Egypt. J. Chem. **Vol. 65**, No. 4 pp. 651 - 663 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Green Synthesis, Cytotoxicity and Antimicrobial Activities of Some New Pyrazolines, Pyrimidines and Naphthyridines Based on 1,3-Di(thien-2-yl)prop-2-en-1-one Using Choline Chloride-Urea Mixture As A Deep Eutectic Solvent

Enas A. Mohamed[a]*, Abdelmotaal Abdelmajeid[a], Mohamed S. Behalo[a], Aly A. Aly[a] and Kaouser A. Hebash[a]

[a] *Chemistry Department, Faculty of Science, Benha University, Benha, P. O. Box 13518. Egypt*

Abstract

An efficient and facile green synthesis of chalcone derivatives **1a-d** was achieved by treatment of aromatic aldehydes with 2-acetylthiophene in the presence of choline chloride-urea mixture as a deep eutectic solvent. Chalcone **1a** was used as a reactive key precursor to design a series of bio-active heterocycles such as pyrimidine, pyrazoline, pyridine, pyridopyrimidine and naphthyridine. The Structural formula of derivatives were confirmed and characterized by their elemental analyses and spectral data (IR, MS, ^{1}H NMR, ^{13}C NMR). In addition, the synthesized derivatives were evaluated for their antimicrobial activities, it was found that compounds **3, 8** and **9a** exhibited potent antifungal activity in comparison with the standard drug. Cytotoxicity against breast cancer (**MCF7**) was screened also and most compounds showed low to moderate activity. The results of the viral screening against HBV of selected compounds indicated that compounds (**9b**, **15), 8**, (**3**, **6**), and **5** showed moderate viral replication inhibition.

Keywords

Chalcone; deep eutectic solvent; pyrazoline; pyrimidine; antitumor activity; antimicrobial activity

Introduction

In recent years, deep eutectic solvents (DESs) have attracted great attention as green solvents in many organic reactions and transformations [1-8]. DESs can be easily formulated *via* hydrogen bonding between two components, the first is hydrogen bond donor which can be acid [9], alcohol [10,11] carbohydrate [12] or amide [13,14] and the second is hydrogen bond acceptor as quaternary ammonium salt [14,15]. Non-flammability, high thermal stability and low volatility of DESs promote their uses as versatile alternatives than conventional solvents [16]. DESs have the additional advantages as inexpensive, nontoxic, biodegradable and recyclable green solvents.

In addition, pyrimidine derivatives are an important group of heterocyclic compounds that possess wide range of pharmacological properties, which include their uses as antimicrobial [17-20], anticancer [21-25], antitumor [26], antioxidant [27], anti-inflammatory [28] and antiviral [29].In view of these observations and in continuation to our ongoing interest in the design of bio-active heterocyclic molecules [30-35], the present work involves synthesis of chalcone derivatives using choline chloride-urea mixture as a deep eutectic solvent and construct a series of new heterocyclic molecules that have remarkable biological activities.

Experimental

The chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). Solvents were commercially available from El-Nasr Chemicals Co. (Egypt) in analytical grade and were used without further purification. Thin-layer chromatography was conducted on precoated silica gel polyester sheets (Kieselgel 60 F254, 0.20 mm, Merck, Kenilworth, NJ).

Melting points were measured using an electro-thermal digital apparatus and are uncorrected. The IR spectra were recorded as KBr pellets using Buck scientific model 500 IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d6 as solvent at 400 MHz, on Varian Gemini NMR spectrophotometer using TMS as internal standard, the chemical shifts are reported as parts per

*Corresponding author e-mail: enas.mohamed@fsc.bu.edu.eg.

Receive Date: 12 September 2021, Revise Date: 05 October 2021, Accept Date: 17 October 2021

DOI: 10.21608/EJCHEM.2021.95621.4488

million (ppm) [Figures and supplementary information]. All reactions were followed and checked by TLC using *n*-hexane/ethyl acetate (1:1) as the mobile phase. The spots were visualized using a UV lamp.Microanalyses were performed at the micro-analytical center, Cairo University and Mansoura University.

General procedures for synthesis of 1a-d:

A mixture of 2-acetylthiophene (0.01 mol), aromatic aldehydes namely, thiophene-2-carboxaldehyde, naphthene-1-carboxaldehyde, benzaldehyde and furan-2-carboxaldehyde (0.01 mol) in choline chloride-urea mixture [prepared by warming a mixture of choline chloride and urea 1:2] (3 mL) and of 10 % sodium hydroxide solution (1 mL) was added and stirred at 0-5º C for 2 h. The precipitate formed was collected by filtration, washed three times by dist. water and recrystallized from ethanol to give pure crystals of chalcones **1a-d.**

(*E*)-1,3-Di(thien-2-yl)prop-2-en-1-one (1a).

Yield: 94 %; M.p. 98-100^{o} C; IR (KBr, ν cm^{-1}): 3098 cm^{-1} (CH aromatic), 1650 (CO)**;** ^{1}H NMR (DMSO-*d*$_6$, δ ppm):7.51 (d, 1H, H$_\alpha$, *J* = 15 Hz), 7.88 (d, 1H, H$_\beta$, *J* = 15 Hz), 7.18-8.25 (m, 6H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 128.3, 129.4, 129.5, 131.1, 131.5, 133.8, 139.7, 141.3 (aromatic carbons)**,** 127.3 (CH$_\alpha$), 134.1 (CH$_\beta$), 181.3 (CO); Anal. calcd. for $C_{11}H_8OS_2$ (220.30): C, 59.97; H, 3.66; Found: C, 59.88; H, 3.56.

(*E*)-3-(Naphthalen-1-yl)-1-(thien-2-yl)prop-2-en-1-one (1b).

Yield: 91 %; M.p. 118-120^{o} C; IR (KBr, ν cm^{-1}): 3064 cm^{-1} (CH aromatic)**,** 1698 cm^{-1} (CO)**;** ^{1}H NMR (DMSO-*d*$_6$, δ ppm): 7.32-8.37 (m, 10H, Ar-H), 7.96 (d, 1H, H$_\alpha$, *J*=15 Hz), 8.54 (d, 1H, H$_\beta$, *J*= 15 Hz); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 122.9, 124.3, 125.6, 125.7, 126.3, 127.2, 128.8, 128.9, 130.9, 131.0, 131.1, 133.3, 133.8, 135.7 (aromatic carbons), 139.0 (CH$_\alpha$), 145.3 (CH$_\beta$), 181.5 (CO)**;** Anal. calcd. for $C_{17}H_{12}OS$ (264.34)**:** C, 77.24; H, 4.58; Found: C, 77.14; H, 4.52.

(*E*)-3-Phenyl-1-(thien-2-yl)prop-2-en-1-one (1c).

Yield: 93 %; M.p. 76-78^{o} C; IR (KBr, ν cm^{-1}): 3081 cm^{-1} (CH aromatic)**,** 1680 cm^{-1} (CO)**;** ^{1}H NMR (DMSO-*d*$_6$, δ ppm); 7.72 (d, 1H, H$_\alpha$, *J* = 15 Hz), 8.07 (d, 1H, H$_\beta$, *J* = 15 Hz), 7.31-8.34 (m, 8H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 127.8, 128.5, 128.6, 129.2, 131.1, 133.7, 135.9, 138.8 (aromatic carbons), 122.4 (CHα), 144.2 (CH$_\beta$), 181.4 (CO); Anal. calcd. for $C_{13}H_{10}OS$ (214.28)**:** C, 72.87; H, 4.70; Found: C, 72.81; H, 4.62**.**

(*E*)-3-(Furan-2-yl)-1-(thien-2-yl)prop-2-en-1-one (1d).

