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EFFICIENT SYNTHESIS OF CHLORO CHALCONES UNDER ULTRASOUND IRRADIATION, THEIR ANTICANCER ACTIVITIES AND MOLECULAR DOCKING STUDIES

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ABSTRACT

Chloro chalcone derivatives **1-5** were synthesized by the conventional and sonochemical method. A significant enhancing effect in reaction time was observed in chalcones synthesis under ultrasound irradiation. Characterization of all chalcones was conducted by GC-MS, FTIR and NMR spectrometers. Anticancer evaluation displayed that chalcone **3** has high activity against breast cancer cell line MCF7 (IC_{50} 0.8 µg/mL), T47D (IC_{50} 0.34 µg/mL), cervical cancer cell line HeLa (IC_{50} 4.78 µg/mL), and colorectal cancer cell line WiDr (IC_{50} 5.98 µg/mL). Docking study revealed the interaction between chalcones and EGFR receptor through hydrogen bonds and π-cation interactions. It was also observed that methoxy substituent on chalcones increased their activity as anticancer.

Keywords: Chalcone, Sonochemical Method, Anticancer, Docking Molecule

INTRODUCTION

Cancer is a class of disease defined by the rapid, uncontrolled and pathological proliferation of abnormal cells[1]. There were up to 18.1 million new cancer cases and 9.6 million cancer deaths estimated in 2018 worldwide. Breast cancer, colorectal cancer, and cervical cancer are some of the dominant cause of cancer deaths[2]. Despite the progress in cancer chemotherapy, the lack of selectivity to hinder the proliferation of cancer cells with the minimum effect on normal cells remains a major problem. Therefore, the discovery of new anticancer agents is an essential purpose in medicinal chemistry.

Chalcones (1,3-diaryl-2-propen-1-ones), which are metabolic precursors of flavonoids and isoflavonoids, are found naturally in fruits and vegetables[3]. Chalcones and their synthetic analogs exhibit wide pharmacological activities, such as anticancer[4], antibacterial[5], antifungal[6], antioxidant[7], antidepressant[8], anti-inflammatory[9] and antimalarial[10]. Chalcones are considered as a promising compound on chemopreventive drugs owing to their properties as an antioxidant, cytotoxic and apoptosis induction[11]. The presence of α,β-unsaturated carbonyl bridge on chalcones is the key factor for their activities[12].

Chalcones, as anticancer agents, inhibit various molecular targets including the EGFR family of receptor tyrosine kinases[13]. Epidermal growth factor receptor (EGFR) family is receptor tyrosine kinases (RTKs) that regulate cellular proliferation, migration, differentiation, and survival. There are four members on EGFR family which are EGFR (HER-1), HER-2, HER-3, and HER-4. Among them, EGFR has been identified as being a significant role in cancer. EGFR overexpression results in the activation of signal transduction pathway that contribute to malignant progression, invasion, and metastasis of numerous types of cancer including those in the breast, lung, ovarian, glioblastomas, colon, head and neck.[14]

Chalcone derivatives containing chloro substituents on ring B were reported to give excellent anticancer activity against several cancer cell lines.[15,16] However, to the best of our knowledge, there is barely any report regarding the effect of chloro chalcones on ring A (Fig.-1). This encouraged us to investigate the

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anticancer activity of chalcones with chloro substituent attached on ring A. Chalcone derivatives containing methoxy were also reported to give excellent anticancer activity against several cancer cell lines.[17,18]
Chalcones are frequently synthesized by base or acid catalyzed aldol condensation reaction between aromatic ketones and aldehydes[19]. However, the acid-catalyzed reaction is undesirable as they mostly need high temperature which drives the formation of side products. On the other hand, the base-catalyzed reactions require longer reaction times and increase the possibility of side reactions occurrences, for instance, degradation and Michael addition[20] under basic media. The usage of ultrasonic irradiation on chalcones synthesis leads to the higher yields, shorter reaction times and/or milder conditions[21,22]. Sonication offers excellent influences on the chemical reactivity by accelerating the reaction, reducing the inducing period and enhancing the catalyst efficiency.[23]

