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Ultrasonic assisted synthesis of novel anticancer chalcones using water as green solvent

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ABSTRACT

The reaction of compound (2) with different aldehydes catalyzed by pulverized NaOH under both ultrasound irradiation using water as green solvent and conventional conditions to afford novel chalcones. It is obvious that the yields and reactions times were improved under ultrasonic irradiation rather than conventional method. All structures were characterized by elemental analysis and spectral data. The synthesized chalcones are evaluated against HepG-2, HCT-116, MCF-7 and Caco-2. Most of compounds showed significant antitumor activities.

Keywords: Ultrasonic, inorganic catalyst, green chemistry, chalcone, anticancer

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INTRODUCTION

The preparation of chalcones has been a long-term mainstay of organic synthesis where Chalcones are key precursors in the synthesis of a large array of biologically important heterocycles [1–3]. Recent studies on biological evaluation of chalcones revealed them to be anti-malarial [4-6], anti-cancer [7], anti-inflammatory [8], antimitotic [9], anti-tuberculosis [10] and anti-hyperglycemic [11] agents. In contrast to the common organic reaction media, water is used as an environmentally benign solvent as it is nontoxic, nonflammable and cheap [12].

Ultrasonic activation is a powerful technique that recently is being used to accelerate and enhance organic reactions particularly, when running these reactions in water as benign medium [13-15]. In continuation of our interest of using green chemistry tools in organic synthesis, here in we studied the reactions of different aldehydes with compound **2** under both sonication and stirring conditions [16,17].

MATERILAS AND METHODS

3.1. Chemistry

3.1.1. General

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VXR-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethyl sulphoxide (DMSO-d6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 e.V. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt.

Reactions carried out under ultrasonic irradiation were performed by fischer sonicator (with frequency of 25 kHz and nominal power 600W).

3.1.2. Synthesis of 1(4-(1-(4-aminophenyl)ethylideneamino)phenyl)ethanone (2)

p-aminoacetophenone (1) (100 mmol) subjected to ultrasound waves at room temperature in 50 ml acetic acid for 2 hours to give yellowish Precipitate which was filtered and recrystalized from acetone to afford 1(4-(1-(4-aminophenyl)ethylideneamino) phenyl) ethanone (2) in 98% Yield .

m.p.=158-159 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O), 3264, 3296 (NH_2);^{1}H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 2.51 (s, 3H, CH_3C=O), 7.69-7.92 (m, 8H, Ar-H), 5.24 (s, 2H, NH_2 D_2O exchangeable); MS (m/z): 252 (Found: C, 76.20; H, 6.36; N, 11.09 $C_{16}H_{16}N_2O$ required: C, 76.16; H, 6.39; N, 11.10.

3.1.3. Synthesis of chalcones

Method A: under sonication

A mixture of (4-(1-(4-aminophenyl)ethylideneamino)phenyl)ethanone (**2**), the appropriate aldehyde 3a-m (5 mmol) and NaOH (1 mmol) in 5 ml distilled water was placed in 50 ml Erlenmeyer flask and subjected to ultrasound waves at room temperature for the appropriate time until completion of the reaction (monitored by TLC). The resulting solid was collected by filtration and purified by crystallization from the appropriate solvent to afford the pure products **4a-m**.

Method B: Convential conditions

To a solution of 1(4-(1-(4-aminophenyl)ethylideneamino)phenyl)ethanone (**2**) (5 mmol) and appropriate aldehyde (5 mmol) in C_2H_5OH (25 ml), 40% of aqueous NaOH (1 mmol) was added. The reaction mixture was stirred at room temperature till completion of reaction (monitored by TLC). Then the reaction mass was poured into ice water and neutralized with aqueous 10% HCl solution. The precipitate was filtered, washed with excess of water, dried and recrystallized from the appropriate solvent to obtain pure chalcones. All structures were confirmed by mass and NMR spectra as discussed below.

3.1.3.1. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-phenylprop-2-en-1-one ***4a***

m.p. 156-157 ^{0}C; IR (KBr) ν/cm^{-1}: 1603 (C=N), 1684 , (C=O) 3200, 3300 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.06 (s, 3H, CH_3-C=N-), 7.41 (d, *J* = 7.5 Hz 1H, COCH=), 8.11 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.72-8.09 (m, 13H, Ar-H), 5.27 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 24., 118, 119, 122 ,129, 130, 132, 135.33, 143, 144, 169.5, 188; MS (m/z): 340 (Found: C, 81.10; H, 5.95; N, 8.25 $C_{23}H_{20}N_2O$ Calculate: C, 81.15; H, 5.92; N, 8.23).