Yield: 91 %; M.p. 80-82^{o} C; IR (KBr, ν cm^{-1}): 3095 cm^{-1} (CH aromatic), 1709 cm^{-1} (CO) absorption; ^{1}H NMR (DMSO-*d*$_6$, δ ppm); 7.46 (d, 1H, H$_\alpha$, *J* = 15 Hz), 7.55 (d, 1H, H$_\beta$, *J* = 15 Hz), 6.69-8.18 (m, 6H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 113.6, 114.7, 129.9, 131.5, 133.5, 138.5, 143.9, 152.2 (aromatic carbons), 121.6 (CH$_\alpha$), 129.2 (CH$_\beta$), 181.4 (CO); Anal. calcd. for $C_{11}H_8O_2S$ (204.24): C, 64.69**;** H, 3.95**;** Found: C**,** 64.61; H, 3.85.

3,5-Di(thien-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3).

Thiosemicarbazide (0.01 mol) was added to a solution of chalcone **1a** (0.01 mol) in ethanol (30 mL) containing sodium hydroxide (0.02 mol) and all were refluxed for 6 h. After cooling, the reaction mixture was poured into cold water and the resulted precipitate was filtered and washed. The crude product was purified to give **3**.

Yield: 91 %; M.p. 121-123^{o} C; IR (KBr, ν cm^{-1}): 3477, 3346 cm^{-1} (NH$_2$)**,** 3100 cm^{-1} (CH aromatic)**,** 2922 cm^{-1} (CH aliphatic)**,** 1642 cm^{-1} (C=N), 1367 cm^{-1} (C=S); ^{1}H NMR (DMSO-*d*$_6$, δ ppm): 3.38 (dd, 1H, Ha, *J*ab = 12 Hz, *J*ax = 4 Hz), 3.86 (dd, 1H, Hb, *J*ab = 16 Hz, *J*bx = 12 Hz), 6.23 (dd, 1H, Hx, *J*ax = 2.4 Hz, *J*bx = 13.2 Hz), 6.92-7.78 (m, 6H, Ar-H), 7.80 (s, 2H, NH$_2$, exchangeable with D$_2$O); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 42.7 (CH$_2$), 58.7 (CH), 124.5, 124.7, 126.5, 128.1, 130.5, 131.3, 133.6, 144.9 (aromatic carbons), 151.5 (C imine) 175.5 (C=S); Anal. calcd. for $C_{11}H_8O_2S$ **(**293.42**)**: C, 49.12; H, 3.78; N, 14.32; Found: C, 49.04; H, 3.71; N, 14.25.

3,5-Di(thien-2-yl)-4,5-dihydro-1*H*-pyrazole (4).

To a solution of chalcone **1a** (0.01 mol) in ethanol (30 mL), hydrazine hydrate (0.01 mol) was added. The reaction mixture was heated under reflux for 4 h. After cooling, the precipitated solid was collected by filtration and purified to give the corresponding pyrazoline **4**.

Yield: 70 %; M.p. 116-118^{o} C; IR (KBr, ν cm^{-1}): 3435 cm^{-1} (NH)**,** 3100 cm^{-1} (CH aromatic)**,** 2931, 2837 cm^{-1} (CH aliphatic), 1633 cm^{-1} (C=N); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 45.1 (CH$_2$), 52.1 (CH), 123.9, 125.9, 127.6, 127.7, 127.9, 128.1, 128.5, 129.9 (aromatic carbons), 154.1 (C imine); Anal. calcd. for $C_{11}H_{10}N_2S_2$ **(**234.34**)**: C, 56.38; H, 4.30; N, 11.95; Found: C, 56.31; H, 4.24; N, 11.87.

1-(3,5-Di(thien-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one (5).

A mixture of chalcone **1a** (0.01 mol) and hydrazine hydrate (0.02 mol) in glacial acetic acid (20 ml) was heated under reflux for 4 h. The reaction mixture was poured into ice and the product obtained was filtered, washed with water, dried and purified.

Yield: 82 %; M.p. 86-88^{o} C; IR (KBr, ν cm^{-1}): 3090 cm^{-1} (CH aromatic)**,** 2928 cm^{-1} (CH aliphatic)**,** 1675 cm^{-1} (C=O), 1641 cm^{-1} (C=N)**;** ^{1}H NMR (DMSO-*d*$_6$, δ ppm): 2.21 (s, 3H, CH$_3$), 3.36 (dd, 1H, Ha, *J*ab = 16

Hz, *J*ax = 8 Hz), 3.80 (dd, 1H, Hb, *J*ab = 20 Hz, *J*bx = 12 Hz), 5.83 (dd, 1H, Hx, *J*ax = 3.6 Hz, *J*bx = 15.2 Hz), 6.93–8.25 (m, 6H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 21.5 **(**CH_3), 42.2 (CH_2), 54.9 (CH), 124.5, 124.9, 126.6, 128.0, 128.9, 129.6, 133.3, 144.5 (aromatic carbons), 150.3 (C imine)**,** 167.1 (C=O); Anal. calcd. for $C_{13}H_{12}N_2OS_2$ **(**276.37**)**: C, 56.50; H, 4.38; N, 10.14; Found: C, 56.42; H, 4.32; N, 10.06.

Ethyl 2-oxo-4,6-di(thien-2-yl)cyclohex-3-ene-1-carboxylate (6).

A mixture of chalcone **1a** (0.01mol) and ethyl acetoacetate (0.01 mol) in choline chloride-urea mixture [prepared by warming a mixture of choline chloride and urea 1:2] (2 mL) was refluxed for 7 h in the presence of 0.8 mL 10% NaOH. The reaction mixture was cooled to room temperature and the solid product obtained was filtered and recrystallized from ethanol.

Yield: 85 %; M.p. 146-148^o C; IR (KBr, ν cm^{-1}): 3098 cm^{-1} (CH aromatic), 2924, 2855 cm^{-1} (CH aliphatic), 1718, 1647 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6, δ ppm): 1.12 (t, 3H, CH_3), 2.71 (m, 1H, $CHCH_2$), 3.06 (d, 1H, CHCOO), 3.44 (d, 2H, CH_2), 4.14 (q, 2H, CH_2CH_3), 6.34 (s, 1H, CHCO) and 6.96-7.80 (m, 6H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 15.2 (CH_3), 27.1 (CH_2), 37.1 (CH_2), 62.2 (CH_2), 65.5 (CH), 134.1 (CH), 125.9, 126.8, 127.5, 127.9, 128.7, 131.1, 137.5, 139.1 **(**aromatic carbons), 156.3 (C), 170.5 (COO), 198.1 (CO); MS; m/z: 332 (M^+, 0.47 %), 333 (M+1, 0.22 %); Anal. calcd. for $C_{17}H_{16}O_3S_2$ **(**332.43**)**: C, 61.42; H, 4.85; Found: C, 61.35; H, 4.77.

4,6-Di(thien-2-yl)pyrimidine-2(1*H*)-thione (7).

A mixture of chalcone **1a** (0.01mol) and thiourea (0.01 mol) in ethanol was refluxed for 18 h in the presence of sodium ethoxide (2mL). The reaction mixture was cooled to room temperature and refrigerated overnight. The solid product obtained was filtered and recrystallized from ethanol to get off white coloured powder.

Yield: 83 %; M.p. 82-84^o C; IR (KBr, ν cm^{-1}): 3381 cm^{-1} (NH)**,** 3030 cm^{-1} (CH aromatic), 1643 cm^{-1} (C=N), 1215 cm^{-1} (C=S); ^{1}H NMR ($CDCl_3$, δ ppm): 3.29 (s, 1H, NH), 6.91-7.75 (m, 7H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 110.8 (CH), 124.9, 126.7, 127.6, 127.8, 127.9, 128.7, 131.9, 137.8 (aromatic carbons), 155.4 (C), 165.7 (C imine), 181.2 (CS); Anal. calcd. for $C_{12}H_8N_2OS_2$ **(**260.33**)**: C, 55.37; H, 3.10; N, 10.76; Found: C, 55.29; H, 3.01; N, 10.65.