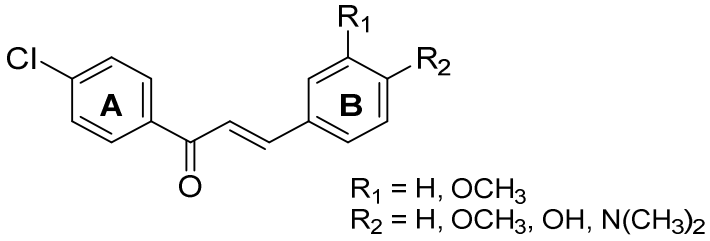

Fig.-1: Chemical Structure of Chalcones 1-5

Given the above facts on the good effect of chloro substituent and the beneficial effects of sonication method, herein we performed a novel study of chalcones **1-5** synthesis by conventional and ultrasonic irradiation methods. Cytotoxicity evaluation of chalcones **1-5** against several cancer cell lines (breast cancer cell lines MCF7 and T47D, cervical cancer cell line HeLa and colorectal cancer cell line WiDr) were conducted as well as their molecular docking study with EGFR receptor.

EXPERIMENTAL

Materials

All starting materials and solvents used were pro analysis grade originated from Merck without further purification, i.e., p-chloroacetophenone, benzaldehyde, p-anisaldehyde, veratraldehyde, vanillin, p-dimethylaminobenzaldehyde, ethanol, methanol, sodium hydroxide, dichloromethane, hexane, hydrochloric acid, chloroform, ethyl acetate and dimethyl sulfoxide. Thin layer chromatography (TLC) was conducted by using aluminum plates 20x20 cm coated by silica gel 60 F_{254} (Merck). Materials used for cytotoxicity evaluation were breast cancer cell line (MCF7 and T47D), cervical cancer cell line (HeLa), colorectal cancer cell line (WiDr), normal cell line (Vero), Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute Medium (RPMI 1640), Medium 199 (M199), HEPES, sodium hydrogen carbonate, Fetal Bovine Serum (FBS), penicillin-streptomycin (Pen-Strep), amphotericin B, trypsin-EDTA solution, phosphate buffer solution (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sodium dodecyl sulfate (SDS).

Instrumentations

All melting points were analyzed by digital melting point apparatus on Electrothermal 9100 (uncorrected). Mass spectra and purity of the products were achieved from GC-MS spectrometer on Shimadzu QP2010S (EI). Infrared spectra were recorded with Shimadzu Prestige-21 using KBr discs. ^{1}H and ^{13}C-NMR spectra were collected on JEOL JNMECA (500 MHz (^{1}H) and 125 MHz (^{13}C)) using TMS as standard internal. The cytotoxicity evaluation were performed using microwell plate 96 (Biologix), micropipette 2-20 µL (VWR brand), micropipette 20-200 µL (VWR brand), micropipette 100-1000 µL (AccuBioTech), laminar air flow (Labconco, Purifier Delta Series Class II), 5% CO_2 incubator (Heraeus), hemocytometer (Neubauer), inverted microscope (Axiovert 25), centrifuge (Janetzki T5) and ELISA reader (BIO-RAD Benchmark).

Synthesis of Chalcones 1-5 Using the Conventional Method

A mixture of aromatic aldehydes (5 mmol) in 10-20 mL absolute ethanol and p-chloroacetophenone (5 mmol) was prepared. Aqueous sodium hydroxide (5 mL, 30-60% w/v) was added dropwise into the

mixture. The reaction was stirred at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice and acidified with HCl 10% (v/v). The formed precipitate was collected using vacuum filtration, washed with cold water and dried in a vacuum desiccator. Purification was done by recrystallization to give target compounds in high yields. The obtained yields are summarized in Table-1.

