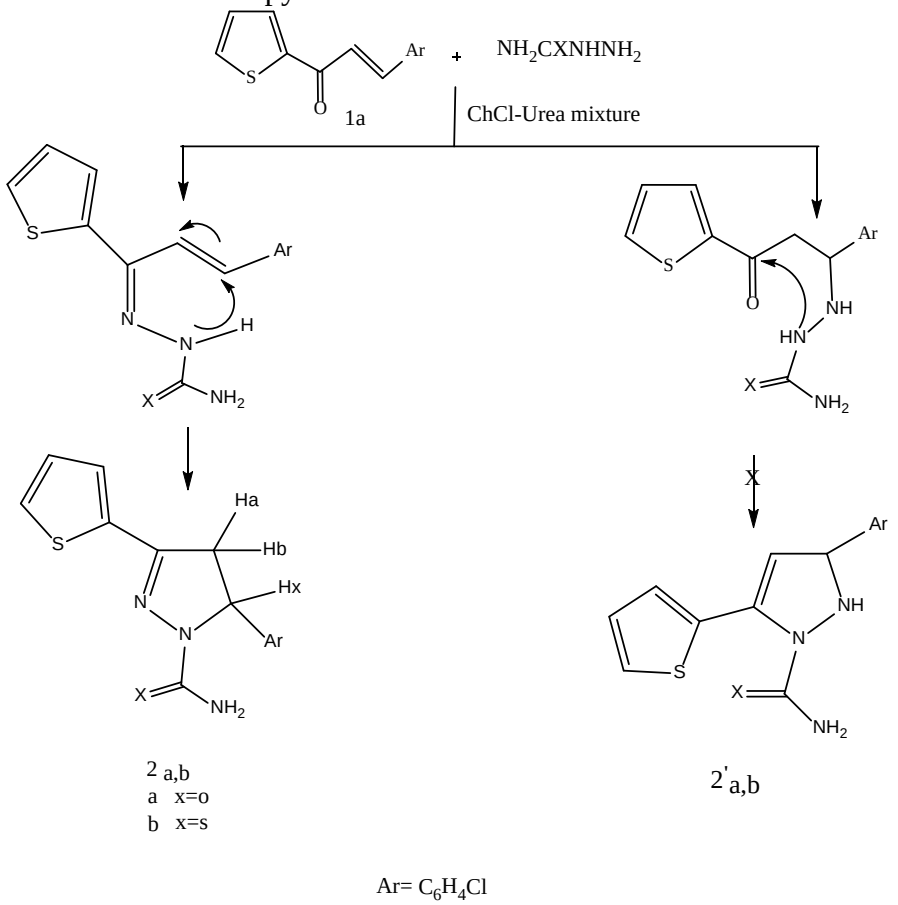
To optimize the reaction conditions and improve the yield, the synthesis of chalcones **1a** was carried out in different DESs. The highest yield was obtained by using choline chloride – urea (1:2), Table (1).

Table(1), chalcone 1a synthesis with different DESs

Entry	Solvent	Time (hr)	Yield (%)
1	ChCl: Urea (1:2)	0.5	93
2	ChCl: glycerol (1:2)	2	87
3	ChCl: oxalic (1:1)	1	79

A number of novel heterocyclic compounds with anticipated biological activity were created using chalcone derivative 1a.Thus, chalcone 1a reaction with semicarbazide as a 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-1*H*-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.