2-Amino-4,6-di(thien-2-yl)nicotinonitrile (8).

A mixture of chalcone **1a** (0.01 mol), malononitrile (0.01 mol) and ammonium acetate (0.02 mol) in ethanol (20 mL) was refluxed for 10 h. The reaction mixture was cooled and poured into ice cold water (50 mL). The precipitate was collected by filtration and recrystallized in ethanol to get off white powder.

Yield: 81 %; M.p. 188-190^o C; IR (KBr, ν cm^{-1}): 3472, 3364 cm^{-1} (NH), 3060 cm^{-1} (CH aromatic)**,** 2206 cm^{-1} (C≡N), 1624 cm^{-1} (C=N)**;** ^{1}H NMR (DMSO-d_6, δ ppm): 6.30 (s, 2H, NH_2, exchangeable with D_2O), 7.18-8.24 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6, δ ppm): 117.5 (C nitrile), 84.4, 92.5, 107.0, 117.6, 120.6, 128.2, 128.8, 129.1, 129.6, 130.0, 130.8, 154.7, 161.3 (aromatic carbons**)**; Anal. calcd. for $C_{14}H_9N_3S_2$ **(**283.37**)**: C, 59.34; H, 3.20; N, 14.83; Found: C, 59.28; H, 3.11; N, 14.75.

General procedures for synthesis of 9a,b:

A mixture of compound **8** (0.01 mol) and urea or thiourea (0.01 mol) was heated on an oil bath at 180^o C for 2 h. On cooling the product solidified, which was recrystallized from DMF-ethanol to give **9a,b**, respectively.

4-Amino-5,7-di(thien-2-yl)pyrido[2,3-*d*]pyrimidin-2(1*H*)-one (9a).

Yield: 76 %; M.p. 150-152^o C; IR (KBr, ν cm^{-1}): 3425-3210 cm^{-1} (NH_2, NH), 3060 cm^{-1} (CH aromatic), 1657 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 6.28 (s, 2H, NH_2, exchangeable with D_2O), 6.83 (s, 1H, NH, exchangeable with D_2O), 6.90-7.95 (m, 7H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 109.8, 112.3, 127.5, 127.6, 128.1, 128.2, 128.7, 128.8, 139.4, 143.2, 145.7, 149.3, 154.1 (aromatic carbons), 148.5 (C amide), 157.2 (C imine); MS; m/z = 326 (M^+, 4.56 %), 327 (M+1, 0.72 %); Anal. calcd. for $C_{15}H_{10}N_4OS_2$ **(**326.39**)**: C, 55.20; H, 3.09; N, 17.17; Found: C, 55.12; H, 3.01; N, 17.08.

4-Amino-5,7-di(thien-2-yl)pyrido[2,3-*d*]pyrimidine-2(1*H*)-thione (9b).

Yield: 74 %; M.p. 250-252^o C; IR (KBr, ν cm^{-1}): 3413-3284 cm^{-1} (NH, NH_2), 3077 cm^{-1} (CH aromatic),1386 cm^{-1} (C=S)**;** ^{1}H NMR (DMSO-d_6, δ ppm): 6.28 (s, 2H, NH_2, exchangeable with D_2O), 6.81-7.73 (m, 7H, Ar-H),7.95 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 109.6, 112.4, 126.3, 126.4, 128.3, 128.4, 128.9, 129.0, 139.1, 142.5, 145.1, 149.3, 153.7 (aromatic carbons), 157.1 (C imine), 181.5 (CS); Anal. calcd. for $C_{15}H_{10}N_4S_3$ **(**342.45**)**: C, 52.61; H, 2.94; N, 16.36; Found: C, 52.55; H, 2.88; N, 16.25.

***N*-acetyl-*N*-(3-cyano-4,6-di(thien-2-yl)pyridin-2-yl)acetamide (10).**

A solution of compound **8** (0.01 mol) in acetic anhydride (10 mL) was heated for 3 hr. After cooling the solid that was separated was recrystallized from ethanol to give **10**.

Yield: 78 %; M.p. 181-183^o C; IR (KBr, ν cm^{-1}): 3079 cm^{-1} (CH aromatic)**,** 2224 cm^{-1} (C≡N),1659 cm^{-}

[1] (CO); ^{1}H NMR (DMSO-d_6, δ ppm):2.73 (s, 3H, CH_3), 2.88 (s, 1H, CH_3), 6.28-7.95 (m, 7H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 27.3 (2 CH_3), 93.2, 119.3, 127.7, 127.8, 128.1, 128.2, 128.8, 128.9, 139.4, 143.1, 150.2, 155.4, 157.3 (aromatic carbons), 115.1 (C nitrile), 173.4 (2C amide); Anal. calcd. for $C_{18}H_{13}N_3O_2S_2$ **(**325.40**)**: C, 58.84; H, 3.57; N, 11.44; Found: C, 58.73; H, 3.49; N, 11.33.

2-((Diethoxymethyl)amino)-4,6-di(thien-2-yl)nicotinonitrile (11).

A mixture of compound **8** (0.01 mol), triethyl orthoformate (3 mL), and acetic anhydride (10 mL) was heated under reflux for 4 h and then allowed to cool. The product was collected and recrystallized from ethanol to give **11**.

Yield: 65 %; M.p. 122-124^o C; IR (KBr, ν cm^{-1}): 3436 cm^{-1} (NH), 3103 cm^{-1} (CH aromatic)**,** 2925, 2854 cm^{-1} (CH_2, CH_3), 2214 cm^{-1} (C≡N), 1639 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 1.19 (t, 6H, $2CH_3$), 3.11 (q, 4H, $2CH_2$), 6.28-8.17 (m, 7H, Ar-H), 9.28 (s, 1H, CH), 10.98 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 16.3 ($2CH_3$), 60.2 ($2CH_2$), 114.6 (C-nitrile), 124.4 (CH), 87.4, 112.2, 127.7, 127.8, 128.2, 128.3, 128.8, 128.9, 137.4, 143.1, 149.3, 156.1, 166.3 (aromatic carbons); Anal. calcd. for $C_{19}H_{19}N_3O_2S_2$ **(**367.44**)**: C, 59.20; H, 4.97; N, 10.90; Found: C, 59.11; H, 4.88; N, 10.82.

4,6-Di(thien-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (12).

A mixture of compound **8** (0.01 mol), hydroxylamine hydrochloride (0.01 mol), and sodium ethoxide (0.01 mol) in ethanol (50 mL) was heated under reflux for 3 h, was allowed to cool, and poured into cold water (60 mL). The solid product was collected and recrystallized from dioxane to give **12**.

Yield: 75 %; M.p. 115-117^o C; IR (KBr, ν cm^{-1}): 3476-3216 cm^{-1} (NH, NH_2), 3056 cm^{-1} (CH aromatic), 1626 cm^{-1} (C=N)**;** ^{1}H NMR (DMSO-d_6, δ ppm): 7.02-7.99 (m, 7H, Ar-H), 7.19 (s, 2H, NH_2, exchangeable with D_2O), 11.93 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 91.1, 122.4, 127.8, 127.9, 129.1, 129. 2, 129.7, 129.8, 142.7, 143.4, 145.8, 152.1, 152.7, 153.2 (aromatic carbons); Anal. calcd. For $C_{14}H_{10}N_4S_2$ **(**298.38**)**: C, 56.36; H, 3.38; N, 18.78; Found: C, 56.28; H, 3.31; N, 18.67.