Synthesis of Chalcones 1-5 Using the Sonochemical Method

A mixture of aromatic aldehydes (5 mmol) in 10-20 mL absolute ethanol and p-chloroacetophenone (5 mmol) was taken into the conical flask in the water bath of an ultrasonic cleaner bath. Aqueous sodium hydroxide (5 mL, 30-60% w/v) was added dropwise into the mixture under ultrasound irradiation. Sonication was continued for 55-250 min. The progress of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice and acidified with HCl 10% (v/v). The formed precipitate was collected using vacuum filtration, washed with cold water and dried in a vacuum desiccator. Purification was done by recrystallization to give target compounds in high yields (Table-1).

Chalcone 1

(*E*)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one as yellowish white crystal was obtained from recrystallization with ethanol, m.p. = 96-97 °C. FTIR (KBr, cm^{-1}): 3055 (C_{sp2}-H str.), 1658 (C=O str.), 1604 & 1489 (Ar C=C str.), 1087 (Ar C-Cl str.), 979 (trans C_{sp2} -H bend.). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7,39 (3H, m, ArH), 7.45 (2H, m, ArH), 7,46 (1H, d, *J* = 16 Hz, C=C**H** trans), 7.62 (2H, m, ArH), 7.79 (1H, d, *J* = 16 Hz, C=C**H** trans), 7.94 (2H, m, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 121.62, 145.53 (C=C), 128.70, 129.12, 129.19, 130.10 (8 CHAr), 130.94 (CHAr), 134.84, 136.65, 139.39 (CAr), 189.34 (C=O). Mass spectrum (EI): m/z 244 (M+2, ^{37}Cl, 20%), 242 (M, ^{35}Cl, 60), 241 (100), 207 (45), 141 (^{37}Cl, 15), 139 (^{35}Cl, 45), 131 (55), 113 (^{37}Cl, 20), 111 (^{35}Cl, 70), 103 (80), 77 (85).

Chalcone 2

(*E*)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one as pale yellow crystal was obtained from recrystallization with ethanol, m.p. = 120-121 °C. FTIR (KBr, cm^{-1}): 3070 (C_{sp2} -H str.), 2939 (C_{sp3}-H str.), 1658 (C=O str.), 1597 & 1512 (Ar C=C str.), 1257 (C-O asymm. str.), 1087 (Ar C-Cl str.), 1033 (C-O sym. str.), 979 (trans C_{sp2} -H bend.). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.86 (3H, s, OCH_3), 6.94 (2H, m, ArH), 7.36 (1H, d, *J* = 15.6 Hz, C=C**H** trans), 7.47, 7.61 (4H, 2m, ArH), 7.79 (1H, d, *J* = 15.55 Hz, C=C**H** trans), 7.95 (2H, m, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 55.58 (CH_3-O), 114.61 (2 CHAr), 119.23, 145.27 (C=C), 127.54 (CAr), 129.01, 129.98, 130.50 (6 CHAr), 136.92, 139.07, 161.99 (CAr), 189.31 (C=O). Mass spectrum (EI): m/z 274 (M+2, ^{37}Cl, 20%), 272 (M, ^{35}Cl, 60), 241 (20), 237 (70), 161 (60), 141 (^{37}Cl, 15), 139 (^{35}Cl, 50), 133 (70), 113 (^{37}Cl, 30), 111 (^{35}Cl, 100), 107 (60).

Chalcone 3

(*E*)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one as light yellow crystal was obtained from recrystallization with ethanol, m.p. =103-104 °C. FTIR (KBr, cm^{-1}): 3070 (C_{sp2}-H str.), 2939 (C_{sp3}-H str.), 1658 (C=O str.), 1589 & 1512 (Ar C=C str.), 1265 (C-O asymm. str.), 1087 (Ar C-Cl str.), 1033 (C-O sym. str.), 987 (trans C_{sp2} -H bend.). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.93, 4.94 (6H, 2s, OCH_3), 6.89, 7.14 (2H, 2d, *J* = 8.4, 1.95 Hz, ArH), 7.23 (1H, dd, *J* = 1.95, 8.45 Hz, ArH), 7.33 (1H, d, *J* = 15.55 Hz, C=C**H** trans), 7.46 (2H, m, ArH), 7.76 (1H, d, *J* = 15.55 Hz, C=C**H** trans), 7.95 (2H, m, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 56.21, 56.18 (CH_3-O), 110.27, 111.29, 123.51 (CHAr), 119.60, 145.71 (C=C), 127.83, 136.93, 139.12, 149.44, 151.78 (CAr), 129.04, 130.01 (4 CHAr), 189.41 (C=O). Mass spectrum (EI): m/z 304 (M+2, ^{37}Cl, 10%), 302 (M, ^{35}Cl, 30), 287 (10), 271 (15), 259 (5), 191 (10), 141 (^{37}Cl, 10), 139 (^{35}Cl, 50), 113 (^{37}Cl, 30), 111 (^{35}Cl, 100).