3.1.3.2. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(2-methoxyphenyl)prop-2-en-1-one ***4b*** m.p.= 165-166 ^{0}C; IR (KBr) ν/cm^{-1}: 1592 (C=N), 1677 (C=O), 2830 (OCH_3), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.06 (s, 3H, CH_3-C=N-),3.84 (s, 3H, CH_3-O), 7.03 (d, *J* = 7.5 Hz 1H, COCH=), 8.08 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.38-8.02 (m, 12H, Ar-H), 5.34 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 24, 56 ,112,118,121, 122, 123, 128, 130, 132, 138, 144, 158, 169, 188; MS (m/z): 370.17 (Found: C, 7.85; H, 5.96; N, 7.55; $C_{24}H_{22}N_2O_2$ Calculate: C, 7.81 H, 5.99; N, 7.56.

3.1.3.3. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(3-methoxyphenyl)prop-2-en-1-one ***4c*** m.p. =134-135 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 , (C=O 2835 (OCH_3), 3269, 3294 (NH_2); ^{1}H NMR (DMSO-d6): δ= 2.05 (s, 3H, CH_3-C=N-),3.79 (s, 3H, CH_3-O), 7.66 (d, *J* = 7.5 Hz 1H, COCH=), 8.13 (d, *J* = 7.5 Hz 1H, COCH=CH), 6.97-7.91 (m, 12H, Ar-H), 5.33 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25, 55,113, 117, 118, 122, 130, 132, 136, 143, 144, 160, 169, 188; MS (m/z): 370 (Found: C, 7.83; H, 5.98; N, 7.55; $C_{24}H_{22}N_2O_2$ Calculate: C, 7.81; H, 5.99; N, 7.56.

3.1.3.4. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one ***4d*** m.p.= 180-181 ^{0}C; IR (KBr) ν/cm^{-1}: 1596 (C=N), 1678 (C=O), 2836 (OCH_3), 3270, 3302 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.05 (s, 3H, CH_3-C=N-), 3.78 (s, 3H, CH_3-O), 7.63 (d, *J* = 7.5 Hz 1H, COCH=), 8.07 (d, *J* = 7.5 Hz 1H, COCH=CH), 6.58-7.86 (m, 12H, Ar-H), 5.25 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51, 56, 121, 145, 114.-159, 165, 189; MS (m/z): 370 (Found: C, 7.83; H, 5.98; N, 7.55; $C_{24}H_{22}N_2O_2$; Calculate: C, 7.81; H, 5.99; N, 7.56.

3.1.3.5. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(4-cyanophenyl)prop-2-en-1-one ***4e*** m.p.= 160-162 ^{0}C;IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O), 2250 (CN) 3264, 3296 (NH_2); 1H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 7.59 (d, 1H, COCH=), 8.03 (d, 1H, COCH =CH), 7.69-7.92 (m, 12H, Ar-H), 5.24 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51, 118, 121, 145, 114.-159, 165, 189; MS (m/z): 365 (Found: C, 78.93; H, 5.22; N, 11.47;$C_{24}H_{19}N_3O$; Calculate: C, 78.88; H, 5.24; N, 11.50.

3.1.3.6. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one ***4f*** m.p.= 227-228 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O),1350,1530 (NO_2), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 7.56 (d, *J* = 7.5 Hz 1H, COCH=), 8.02 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.69-7.92 (m, 12H, Ar-H), 5.24 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51, 121, 145, 115.-158, 165, 189; MS (m/z): 386 (Found: C, 71.72; H, 4.94; N, 10.88 $C_{23}H_{19}N_3O_3$ Calculate: C, 71.67; H, 4.97; N, 10.90.

3.1.3.7. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(4-flourophenyl)prop-2-en-1-one ***4g*** m.p.= 205-207 ^{0}C; IR (KBr) ν/cm^{-1}: 1600 (C=N), 1660 (C=O), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.06 (s, 3H, CH_3-C=N-), 7.60 (d, *J* = 7.5 Hz 1H, COCH=), 8.04 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.69-7.92 (m, 12H, Ar-H), 5.21(s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51 , 121 , 145 , 114.-159 , 165 , 189 ; MS (m/z): 359 (Found: C, 77.12; H, 5.32; F, 5.29; N, 7.81; $C_{23}H_{19}FN_2O$; Calculate: C, 77.08; H, 5.34; F, 5.30; N, 7.82.