4-Amino-2-oxo-5,7-di(thien-2-yl)-1,2-dihydro-1,8-naphthyridine-3-carbonitrile (13).

A mixture of compound **8** (0.01 mol), ethyl cyanoacetate (0.01 mol) and piperidine (0.5 mL) in dimethylformamide (10 mL) was heated under reflux for 3 h. The solid product was collected and recrystallized from ethanol to give **13**.

Yield: 81 %; M.p. 162-164^o C; IR (KBr, ν cm^{-1}): 3434 cm^{-1} (NH, NH_2), 3093 cm^{-1} (CH aromatic)**,** 2208 cm^{-1} (C≡N)**,** 1680 cm^{-1} (CO), 1641 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6, δ ppm):4.73 (s, 2H, NH_2, exchangeable with D_2O), 6.97-8.05 (m, 7H, Ar-H), 8.09 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR spectrum (DMSO-*d6,* δ ppm): 116.7 (C nitrile), 82.1, 112.3, 114.1, 127.8, 127.9, 128.1, 128. 2, 128.8, 128.9, 139.1, 143.5, 146.1, 150.3, 152.5 (aromatic carbons), 169.4 (C amide), 177.3 (C–NH_2); Anal. calcd. for $C_{17}H_{10}N_4OS_2$ **(**350.41**)**: C, 58.27; H, 2.88; N, 15.99; Found: C, 58.17; H, 2.76; N, 15.87.

4-Amino-2-phenyl-5,7-di(thien-2-yl)-1,8-naphthyridine-3-carbonitrile (15).

A mixture of compound **8** (0.01 mol), benzylidene malononitrile (0.01 mol) and piperidine (0.01 mol) in ethanol (50 mL) was heated under reflux for 2 h, allowed to cool, and poured into ice/H_2O and acidified with HCl. The solid product was collected and recrystallized from dioxane to give **15**.

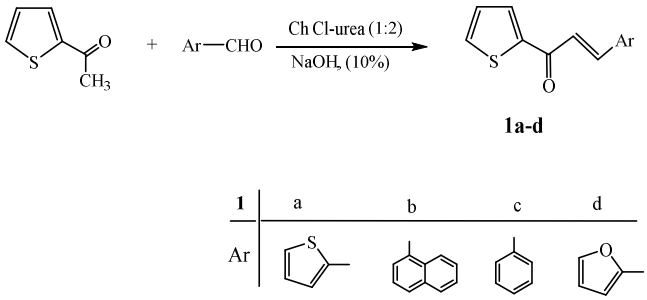
Yield: 87 %; M.p. 70-72^o C; IR (KBr, ν cm^{-1}): 3437 cm^{-1} (NH), 3065 cm^{-1} (CH aromatic)**,** 2205 cm^{-1} (C≡N); 1639 cm^{-1} (C=N)**;** ^{1}H NMR (DMSO-d_6, δ ppm): 7.01 (s, 2H, NH_2, exchangeable with D_2O), 7.18-8.24 (m, 12H, Ar-H); ^{13}C NMR spectrum (DMSO-*d6*, δ ppm): 106.3, 120.2, 127.9, 128.4, 128.5, 128.8, 128.9, 129.0, 129.3, 130.6, 133.0, 133.4, 135.5, 136.0, 139.5, 145.2 (aromatic carbons); MS; m/z = 410 ($M^{\cdot+}$, 0.21%), 411 (M+1, 0.02%); Anal. calcd. for $C_{23}H_{14}N_4S_2$ **(**410.51**)**: C, 67.29; H, 3.44; N, 13.65; Found: C, 67.21; H, 3.32; N, 13.55.

Results and discussion

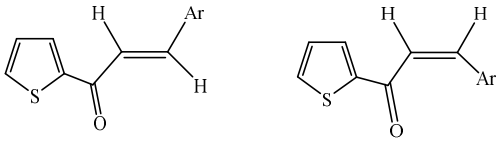
Herein, we report effective and facile methods for the synthesis of chalcone derivatives *via* reaction of aromatic aldehydes and 2-acetylthiophene in the presence of choline chloride-urea mixture as a deep eutectic solvent.

The reaction involves the treatment of aromatic aldehydes namely, thiene-2-carboxaldehyde, naphthene-1-carboxaldehyde, benzaldehyde and furan-2-carboxaldehyde with 2-acetylthiophene in choline chloride-urea mixture (1:2) and 10 % sodium hydroxide solution was added and stirred at 0-5º C to afford chalcone derivatives **1a-d** in high yields than conventional method, some of these compounds (1a,c and d) were prepared by conventional method [36] Scheme 1.

Also, chalcones **1a-d** can have two stereoisomeric structures, *Z* and *E* forms, but on the basis of the ^{1}H NMR spectrum that showed two doublet signals for the two olefinic protons at 7.49 and 8.56 ppm with the coupling constant value J = 15 Hz, it seems to exist predominately in the *E* form.



Scheme 1: Synthesis of chalcone derivatives **1a-d** using choline chloride-urea mixture (ChCl-Urea, 1:2).



E ***Z***

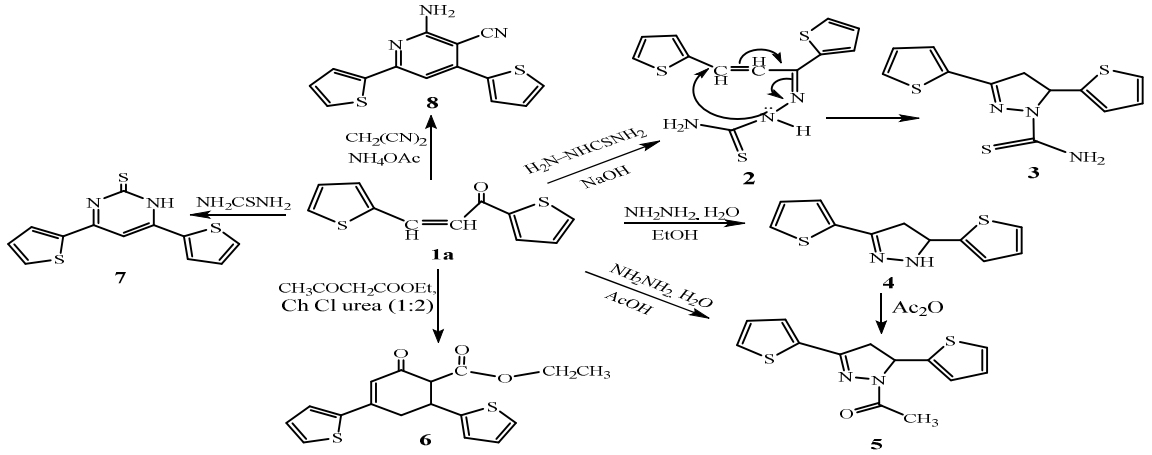
The chemical structures of chalcone derivatives **1a-d** were elucidated on the basis of their spectral analyses. IR spectra exhibited absorption bands at 3098-3026, 1709-1650 cm^{-1} corresponding to functional groups C-H olefinic and CO respectively. ^1H NMR spectra showed also characteristic signals of the two olefinic α and *β* protons.

On the other hand, chalcone derivative **1a** was used as a reactive key precursor to construct a series of new heterocyclic molecules that have remarkable biological activities. Chalcone **1a** reacted with some nitrogen and carbon nucleophiles *via* aza and carba Michael addition.

Treatment of chalcone **1a** with thiosemicarbazide as a nitrogen nucleophile in refluxing ethanol and sodium hydroxide gave pyrazoline derivative **3** (formed through hydrazone intermediate **2** followed by the addition of NH to the olefinic double bond). Similarly, the reaction of chalcone **1a** with hydrazine hydrate afforded the corresponding product **4**.