Chalcone 4

(*E*)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one as yellow crystal was obtained from recrystallization with methanol-water, m.p. = 81-83 °C. FTIR (KBr, cm^{-1}): 3425 (O-H str.), 3070 (C_{sp2}-H str.), 2924 (C_{sp3}-H str.), 1651 (C=O str.), 1589 & 1519 (Ar C=C str.), 1280 (C-O asymm. str.), 1087

(Ar C-Cl str.), 1033 (C-O sym. str.), 979 (trans C_{sp2} -H bend.). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.96 (3H, 1s, OCH_3), 5.95 (1H, s, OH), 6.96, 7.12 (2H, 2d, *J* = 7.8, 1.9 Hz, ArH), 7.22 (1H, dd, *J* = 1.6, 8.1 Hz, ArH), 7.32 (1H, d, *J* = 15.6 Hz, C=C**H** trans), 7.47 (2H, m, ArH), 7.75 (1H, d, *J* = 15.55 Hz, C=C**H** trans), 7.95 (2H, m, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 56.21 (CH_3-O), 110.27, 115.12, 123.70 (CHAr), 119.34, 145.96 (C=C), 127.46, 136.95, 139.14, 147.02, 148.68 (CAr), 129.05, 130.03 (4 CHAr), 189.52 (C=O). Mass spectrum (EI): m/z 290 (M+2, ^{37}Cl, 30%), 288 (M, ^{35}Cl, 90), 271 (30), 253 (75), 177 (30), 145 (60), 141 (^{37}Cl, 20), 139 (^{35}Cl, 65), 124 (45), 113 (^{37}Cl, 30), 111 (^{35}Cl, 100).

Chalcone 5

(*E*)-1-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one as yellow crystal was obtained from recrystallization with ethanol, m.p. = 138-140 °C. FTIR (KBr, cm^{-1}): 3070 (C_{sp2}-H str.), 2924 (C_{sp3}-H str.), 1651 (C=O str.), 1581 & 1527 (Ar C=C str.), 1342 (Ar C-N str.), 1188 (C-N str.), 1087 (Ar C-Cl str.), 979 (trans C_{sp2} -H bend.). ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.05 (6H, s, CH_3), 6.69 (2H, m, ArH), 7.27 (1H, d, *J* = 15.6 Hz, C=C**H** trans), 7.45, 7.54 (4H, 2m, ArH), 7.79 (1H, d, *J* = 15.55 Hz, C=C**H** trans), 7.94 (2H, m, ArH). ^{13}C-NMR (125 MHz, $CDCl_3$, ppm): δ 40.28 (2 CH_3-N), 111.99 (CHAr), 116.42, 146.53 (C=C), 122.63, 137.59, 138.60, 152.35 (CAr), 128.90, 129.90, 120.71 (6 CHAr), 189.43 (C=O). Mass spectrum (EI): m/z 287 (M+2, ^{37}Cl, 25%), 285 (M, ^{35}Cl, 75), 250 (20), 174 (50), 146 (50), 141 (^{37}Cl, 10), 139 (^{35}Cl, 30), 121 (50), 113 (^{37}Cl, 30), 111 (^{35}Cl, 100).