3.1.3.8. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(4-chlorophenyl)prop-2-en-1-one ***4h*** m.p.= 159-160 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 7.55 (d, *J* = 7.5 Hz 1H, COCH=), 8.01 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.69-7.92 (m, 12H, Ar-H), 5.21 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51 , 121 , 145 , 114.-159 , 165 , 189 ; MS (m/z): 375 (Found: C, 73.65; H, 5.13; Cl, 9.45; N, 7.50 $C_{23}H_{19}ClN_2O$ Calculate: C, 73.69; H, 5.11; Cl, 9.46; N, 7.47.

3.1.3.9. 4-(4-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-4-oxoprop-2-en-1-yl)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one ***4i***
m.p.= 245-246 ^{0}C;IR (KBr) ν/cm^{-1}: 1594 (C=N), 1656 (C=O), 3264, 3296 (NH_2);^{1}H NMR (DMSO-d_6): δ= 2.04 (s, 3H, CH_3-C=N-), 2.42 (s, 3H, CH_3 pyrazolone), 3.27 (s, 3H, CH_3- N- pyrazolone), 7.85 (d, *J* = 7.5 Hz 1H, COCH=), 8.03 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.33-7.69 (m, 13H, Ar-H), 5.23 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 13.5 , 25.51 , 35 , 109 , 129 , 144 , 163 , 145 , 114.-159 , 165 , 189 ; MS (m/z): 450; (Found: C, 74.69; H, 5.80; N, 12.42; $C_{28}H_{26}N_4O_2$; Calculate: C, 74.65; H, 5.82; N, 12.44.

3.1.3.10. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one ***4j*** m.p.= 147-149 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O), 3200 (NH), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 7.59 (d, *J* = 7.5 Hz 1H, COCH=), 8.03 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.52 (s, 1H, indole), 7.69-7.92 (m, 12H, Ar-H), 5.10 (s, 1H, NH D_2O exchangeable), 5.24(s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51 , 115 , 121 , 130 , 145 , 111.-156 , 165 , 189 ; MS (m/z): 380 (Found: C, 79.18; H, 5.55; N, 11.05; $C_{25}H_{21}N_3O$; Calculate: C, 79.13; H, 5.58; N, 11.07.

3.1.3.11. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-4-(thiophen-2-yl)prop-2-en-1-one ***4k*** m.p.= 119-120 ^{0}C; IR (KBr) ν/cm^{-1}: 1591 (C=N), 1675 (C=O), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.08 (s, 3H, CH_3-C=N-), 7.59 (d, *J* = 7.5 Hz 1H, COCH=), 8.03 (d, *J* = 7.5 Hz 1H, COCH=CH), 7.52 (t, 1H, thiophene), 7.91 (d, 1H, thiophene), 8.10 (d, 1H, thiophene), 7.55-7.88 (m, 8H, Ar-H), 5.30 (s, 2H, NH_2 D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 25.51 , 121 , 130 , 142 , 145 , 111.-160 , 165 , 189 ;MS (m/z): 345 (Found: C, 72.85; H, 5.20; S 9.28 N, 8.06;$C_{21}H_{18}N_2OS$ Calculate: C, 72.80; H, 5.24; S, 9.26; N, 8.09.

3.1.3.12. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(pyridin-3-yl)prop-2-en-1-one ***4l*** m.p.= 221-222 ^{0}C; IR (KBr) ν/cm^{-1}: 1520 (C=N), 1591 (C=N)1675 (C=O), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d6): δ= 2.08 (s, 3H, CH_3-C=N-),7.52 (t, 1H, pyridine), 7.98 (d, 1H, pyridine), 8.33 (d, 1H, pyridine), 8.85 (s, 1H, pyridine), 7.59 (d, 1H, COCH=), 8.03 (d, 1H, COCH=CH), 7.69-7.92 (m, 8H, Ar-H), 5.24 (s, 2H, NH_2 D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 25.51 , 121 , 124 , 130 , 145 , 149 , 112.-155 , 165 , 189 ; MS (m/z): 341(Found C, 77.36; H, 5.62; N, 12.34$C_{22}H_{19}N_3O$ Calculate: C, 77.40; H, 5.61; N, 12.31).