On the other hand, the reaction of chalcone **1a** with hydrazine hydrate in glacial acetic acid afforded the corresponding *N*-acetylpyrazoline **5**. Also compound **5** can be formed also from acetylation of pyrazoline **4** with acetic anhydride.

Treatment of chalcone **1a** with ethylacetoacetate using choline chloride-urea mixture as a deep eutectic solvent in the presence of basic catalyst furnished cyclohexenone **6**. The reaction probably proceed *via* the intramolecular cyclocondensation of the methyl group originating from ethyl acetoacetate and the ketone function of the initial chalcone **1a**. When chalcone **1a** was allowed to react also with thiourea under basic condition yielded pyrimidine derivative **7**. Moreover, the reaction of chalcone **1a** with malononitrile in absolute ethanol in presence of ammonium acetate afforded cyanopyridine derivative **8**. Scheme 2. The formation of cyclohexenone **6** could be explained according to the following proposed mechanism as shown in Fig. (1).



Scheme 2**:** Synthesis of derivatives **3-8.**

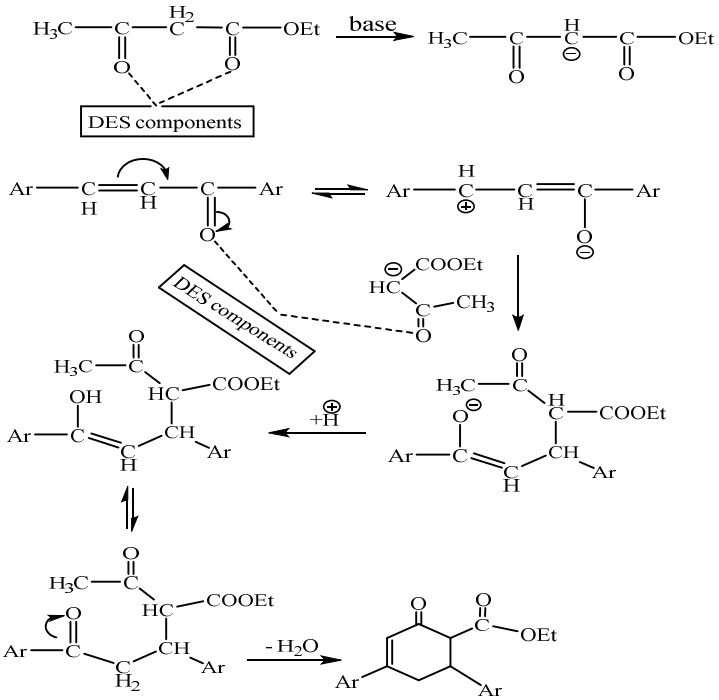


Fig (1): Formation of the cyclohexenone **6.**

Aminonicotinonitrile **8** was used as a reactive key precursor towards variety of chemical reagents to synthesize a series of heterocyclic molecules with potent pharmaceutical activity. Thus, fusion of compound **8** with urea or thiourea furnished the pyridopyrimidine derivatives **9a,b**.

On the other hand, synthesis of amide derivative **10** was achieved by the refluxing of amino nicotinonitrile **8** with acetic anhydride for 3 h. Refluxing of compound **8** with triethylorthoformate in the presence of acetic anhydride yielded the ethoxymethyleneamino derivative **11**. Pyrazolopyridine **12** can be formulated also through the addition of the amino group of hydroxylamine to the cyano function group of compound **8** followed by intramolecular cyclization by the elimination of water.

Naphthyridine derivatives were reported to be biologically interesting molecules that have established utility in the pharmaceutical industries [37, 38]. This encouraged us to synthesize new derivatives of naphthyridine attached with thiophene moities aiming to get reactive biomolecules. Thus, the reaction of **8** with ethyl cyanoacetate in dimethylformamide in the presence of a few drops of piperidine under reflux yielded the corresponding naphthyridine derivative **13**. Compound **8** was cyclocondensed with benzylidene malononitrile **14** in ethanol in the presence of a catalytic amount of piperidine under reflux and provided 4-amino-2-phenyl-5,7-di(thien-2-yl)-1,8-naphthyridine-3-carbonitrile **15**. The conversion of compound **8** to **15** could be explained on the basis of an initial Michael addition of the amino function group in compound **8** to the double bond of benzylidene malononitrile followed by intramolecular cyclization, which loses hydrogen cyanide and tautomerize to give **15**. Scheme 3.

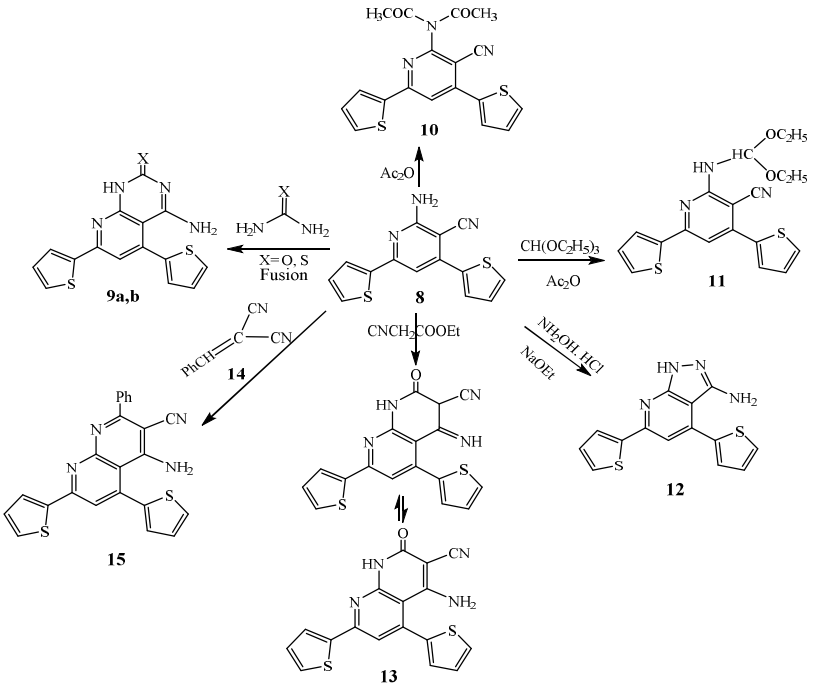
Biological evaluation

Antitumor Activity

***In vitro* Antitumor Activity**

Measurement of Potential Cytotoxicity by SRB assay:

Some of the newly synthesized compounds have been evaluated for their Potential Cytotoxicity testing against breast cancer (**MCF7**) using the method of skehan and Storeng [39]. Cells were plated in 96-multiwell plate (10^4 cells / well) for 24 hrs before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentration of the compound under test (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37° C and in atmosphere of 5 % CO_2. After 48 hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.



Scheme 3**:** Synthesis of derivatives **9-13, 15.**

Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color Intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specific compound. The IC_{50} percent control of infected and uninfected response values were calculated for the various active compounds were reported in Table 1. Doxirubsin (DOX) fig. (2), was used as positive standard. Compounds having IC_{50}< 5 µg/ml are considered potentially active and exposed to further in *vivo* studies.

Table 1. The IC_{50} (µg/mL) of some of the selected new compounds against Breast cancer cell line (**MCF7**).

Compound	IC_{50} µg/ml	Compound	IC_{50} µg/ml
DOX	2.97	**DOX**	2.97
1a	5.50	**9a**	5.54
3	5.50	**9b**	4.71
5	4.42	**10**	5.15
6	4.44	**11**	4.02
7	5.88	**12**	5.55
8	5.57	**15**	4.00

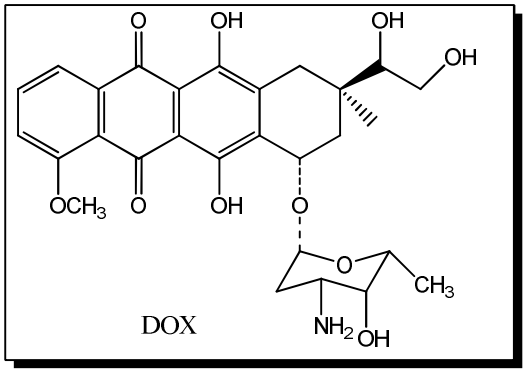


Fig. (2): Chemical structure of DOX.