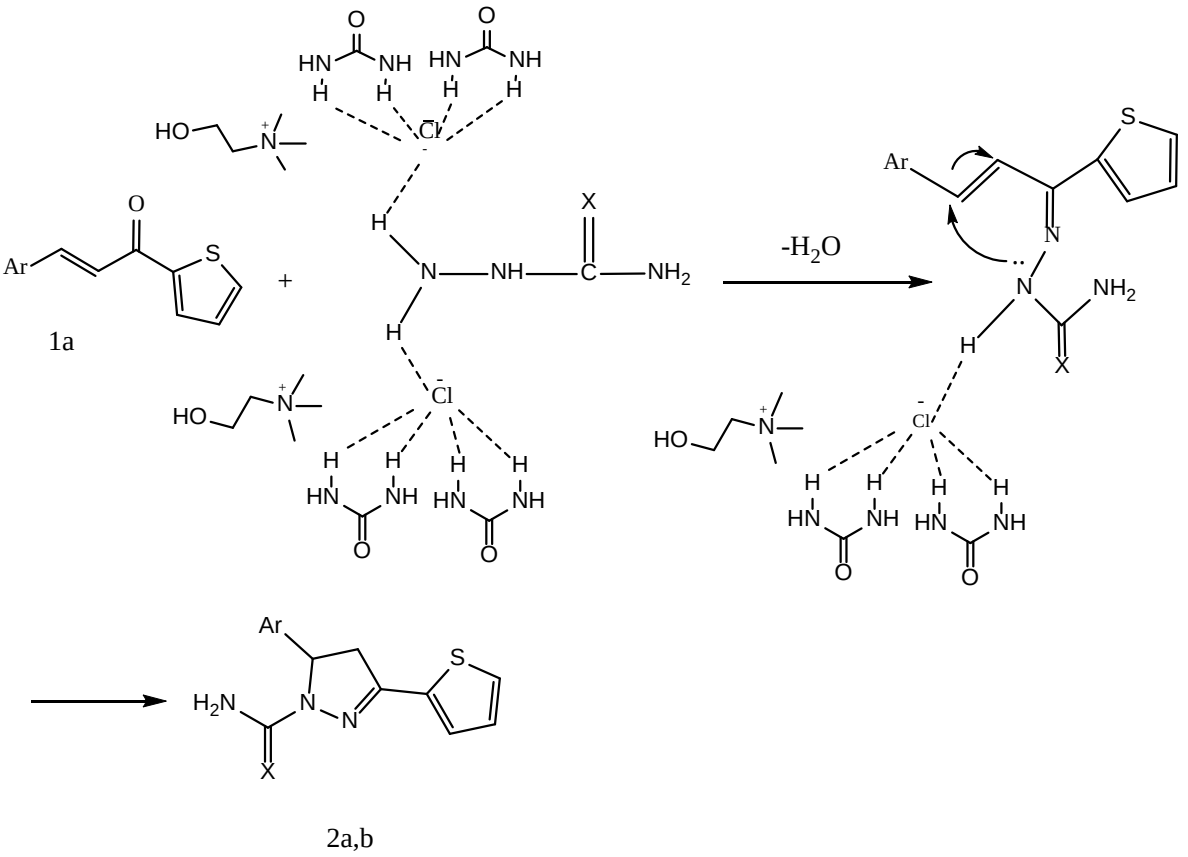
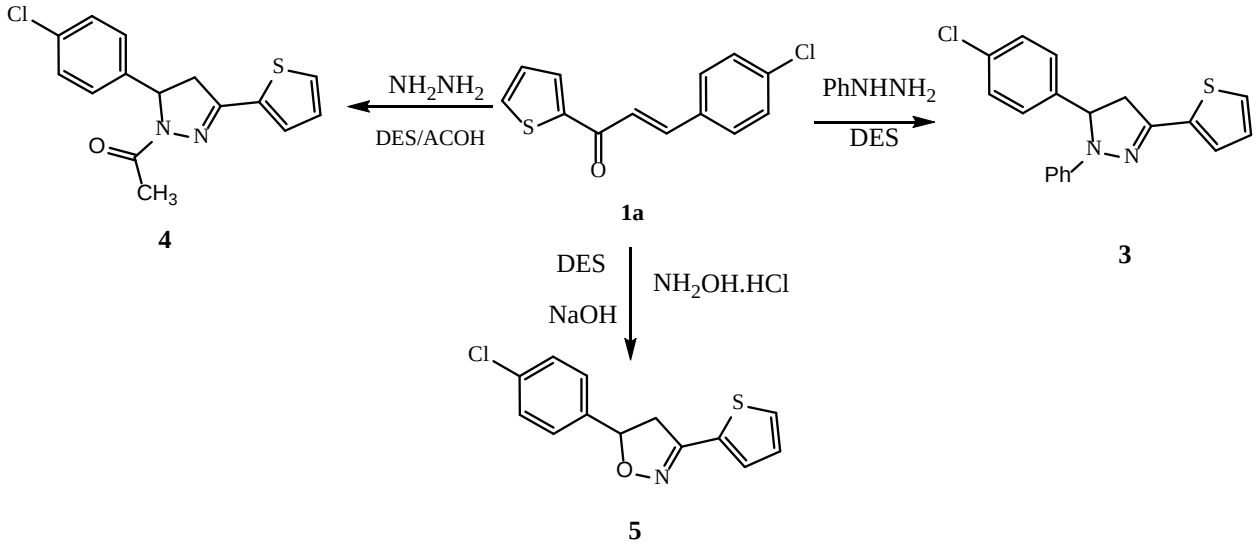


Fig (1): Proposed mechanism for the synthesis of the pyrazoline derivatives **2a,b.**

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.