3.1.3.13. 1-(4-(1-(4-aminophenyl)ethylidene)amino)phenyl)-3-(furan-2-yl)prop-2-en-1-one ***4m*** m.p.= 78-79 ^{0}C IR (KBr) ν/cm^{-1}: 1520 (C=N), 1675 (C=O), 3264, 3296 (NH_2); ^{1}H NMR (DMSO-d_6): δ= 2.05 (s, 3H, CH_3-C=N-),6.82 (t, 1H, furan), 7.88 (d, 1H, furan), 8.22 (d, 1H, furan), 7.59 (d, 1H, COCH=), 8.03 (d, 1H, COCH=CH), 7.69-7.92 (m, 8H, Ar-H), 5.24 (s, 2H, NH_2 D_2O exchangeable). ^{13}C NMR (DMSO-d6): δ 25.51, 115 , 121 , 144 , 145 , 155 , 114.-159 , 165 , 189 ; MS (m/z): 331 (Found C, 76.39; H, 5.46; N, 8.46 $C_{21}H_{18}N_2O_2$Calculate C, 76.34; H, 5.49; N, 8.48).

3.2. Biological Evaluation

3.2.1. In-vitro anticancer activity

The four human cell lines, liver carcinoma (Hep-G2); breast carcinoma (MCF-7); Colorectal adenocarcinoma (Caco-2); colorectal carcinoma (HCT116) were supplied by Applied Research Sector, VACSERA-Egypt and maintained in RPMI-1640 that was supplemented with 10% heat-inactivated FBS, 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

3.2.2. Lactate dehydrogenase (LDH) assay

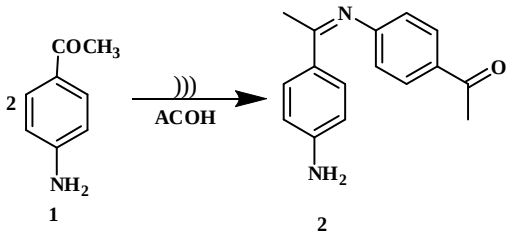
The effect of the selected synthesized compounds on membrane permeability of the four human cancer cell lines, HepG2; MCF-7; Caco-2; HCT-116, was measured using lactate dehydrogenase (LDH) release assay [20]. The cells were cultured in 24-well culture plates at a density of 5×10^5 cells/well in 500 µL media and allowed to grow for 18h before treatment with a series of different concentrations of the tested compounds in DMSO. Doxorubicin® was used as positive control. The plates were incubated for 48h. Then, the supernatant (40 µL) was transferred to a new 96 well to determine LDH release in each media and 6% triton X-100 (40 µL) was added to the original plate for determination of total LDH. 100 µL of 0.1 M potassium phosphate buffer (pH 7.5) containing 4.6 mM pyruvic acid was mixed to the supernatant. Then, 100 µL of 0.1 M potassium phosphate buffer (pH 7.5) containing 0.4 mg/mL reduced β-NADH was added to each well. The absorbance changes, at wavelength 340 nm, were read using SpectraMax® Paradigm® Multi-Mode microplate reader (Molecular Devices). This procedure was repeated with 40 µL of the total cell lysate to determine total LDH. The percentage of LDH release was determined by dividing the LDH released into the media by the total LDH following cell lysis in the same well [21].

Structure–activity relationships in structures 4a,4c,4d,4f,4g,4k,4l and 4m demonstrated that compounds with para electron-withdrawing substituent showed more anticancer activities than those with meta and ortho position or electron-donating substituent. A comparison of addition of thiophene, pyridine and pyrole demonstrated more anticancer activities than 2-O-CH_3-C_6H_4 and 4-CN-C_6H_4.

RESULTS AND DISCUSSION

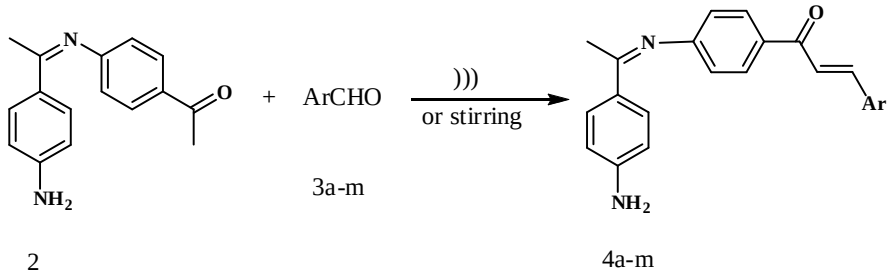
2.1 Chemistry

Compound **2** is obtained in excellent yield (98%) under ultrasonic irradiation while under reflux conditions the yield was 85% [18]. The structure of compound **2** was confirmed depending on its analytical and spectroscopic data (scheme 1).