Hepatitis B Activity

Hepatitis B virus (HBV) is a DNA virus that causes acute hepatitis and lead to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [40]. Approximately 300 million HBV carriers are infected and more than one million deaths world-wide are reported every year due to HBV-related complications [41]. Although effective vaccination has been successfully used of the prevention of HBV infection, the availability of selective antiviral drug against HBV replication is still needed [42]. A variety of drugs have been evaluated but only alpha interferon has demonstrated some clinical benefit in selected patients [43, 44].

The potential target for antiviral chemotherapy is the reverse transcription step in HBV life cycle. The minus strand of HBV is synthesized by reverse transcription of the pregenome using the endogenous viral reverse transcriptase. It is shown that reverse transcriptase enzyme leads to incorporate nucleotide analogues more efficiency than cellular DNA polymerase [45]. These nucleotide analogues are competitive inhibitors of the reverse transcriptase with the nucleosides pool in the cells cytoplasm in minus strand synthesis.

The recent development of heterocyclic analogues has represented a breakthrough the research for selective antiviral activities. Among these agents e.g. Lamivudine acts as a retroviral inhibitor [46]. It has activity against HBV replication both in *vitro* and *vivo*.

Preparation and culture of Hep G2 2.2.15 cells

The required cell line was made by transfection of Hep G2-cells with a plasmid containing multiple tandem copies of HBV genome (subtype ayw) [47]. The 2.2.15 cell line was maintained in RPMI-1640 (Glutamax) culture media containing 100 IU/ml nystatin and 380 µg/ml G418 (geneticin). The transferred HEP G2-2.2.15 cell line was kept in tissue culture flask at 37° C + 5% CO_2. Subcultures were set up after a week by aspiration of the media from culture flask and washing the cells twice by PBS. A 10% versene/trypsin was added and the cells were incubated for 1 min. at 37° C.

The drug Lamivudine which is a potent selective inhibitor of HBV replication [47] has been used as a standard for the comparative studies.

DNA Extraction

HBV-DNA extraction was done by mixing 10 µl of diluted supernatant (1:5 with PBS) in reaction tube with 10 µl of 0.2 *M* NaOH and incubated at 37° C for one hour. Carefully, 9.6 µl of 0.2 *M* HCl was added followed by 90 µl of TE buffer solution.

PCR-Ellisa

The PCR reaction mixture contained 14 µl extracted supernatant, 4 mmol/l $MgCl_2$, 10 µmol/l DIG-11-dUTP, 190 µmol/l dTTP, 200 µmol/l dATP, dGTP, dCTP, 1.5 U Taq polymerase, 20 mmol/l HCl (pH 8.4), 50 mmol/l KCl, 1 µmol/l HCID-1 primer (5′GGA AAG AAG TCA GAA GGC A3′) and 1 µmol/l HCID-2 (5′TTG GGG GAG GAG ATT AGG TT3′), in total volume 50 µl. PCR reaction conditions were 32 cycles of 1 min. at 94° C, 30 sec. at 58° C and 30 sec. at 72° C + 3 sec. for each cycle in a thermal circler as described in literature [48].

Cytotoxicity Assay

A colorimetric assay for living cells utilized the colorless substrate 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) that is modified to colored product by any living cells, but not by dead cells or tissue culture medium. The cytotoxic effect of the compounds was accessed by culturing the Hep G2-2.2.15 cells in the presence of compounds using a MTT-assay [49].

Calculation of IC_{50}, CC_{50} and SI

The 50% inhibitory concentration of antiviral drugs (IC_{50}) was determined by interpolation from the plots of amount of DNA copies versus antiviral drug concentration. The 50% cytotoxic effect (CC_{50}) was calculated from the average viability of the cells with

concentration of drugs. The selective index (SI) could be calculated as CC_{50}/IC_{50} [49].

The results of the viral screening against HBV of selected compounds indicated that some compounds showed moderate viral replication inhibition and mild cytotoxicity.

The results of the viral screening against HBV of selected compounds indicated that compounds (**9b**, **15**), **8**, (**3**, **6**), and **5** showed moderate viral replication inhibition and mild cytotoxicity due to the presence of thiophene ring, carbonyl group and thioxo carbonyl group with selective index >151, >625, >370, >500 and >400, respectively. On the other hand compounds **7**, (**11**, **1a**), and **10** showed very low inhibition and high cytotoxicity with selective index >80, >64, >73 and >84, respectively (Table 2).

Table 2. Cytotoxic effect (CC_{50}), inhibitory concentration (IC_{50}) and selective index (SI) of selected compounds.

Compd.	HBV DNA IC_{50} (μM)	Hep G2 2.2.15 CC_{50} (μM)	SI
Lamivudine	<0.1	>100	>1000
1a	1.37	>100	>73
3	0.20	>100	>500
5	0.25	>100	>400
6	0.20	>100	>500
7	1.56	>100	>64
8	0.27	>100	>370
9a	1.37	>100	>73
9b	0.66	>100	>151
10	1.19	>100	>84
11	1.37	>100	>73
12	1.56	>100	>64
15	0.66	>100	>151

Antimicrobial Activity

The agar diffusion method reported by Cruickshank *et al* [50] was used for the screening process. The bacteria and fungi were maintained on nutrient agar and Czapek's-Dox agar media, respectively. The assay medium flasks containing 50 mL of nutrient agar for bacteria and Czapek's-Dox agar medium for fungi respectively were allowed to reach 40-50^{o} C to be inoculated with 0.5 mL of the test organism cell suspension.

The flasks were mixed well and poured each into a Petri dish (15 x 2 cm) and allowed to solidify. After solidification, holes (0.6 cm diameter) were made in the agar plate by the aid of a sterile cork poorer (diameter 6 mm).

The synthesized target compounds were dissolved each in 2 mL DMSO. In these holes, 100μl of each compound was placed using an automatic micropipette.

The Petri dishes were left at 5° C for 1 h to allow diffusion of the samples through the agar medium and retard the growth of the test organism. Plates were incubated at 30^{o} C for 24 h for bacteria and 72 h of incubation at 28^{o} C for fungi. DMSO showed no inhibition zones.

The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin [51, 52] (50 μg/mL) and fusidic acid [53] (50 μg/mL) were used as standard for antibacterial and antifungal activity respectively. The observed zones of inhibition are presented in Table 3

Table 3. In vitro antimicrobial activity by agar diffusion method of the tested compounds.

Comp. No.	Zone of Inhibition (mm) of Microorganisms			
	Bacillus subtilis	*Escherichia coli*	*Candida albicans*	*Aspergillus flavus*
1a	45	30	12	30

3	47	35	14	39
5	30	25	10	20
6	32	22	10	26
7	25	18	10	25
8	30	34	12	33
9a	39	34	14	35
9b	25	20	12	30
10	30	30	10	30
11	30	20	10	20
12	30	30	10	30
15	22	25	10	27
Penicillin	50	45	-	-
Terbinafine	-	-	15	35

The synthesized compounds were screened *in vitro* for their antimicrobial activities [51-53] against *Escherichia coli* NRRL B-210 (Gram -ve bacteria), *Bacillus subtilis* NRRL B-543 (Gram +ve bacteria), *Aspergillus flavus* and *Candida albicans* NRRL Y-477 (Fungi).