Table-1: The Results of Chalcones Synthesis by Conventional and Sonochemical Methods

Entry	R_1	R_2	Molecular Formula	Conventional Method		Sonochemical Method		Melting Point (°C)
				Time (h)	Yield (%)	Time (h)	Yield (%)	
1	H	H	$C_{15}H_{11}OCl$	26.5	90.91	4.2	93.39	96-97
2	H	OCH_3	$C_{16}H_{13}O_2Cl$	4.2	97.06	1.3	96.32	120-121
3	OCH_3	OCH_3	$C_{17}H_{15}O_3Cl$	4.0	96.03	1.1	96.69	103-104
4	OCH_3	OH	$C_{16}H_{13}O_3Cl$	24.0	53.47	2.5	47.22	81-83
5	H	$N(CH_3)_2$	$C_{17}H_{16}ONCl$	1.75	93.01	0.9	90.21	138-140

Cytotoxicity Evaluation

All cell lines were cultured in 5% CO_2 water-saturated atmosphere at 37 °C with medium DMEM/10% FBS and RPMI/10% FBS for cancer cell lines (MCF7, T47D, HeLa, WiDr) and M199/10% FBS for normal cell line (Vero). Cell suspensions (10^6/mL) were prepared and 100 µL/well dispensed into 96-well plate giving 10^4 cells/well. The plates were returned to the incubator for 24 h to allow cells to reattach. Chalcones were initially prepared at 10^5 µg/mL in DMSO. These samples were diluted into culture medium giving 6 serial concentration: 200, 100, 50, 25, 12.5, 6.25, 3.125 µg/mL. Aliquots (100 µL) of each concentration were added to the wells. After further incubation for 24 h, the cell viability was assessed by MTT assay. The culture medium on plates was removed and washed with PBS. A solution of MTT in PBS was prepared at 50 mg/10mL. Aliquots (1 mL) of MTT solution were diluted with 9.5 mL culture medium. Aliquots (100 µL) of diluted MTT was added to the wells and incubated for 4 h. A total of 100 µL SDS stopper 10% in 0.1 N HCl was added into each well and left overnight. Absorbance readings were performed by ELISA reader at 595 nm. IC_{50} values were calculated.

Molecular Docking Study

The three-dimensional structure of chalcone **1-5** was drawn using GaussView 5.0.8 and optimized using Gaussian 09 [24] with DFT/B3LYP method and 6-31G basis set. The three-dimensional crystal structure of EGFR domain bound to Erlotinib was acquired from the protein data bank (PDB ID: 1M17). Preparation of ligand and protein was conducted using UCSF Chimera. Redocking and docking were performed with Autodock Tools[25] and Autodock 4 in grid box of 45 x 45 x 45 Å with a spacing of 0.375 Å using a Lamarckian Genetic Algorithm (LGA). The redocking analysis was successfully performed when the

RMSD value was less than 2 Å [26]. Ten molecular docking poses for each ligand were ranked based on their docking score. The scoring function in AutoDock was used to predict the binding affinity of the ligand to the receptor. The conformation with the lowest binding energy was chosen as the most suitable conformation.

RESULTS AND DISCUSSION

Chemistry

The synthesis of chloro chalcones **1-5** was conducted by a base-catalyzed aldol condensation reaction using sodium hydroxide between p-chloroacetophenone and several aromatic aldehydes (Fig.-2).

The conventional method was carried out according to literature[27] with slight modification. The synthesis result using both conventional and sonochemical method were collected in Table-1. The reaction times were determined by TLC to monitor the consumption of starting materials. It was observed that the reaction times by sonochemical method was shorter than the conventional ones. The conventional method required 1.75-26.5 h for completion of the reaction, while the sonochemical method took 0.9-4.2 h (55-250 min). It was evident that the acoustic energy made a great impact on reducing the processing time. It was also observed that the presence of electron-donating groups on aldehydes shortened the time of reaction (compare chalcone **1** with **2**, **3**, **4**, or **5**).