Scheme 1: Synthesis of compound 2

The reaction of compound **2** with different aldehydes was performed by stirring at room temperature or under ultrasonic irradiation to yield novel chalcones (scheme 2).



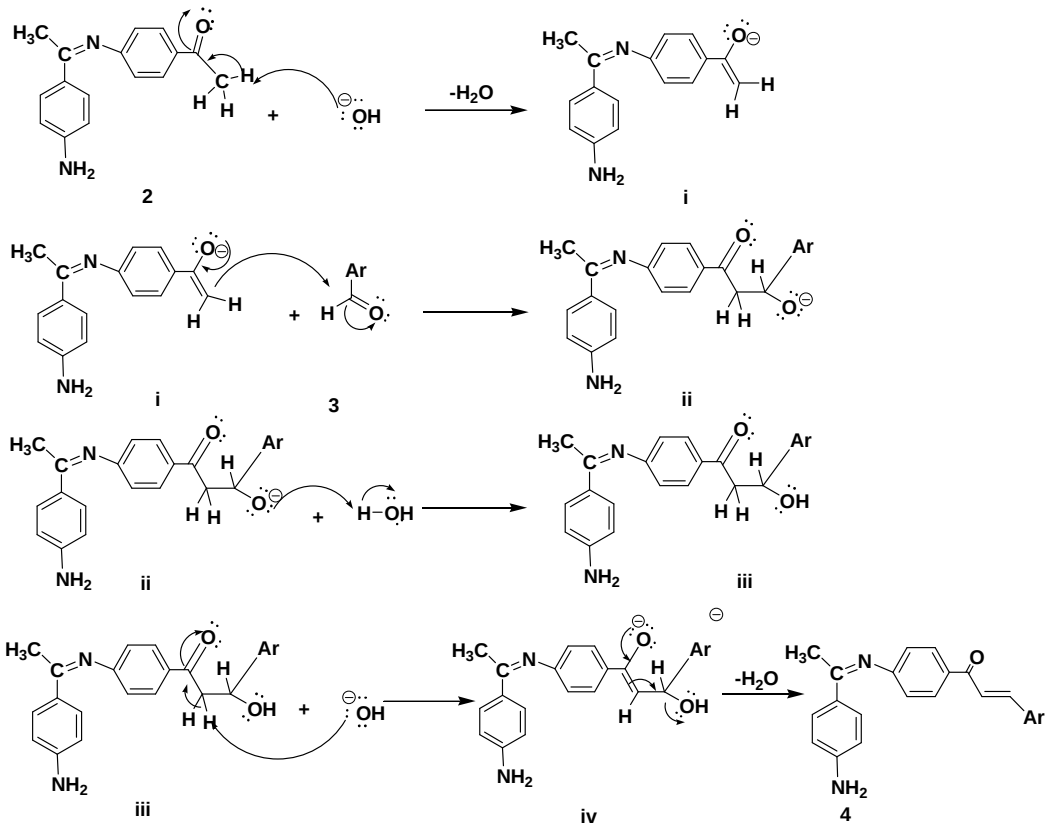
Scheme 2: Synthesis of compounds 4a-m

To find optimum conditions for the reaction under ultrasonic irradiation we took as a model the reaction of benzaldehyde **3a** with compound **2** in presence of different inorganic catalysts (Table 1).

Table 1: The effect of some inorganic catalysts on the yield and time of the reaction of 3a with compound 2 under ultrasonic irradiation

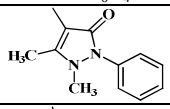
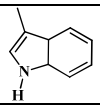
Catalyst	Time (h)	Yield
Molecular iodine	4	-
Basic Al_2O_3	4	-
Al_2O_3/KF	4	30
KF	4	43
Na_2CO_3	4	82
NaOH	2	95

From these results, it is obvious that, using molecular iodine or basic alumina no reaction has been occurred (TLC).The maximum yield for chalcone 4a (95%) was obtained when the reaction is catalyzed by sodium hydroxide, thus compound **2** reacts with different aldehydes under ultrasonic irradiation in presence of sodium hydroxide.