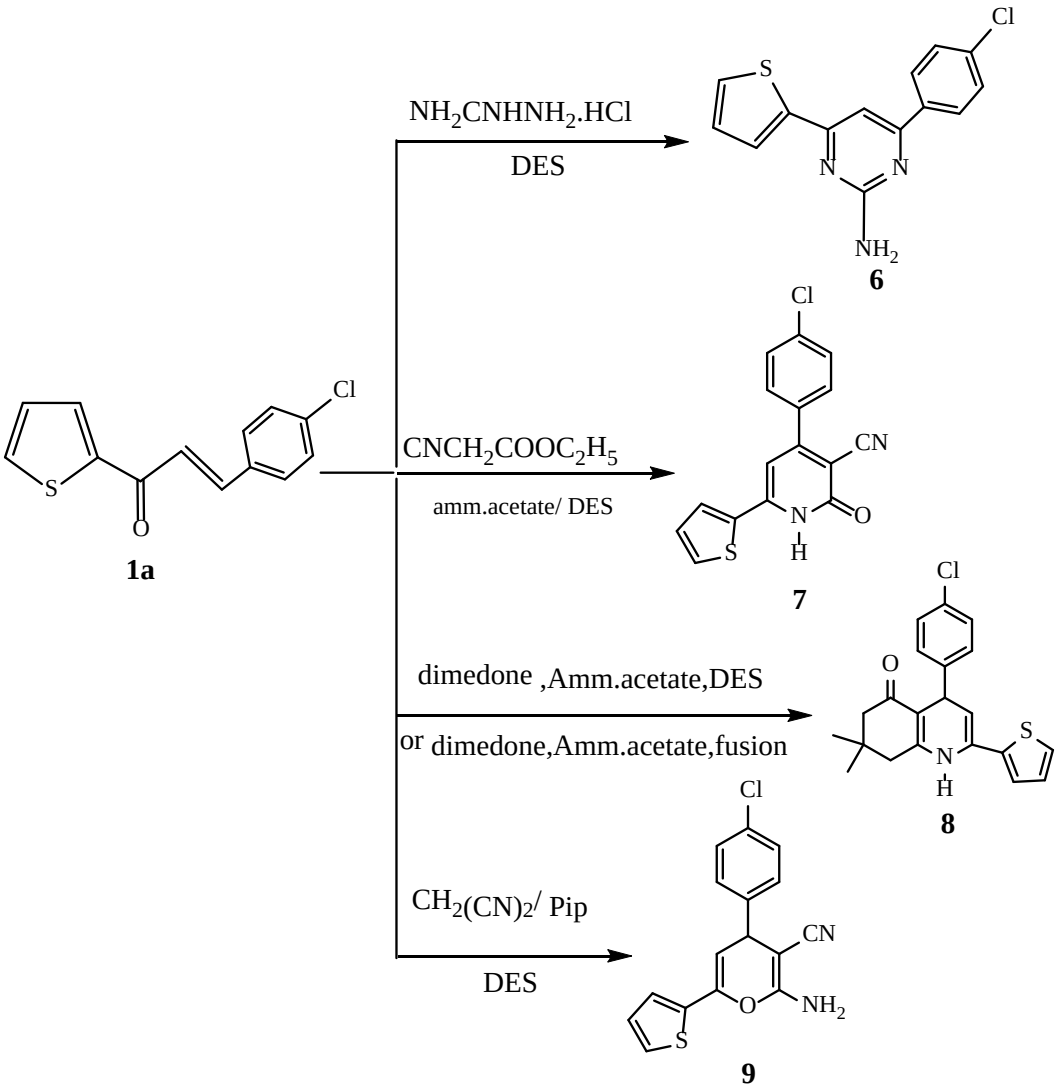
Scheme 3: Synthesis of derivatives **3-5.**

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(1*H*)-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 NH_2 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.**