The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Penicillin and Terbinafine were used as standard antibacterial and antifungal, the observed zones of inhibition are presented in Table 3.

The results indicated generally that tested compounds did not show high activity against bacteria under test (*Escherichia coli* and *Bacillus subtilis*) while some compounds as **3, 8** and **9a** revealed high activity against fungi. All new compounds were active against the microorganisms.

Acknowledgement

This work was supported by the financial assistance from Science & Technology Development Fund (STDF), Egypt through project 25410 and Faculty of Science, Benha University, Egypt.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Conclusion

In conclusion, the antimicrobial screening suggests that all the newly synthesized compounds showed moderate to good activity against the tested organisms.

Hence the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar analogues is clearly warranted.

References

[1] S.M. Shahcheragh, A. Habibi, S. Khosravi, Straightforward Synthesis of Novel Substituted 1,3,4-Thiadiazole Derivatives in Deep Eutectic Solvent, Tetrahedron Lett. **58**, 855–859 (2017)

[2] K.M. Taylor, Z.E. Taylor, S.T. Handy, Rapid synthesis of aurones under mild conditions using a combination of microwaves and deep eutectic solvents, Tetrahedron Lett. **58**, 240–241 (2017)

[3] J. Wang, Y. Liu, Z. Zhou, Y. Fu, J. Chang, Epoxidation of Soybean Oil Catalyzed by Deep Eutectic Solvents Based on the Choline Chloride–Carboxylic Acid Bifunctional Catalytic

System, Ind. Eng. Chem. Res. **56(29),** 8224–8234 (2017)

[4] F. Messa, S. Perrone, M. Capua, F. Tolomeo, L. Troisi, V. Capriati, A. Salomone, Towards a sustainable synthesis of amides: chemoselective palladium-catalysed aminocarbonylation of aryl iodides in deep eutectic solvents, Chem. Commun. **54**, 8100-8103 (2018)

[5] S. Perrone, M. Capua, F. Messa, A. Salomone, L. Troisi, Green synthesis of 2-pyrazinones in deep eutectic solvents: From *α*-chloro oximes to peptidomimetic scaffolds, Tetrahedron **73**, 6193-6198 (2017)

[6] P. Vitale, V.M. Abbinante, F.M. Perna, A. Salomone, C. Cardellicchio, Unveiling the hidden performance of whole cells in the asymmetric bioreduction of aryl-containing ketones in aquoes deep eutectic solvents, Adv. Synth. Catal. **359**, 1049-1057 (2017)

[7] H. Lanfang, L. Juan, L. Dan, C. Qiang, Urea decomposition: Efficient synthesis of pyrroles using the deep eutectic solvent choline chloride/urea, Tetrahedron Lett. **59**, 1698-1701 (2018)

[8] M.S. Behalo, A. Abdelmajeid, A.A. Aly, K.A. Hebash, E.A. Mohamed, Green and Facile Synthesis of Pyrimidine Derivatives Using Choline Chloride-urea Mixture as a Deep Eutectic Solvent or Cerium (IV) Ammonium Nitrate, Curr. Org. Chem. **23**, 1771-1777 (2019)

[9] A.P. Abbott, D. Boothby, G. Capper,D.L. Davies, R.K. Rasheed, Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids, J. Am.Chem. Soc. **126**, 9142-9147 (2004)

[10] A.P. Abbott, R.C. Harris, K.S. Ryder, C.D. Agostino, L.F. Gladden, M.D. Mantle, Glycerol eutectics as sustainable solvent systems, Green Chem. **13**, 82-90 (2011)

[11] R. Mancuso, A. Maner, L. Cicco, F.M. Perna, V. Capriati, B. Gabriele, Synthesis of thiophenes in a deep eutectic solvent: heterocyclodehydration and iodocyclization of 1-mercapto-3-yn-2-ols in a choline chloride/glycerol medium, Tetrahedron **72(29),** 4239-4244 (2016)

[12] A. Paiva, R. Craveiro, I. Aroso, M. Martins, R.L. Reis, A.R.C. Duarte, Natural Deep Eutectic Solvents–Solvents for the 21st Century, ACS Sustainable Chem. Eng. **2(5)**, 1063-1071 (2014)

[13] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep eutectic solvents (DESs) and their applications, Chem. Rev. **114**, 11060-11082 (2014)

[14] S.B. Phadtare, G.S. Shankarling, Halogenation reactions in biodegradable solvent: Efficient bromination of substituted 1-aminoanthra-9, 10-quinone in deep eutectic solvent (choline chloride: urea), Green Chem. **12**, 458-462 (2010)

[15] M. Francisco, A. Bruinhorst, C. Maaike, Low-Transition-Temperature Mixtures (LTTMs): A New Generation of Designer Solvents, Angew. Chem. Int. Ed. **52**, 3074-3085 (2013)

[16] M. Capua, S. Perrone, F.M. Perna, P. Vitale, L. Troisi, A. Salomone, An expeditious and greener synthesis of 2-aminoimidazoles in deep eutectic solvents, Molecules **21**, 924-935 (2016)

[17] E. Abdelghani, S.A. Said, M.G. Assy, A.M. Abdel Hamid, Synthesis and antimicrobial evaluation of some new pyrimidines and condensed pyrimidines, Arab. J. Chem. **10**, 2926-2933 (2017)

[18] N.N.A. Jafar, I.M.A. Mahdi, M.H. Hadwan, A.A. Alameri, The Antifungal Effect of some 4-Chloro-6-Methoxy-*N*,*N*-Dimethylpyrimidin-2-Amine Derivatives Containing a Heterocyclic Compound on the Important types of Fungi, J. Young Pharm. **9(4**), 463-467 (2017)

[19] S. Fandakli, N. Kahriman, T.B. Yucel, S.A. Karaoglu, N. Yayli, Biological evaluation and synthesis of new pyrimidine-2(1*H*)-ol/-thiol derivatives derived from chalcones using the solid phase microwave method, Turk. J. Chem. **42**, 520-535 (2018)

[20] T. Venkatesh, Y.D. Bodke, K. Nagaraj, K.S. Ravi, One-Pot Synthesis of Novel Substituted Phenyl-1,5-dihydro-2*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimido[4,5-*d*]pyrimidine Derivatives as Potent Antimicrobial Agents, Med. Chem. **8(1)**, 1-7 (2018)

[21] B.H. Naguib, H.B. El-Nassan, T.M. Abdelghany, Synthesis of new pyridothienopyrimidinone derivatives as Pim-1 inhibitors, J. Enzyme Inhib. Med. Chem. **32**, 457-467 (2017)

[22] A. Abdelmajeid, M.S. Amine, R.A. Hassan, Fatty Acids in Heterocyclic Synthesis: Part XIX Synthesis of Some Isoxazole, Pyrazole, Pyrimidine and Pyridine and Their Surface, Anticancer and Antioxidant Activities, Am. J. Heterocycl. Chem. **4(2)**, 30-41 (2018)

[23] M. Maher, A.E. Kassab, A.F. Zaher, Z. Mahmoud, Novel pyrazolo[3,4-*d*]pyrimidines: design, synthesis, anticancer activity, dual EGFR/ErbB2 receptor tyrosine kinases inhibitory activity, effects on cell cycle profile and caspase-3-mediated apoptosis, J. Enzyme Inhib. Med. Chem. **34(1)**, 532-546 (2019)

[24] M.N.M. Yousif, W.A. El-Sayed, H.S. Abbas, H.M. Awad, N.M. Yousif, Anticancer Activity of New Substituted Pyrimidines, Their Thioglycosides and Thiazolopyrimidine Derivatives, J. App. Pharm. Sci. **7 (11)**, 021-032 (2017)