Scheme 3: Mechanism of chalcone synthesis

Table 2: Comparison between reaction times and yields for conventional and ultrasonic irradiation methods for syntheses of chalcones

Entry	Ar	Stirring		Ultrasonic Irradiation	
		Time (min.)	Yield (%)	Time (min.)	Yield (%)
4a	$-C_6H_5$	240	80	60	95
4b	$2\text{-}CH_3O\text{-}C_6H_4$	180	81	30	93
4c	$3\text{-}CH_3O\text{-}C_6H_4$	180	75	30	88
4d	$4\text{-}CH_3O\text{-}C_6H_4$	180	80	30	95
4e	$4\text{-}CN\text{-}C_6H_4$	180	82	30	93
4f	$4\text{-}NO_2\text{-}C_6H_4$	180	83	30	92
4g	$4\text{-}F\text{-}C_6H_4$	180	81	30	93
4h	$3\text{-}Cl\text{-}C_6H_4$	240	84	60	95
4i		240	77	60	87
4j		240	82	30	90
4k		240	85	60	90
4l		300	77	120	85
4m		180	81	30	90

The structure of novel chalcones were confirmed in terms of elemental analysis and spectral data (*cf. experimental part*). Chalcones are generally synthesized via Claisen–Schmidt condensation carried out in basic or acidic media under homogeneous conditions [19]. In the case of acid catalyzed Claisen–Schmidt condensation, protonating of aldehydetakes place which is attacked by the enolic ketone then dehydration of the condensed product to form chalcones. But in our case sodium hydroxide catalyzed the formation of the anion of compound **2** which is attacked by aldehyde then dehydration of the condensed product to form chalcone (scheme 3).

2.2. Biological activity

In vitroantitumor activity

From Figure 1, comparing to the starting compound **2** as well as doxorubicin for the substituted compounds, antitumor activities against HepG-2 and HCT-116, it is obvious that eight out of thirteen synthesized compounds showed significant antitumor activity. These compounds are: **4a, 4c, 4d, 4f, 4g, 4k, 4l** and **4m**. The five compounds which showed week antitumor activities are: **4b, 4e, 4h, 4i** and **4j**. In addition, from figure 2, comparing to the starting compound **2** as well as doxorubicin, the results in the figure 2 reveal that, eleven out of the thirteen substituted compounds showed better antitumor activities against MCF-7 and Caco-2 human cancer cell lines. Only two compounds did not show significant antitumor activities. These compounds are: **4h** and **4i**.

Further experiments have been carried out to calculate the IC_{50} concentrations for the most promising compounds according to their activities. Table 3 showed the IC_{50} values for the most promising highly active compounds.