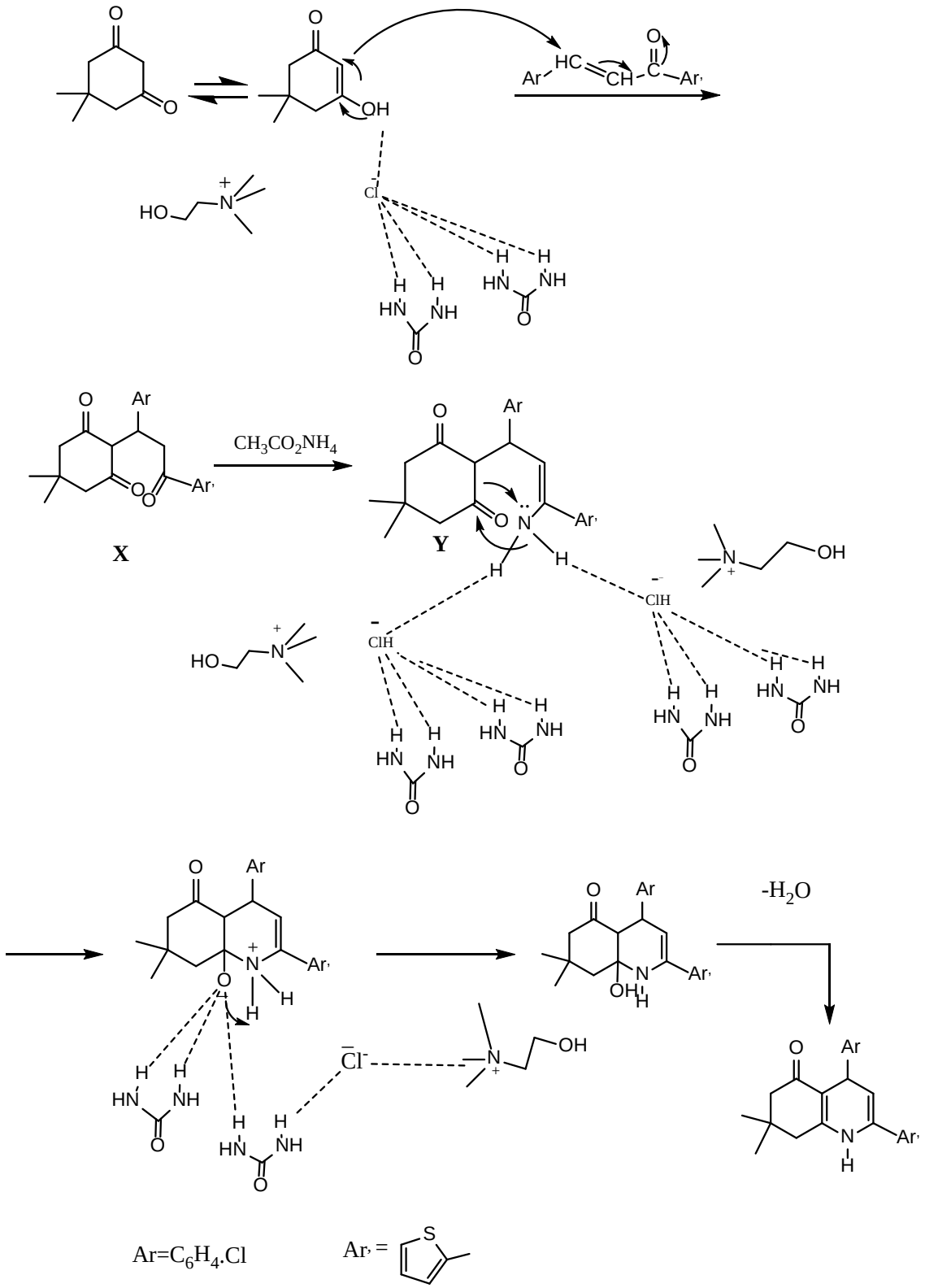


Fig 2: Proposed mechanism for treatment of chalcone **1a** with dimedone

3.2. Antitumor activity:

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 colorectala denocarcinoma (Caco-2).(40, 41). 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 IC_{50} 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 colorectala denocarcinoma (Caco-2) and mammary gland (MCF-7) with IC_{50} 45.42 and 40.53 µM, respectively. On the other hand, thiocarbamoylpyrazoline **2b** exhibited moderate activity with IC_{50} = 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].

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

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:

$A_0 - A_1 / A_0 \times 100$.

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/m, respectively.

Table 3: Antioxidant assay (DPPH).

	Sample (2b)		Sample (2a)		Sample (3)	
Conc. ug/ml)	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

	Sample (4)		Sample (6)		Sample (7)	
Conc. ug/ml)	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

7.8125	1.246	22.8	1.385	34.2	1.195	55.9
3.9	1.309	18.9	1.451	20.1	1.287	50.3
1.95	1.368	15.2	1.503	16.9	1.333	27.4

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.

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