[25] M.S. Behalo, I.G. El-karim, R. Rafaat, Synthesis of Novel Phthalazine Derivatives as Potential Anticancer and Antioxidant Agents Based on 1-

Chloro-4- (4-phenoxyphenyl)phthalazine, J. Heterocycl. Chem. **54(6)**, 3591-3599 (2017)
[26] E.R. Kotb, E.M.H. Morsy, M.M. Anwar, E.M. Mohi El-Deen, H.M. Awad, New Pyrimidine Derivatives: Synthesis, Antitumor and Antioxidant Evaluation, International Journal of Pharmacy & Technology **7**, 8061-8085 (2015)
[27] M. Kachroo, R. Panda, Y. Yadav, Synthesis and biological activities of some new pyrimidine derivatives from chalcones, Der Pharma Chemica **6(2),** 352-359 (2014)
[28] S.A. Bahashwan, Pharmacological activities of some triazinopyrazolothieno pyrimidine derivatives, Acta Pharm. **67**, 407-414 (2017)
[29] D.A. DeGoey, D.A. Betebenner, D.J. Grampovnik, D. Liu, J.K. Pratt, M.D. Tufano, W. He, P. Krishnan, T.J. Pilot-Matias, K.C. Marsh, A. Molla, D.J. Kempf, C.J. Maring, Discovery of pyrido[2,3-*d*]pyrimidine-based inhibitors of HCV NS5A, Bioorg. Med. Chem. Lett. **23**, 3627-3630 (2013)
[30] M.S. Behalo, Synthesis of some novel thiazolo[3,2-*a*]pyrimidine and pyrimido[2,1-*b*][1,3]thiazine derivatives and their antimicrobial evaluation, J. Heterocycl. Chem. **55**, 1391-1397 (2018)
[31] M.S. Behalo, Facile synthesis of novel amino acids derivatives as potential antibacterial agents using sustainable materials, J. Chin. Chem. Soc. **64**, 1181-1189 (2017)
[32] M.S. Behalo, Synthesis and antimicrobial activities of some novel pyrido[2,3-d]pyrimidine derivatives. Phosphorus Sulfur Silicon Relat. Elem., **184 (1)**, 206-219 (2009)
[33] M.S. Behalo, I.G. El-Karim, Y.A. Issac, M.A. Farag, Synthesis of novel pyridazine derivatives as a potential antimicrobial agents, J. Sulf. Chem. **35(6)**, 661-673 (2014)
[34] M.S. Behalo, M.S. Amine, I.M. Fouda, Regioselective synthesis, antitumor and antioxidant activities of some 1,2,4-triazole derivatives based on 4-phenyl-5-(quinolin-8-yloxy)methyl-4*H*-1,2,4-triazole-3-thiol, Phosphorus, Phosphorus Sulfur Silicon Relat. Elem., **192 (4),** 410-417 (2017)
[35] M.S. Behalo, E. Bloise, L. Carbone, R. Del Sole, D. Lomonaco, S.E. Mazzetto, G. Mele, L. Mergola, Cardanol-based green nanovesicles with antioxidant and cytotoxic activities, J. Experimental Nanoscience **11(16),** 1274-1284 (2016)
[36] C.S. Marvel, J.M. Quinn, J.S. Showell, Copolymerizations of conjugated unsaturated carbonyl systems containing thiophene units, J. Org. Chem. **18**, 1730-1738 (1953)
[37] M.S. Behalo, G. Mele, Synthesis and evaluation of pyrido[2,3-*d*]pyrimidine and 1,8-naphthyridine derivatives as potential antitumor agents, J. Heterocycl. Chem. **54(1)**, 295-300. (2017)
[38] M. Di Braccio, G. Grossi, S. Alfei, V. Ballabeni,; M. Tognolini, L. Flammini, C. Giorgio, S. Bertoni, E. Barocelli, 1,8-Naphthyridines IX. Potent anti-inflammatory and/or analgesic activity of a new group of substituted 5-amino[1,2,4]triazolo[4,3-*a*][1,8]naphthyridine-6-carboxamides, of some their Mannich base derivatives and of one novel substituted 5-amino-10-oxo-10*H*-pyrimido[1,2-*a*][1,8]naphthyridine-6-carboxamide derivative. Eur. J. Med. Chem. **86**, 394-405 (2014)
[39] P. Skehan, R. Storeng, New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening, J. Natl. Cancer Inst. **82 (13)**, 1107-1112 (1990)
[40] R.P. Beasley, L.Y. Hwang, C.C. Lin, C. S. Chien, Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan, Lancetii **2**, 1129-1133 (1981)
[41] E.A. Ayoola, M.S. Balayan, F. Deinhardt, I.Gust, A.W. Kureshi, J.E. Maynard, D.W. Brodley, M.Ferguson, J. Melnick, R.H.Purcell, A.J. Zuckerman, Progress in the control of viral hepatitis: Memorandum from a WHO Meeting. Bull. World Health Org. **66**, 443-455 (1988)
[42] J.P. Sommadossi, Treatment of hepatitis B by nucleoside analogues: still a reality, Current Opinion in Infection Diseases **7**, 678-682 (1994)
[43] G.L. Davis, J.H. Hoofnagle, Interferon in viral hepatitis: role in pathogenesis and treatment, Hepato **l6**, 1038-1041 (1986)
[44] R.P. Perrillo, Interferon therapy for chronic type B hepatitis: The promise comes of age, Gastroentero **l96**, 532-536 (1989)
[45] K. Ueda, T. Tsurimoto, T. Nagahata, O. Chisaka, K. Matsubara, An in vitro system for screening anti-hepatitis B virus activity and susceptibility to other nucleosides of AZT-resistant variants of HIV-1, J. Virol. **169(1)**, 213-216 (1989)
[46] S.L. Doong, C.H. Tsai, R.F. Schinazi, D.C. Liotta, Y.C. Cheng, Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiacytidine and related analogues, Proc. Nat. Acad. Sci. USA **88**, 4895-4904 (1991)
[47] M.A. Sells, A.Z. Zelent, M. Shvartsman, G. Acs, Integration of viral DNA encoding HBxAg during chronic infection results in Replicative intermediates of hepatitis, J. Virol. **62(8),** 2836-2844 (1988)
[48] B.E. Korba, J.L. Gerin, Use of a standardized cell culture assay to assess activities of nucleoside analogs against hepatitis B virus replication, Antiviral Res. **19(1),** 55-70 (1992)

[49] T. Fouad, C. Nielsen, L. Brunn E.B. Pederson, Use of some potent anti-HIV nucleosides analogues against Hepatitis *B* virus replication, J. Az. Med. Fac. (GIRLS) **19**, 1173-1187 (1998)
[50] R. Cruickshank, J.P. Duguid, B.P. Marion, R.H.A. Swain, The Practice of medical microbiology, Medicinal Microbiology, twelfth ed. Churchill Livingstone, London, **vol. II** 196-202 (1975)
[51] R. Dahiya, Synthesis, Characterization and Antimicrobial Studies on Some Newer Imidazole Analogs, Sci. Pharm **76(2),** 217-240 (2008)
[52] H.C. Su, K. Ramkissoon, J. Doolittle, M. Clark, J. Khatun, A. Secrest, M.C. Wolfgang, M.C. Giddings, The development of ciprofloxacin resistance in Pseudomonas aeruginosa involves multiple response stages and multiple proteins, Antimicrob. Agents Chemother. **54(11)**, 4626-4635 (2010)
[53] T.F. Poyner, B.K. Dass, Comparative efficacy and tolerability of fusidic acid/hydrocortisone cream (FucidinH cream) and miconazole/hydrocortisone cream (Daktacort cream) in infected eczema, J. Eur. Acad. Dermatol. Venereol. **7,** S23-S30 (1996)