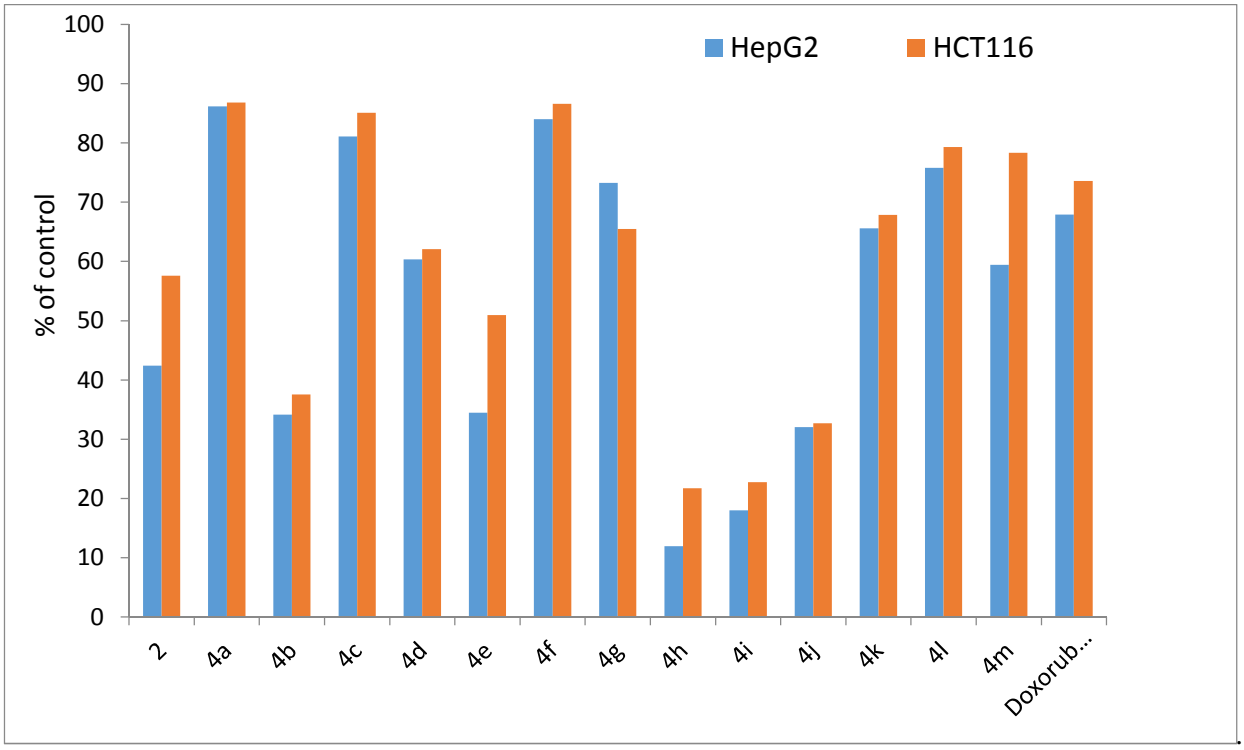


Figure 1: The antitumor activities of the substituted compounds against HepG2 and HCT 116 in comparison with the start compound 2 and doxorubicin using LDH assay

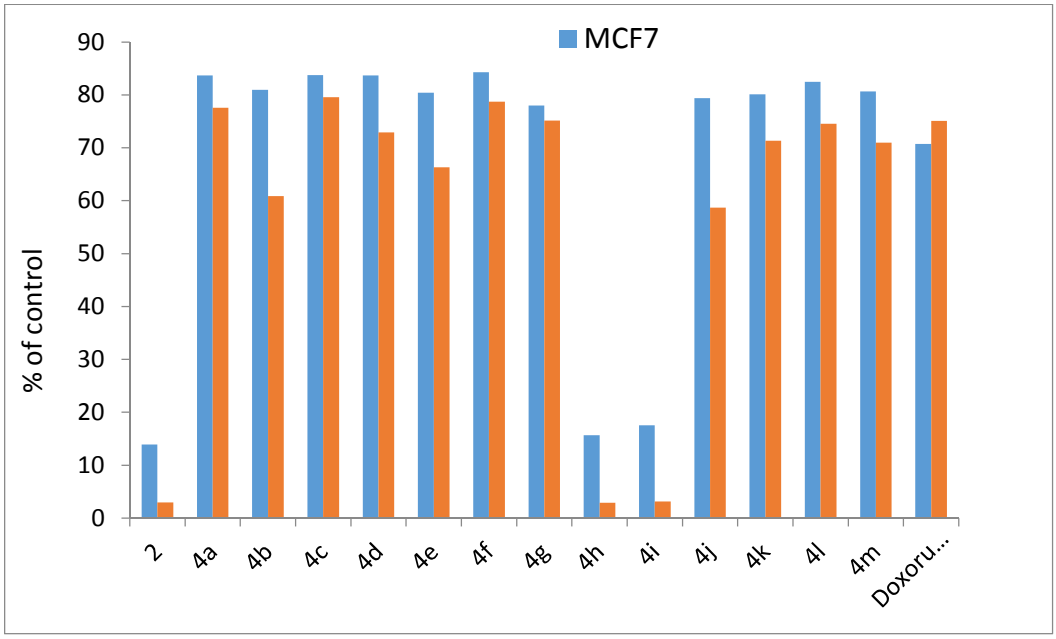


Figure 2: The antitumor activity of the synthesized compounds against MCF-7 and Caco-2 in comparison with the start compound 2 and doxorubicin using LDH assay

Table 3: The IC_{50} values of the selected compounds tested for their antitumor activities against HepG-2, HCt-116, MCF-7 and Caco-2 using LDH assay

Compound No.	IC_{50}			
	HepG-2	HCT-116	MCF-7	Caco-2
4a	53.5	52.7	12.8	38.2
4b	95.4	91.4	34.2	53.6
4c	53.9	53.8	19.1	35.1
4d	62.7	84.1	41.1	55.8
4e	94.7	89.3	58.7	72.9
4f	57.7	59.8	21.3	53.2
4g	76.8	90.7	29.9	51.9
4j	91.6	84.7	60.7	72.9
4k	62.1	68.2	28.3	58.7
4l	63.9	69.8	33.9	51.4
4m	60.9	54.2	36.9	55.6

Exploring the results of the antitumor activities of the compounds against all the four human cancer types, which mentioned above, it is obvious that, the antitumor activities of compound **2** highly increased by attaching the following groups to it: phenyl; 3-CH_3-O-C_6H_4; 4-CH_3-O-C_6H_4; 4-NO_2-C_6H_4, 4-F-C_6H_4; thiophen-2-yl; pyridin-3-yl; furan-2-yl.

In addition, substitution of compound **2** with the two groups: 2-CH_3O-C_6H_4; 4-CN-C_6H_4, slightly decreased its antitumor activities only in case of both HepG2 and HCT 116 human cancer types. The substitution with group indol-3-yl caused a high drop in the antitumor activity of compound 2 against the same two cancer types.

However, when compound **2** was substituted by both groups: 3-Cl-C_6H_4 and 2-phenyl--pyrazol-3-one-4-yl, its antitumor activity either disappeared as in case of HepG2 and HCT 116 cancer types, or retained without any significant change as in case of MCF-7 and Caco-2 cancer types.

CONCLUSION

The results showed that the yields and reactions times were improved under ultrasonic irradiation rather than conventional method. As well as eight of thirteen synthesized compounds showed high significant antitumor activity.

REFERENCES

[1] AL. Marzinzik, ER. Felder, *J. Org. Chem.* **1998**, 63, 723.

[2] M. Bennett, AJ. Burke, WI. O'Sullivan, *Tetrahedron* **1996**, 52, 7163.
[3] G. Bose, E. Mondal, AT. Khan, MJ. Bordoloi, *Tetrahedron Lett*. **2001**, 42, 8907.
[4] M. Liu, P. Wilairat, ML. Go, *J. Med. Chem.* **2001**, 44, 4443.
[5] JN. Dominguez, JE. Charris, G. Lobo, NG. de Dominguez, MM. Moreno, F. Riggione, E. Sanchez, J. Olson , PJ. Rosenthal, *Eur. J. Med. Chem*. **2001**, 36, 555.
[6] VJ. Ram, AS. Saxena, S. Srivastava, S. Chandra, *Bioorg. Med. Chem. Lett.* **2000**, 10, 2159.
[7] Y. Xia, ZY. Yang, P. Xia, KF. Bastow, Y. Nakanishi, KH Lee, *Bioorg. Med. Chem. Lett.* **2000**, 10, 699.
[8] F. Herencia, ML. Ferrandiz, A. Ubeda, JN. Dominguez, JE Charris, GM. Lobo, MJ. Alcaraz, *Bioorg. Med. Chem. Lett.* **1998**, 8, 1169.
[9] S. Ducki, R. Forrest, JA. Hadfield, A. Kendall, NJ. Lawrence, AT. Gown, D. Rennison, *Bioorg. Med. Chem. Lett.***1998**, 8, 1051.
[10] LM. Lin, Y. Zhou, MT. Flavin, LM. Zhou, W. Nie, FC. Chen, *Bioorg. Med. Chem.* **2002**, 10, 2795.
[11] M. Satyanarayana, P. Tiwari, BK. Tripathi, AK. Srivastava, R. Pratap, *Bioorg. Med. Chem.* **2004**, 12, 883.
[12] IT. Horvath, *Green Chem*. **2008**, 10, 102.
[13] CP. Frizzo, E. Scapin, MR. Marzari, TS. München, N. Zanatta, HG. Bonacorso, L Buriol, MA. Martins, *Ultrasonics Sonochem.,* **2014**, 21, 958.
[14] R. Ghahremanzadeh, Z. Rashid, AH. Zarnani, H. Naeimi, *Ultrasonics Sonochem*., **2014**, 21, 1451.
[15] B. Drawanz, CS. Ribeiro, HG. Masteloto, P. Neuenfeldt,CM. Pereira, GM. Siqueira, W. Cunico, *Ultrasonics Sonochem*., **2014**, 21, 1615.
[16] AA. El-Kateb, NM. Abd El-Rahman, TS. Saleh, AS. Elhaddad, AY. El-Dosoky, *Nature and Sci.,* **2012**, 10,77-86.
[17] U. Fathy, AY. Younis, HM. Awad, *J. Chem. and Pharm. Res*., **2015**, 7, 4.
[18] A. El-Kateb, NM. Abd El-Rahman, TS. Saleh, AM. Hassan, AY. El-Dosoky, G. Awad, *J. of Appl. Sci. Res.* **2012**, 8, 3393.
[19] DN. Dhar. Chemistry of Chalcones and Related Compounds; Wiley, New York, **1981**.
[20] NA. Hamdy, MM. Anwar, KM. Abu-Zied, HM. Awad, *Acta Poloniae Pharma. - Drug Res.,* **2013**, 70, 987.
[21] FN. Almajhdi, H. Fouad , KA. Khalil, HM. Awad, SH. Mohamed, T. Elsarnagawy, AM. Albarrag , FF. Al-Jassir, HS. Abdo HS, *J. Mater. Sci.* **2014**, 25, 1.