Elucidation of all chalcone structures was conducted by MS, FTIR and NMR spectrometers. Mass spectra approved the molecular weight of the desired chalcones as well as their characteristic molecular ion peaks (M^+ and M+2) with the height ratio of 3:1 indicating the presence of two chlorine isotopes (^{35}Cl and ^{37}Cl). The synthesis of chalcones **1-5** via Claisen-Schmidt condensation formed E-configuration on their C=C bond as proved by IR and ^{1}H-NMR spectroscopy. The IR spectra displayed the absorption bands at 979 cm^{-1} for C_{sp2}–H bending band corresponding to trans-disubstituted alkene[28]. The ^{1}H-NMR spectra showed two doublet signals at 7.2-7.8 ppm with coupling constant (*J*) value of 15.55 Hz for trans alkene protons[29].

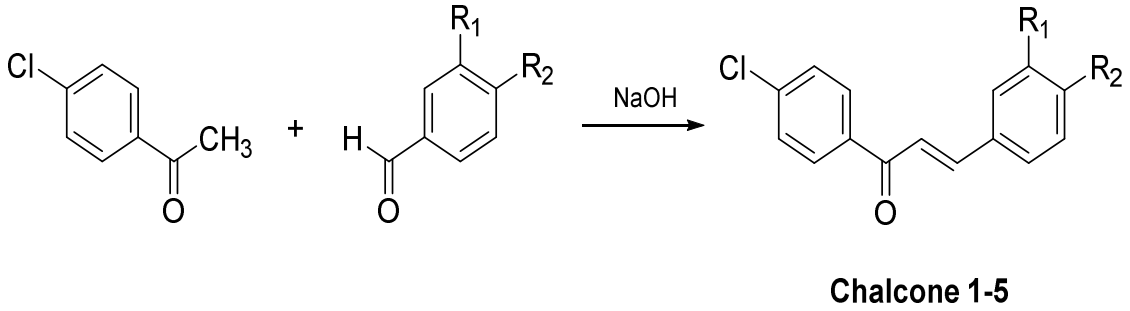

Fig.-2: General Synthesis of Chalcones **1-5**

Cytotoxicity Evaluation

Cytotoxicity evaluation of the synthesized chalcones **1-5** and several commercial drugs was accomplished by MTT method against breast cancer cell lines (MCF7 and T47D), cervical cancer cell line (HeLa), colorectal cancer cell line (WiDr) and normal cell line (Vero). Three different commercial drugs were used for different type of cancer. Doxorubicin was used for breast cancer treatment caused by MCF7 [30] and T47D [31], cisplatin was used for cervical cancer caused by HeLa [32], and 5-fluorouracil was used for colorectal cancer caused by Widr [33].

The IC_{50} values and selectivity index of all tested sample are presented in Table-2. Compounds activity on their cell growth inhibition was reported to be classified into three categories, i.e., active (IC_{50}<20 µg/mL), moderate (IC_{50} 20-100 µg/mL), and inactive (IC_{50} >100 µg/mL)[34]. According to that, it can be said that chalcone **1** is inactive in the inhibition of all tested cancer cell lines. Chalcone **2** has moderate activity against the tested cancer cell lines except for T47D. Chalcone **3** and **4** have high activity against all tested cancer cell lines. Chalcone **5** has moderate activity against HeLa, but inactive against the other cell lines. The best compound to inhibit breast cancer cell lines (MCF7 and T47D) is chalcone **3**, while the HeLa and WiDr cell lines are highly inhibited by chalcone **4**.

Table-2: IC_{50} Values and Selectivity Index of Chalcones **1-5**

Entry	IC_{50} (µg/mL)						SI			
	MCF7	T47D	HeLa	WiDr	Vero		MCF7	T47D	HeLa	WiDr
1	>100	>100	>100	>100	>100		>100	>100	>100	>100
2	99.51	>100	24.97	24.73	>100		>100	>100	>100	>100
3	0.80	0.34	4.78	5.98	>100		>100	>100	>100	>100
4	7.58	5.61	3.34	4.25	7.03		0.93	1.25	2.10	1.65
5	>100	>100	87.37	>100	>100		52.94	14.77	>100	>100
Doxorubicin	3.04	10.58	-	-	-		-	-	-	-
Cisplatin	-	-	18.39	-	-		-	-	-	-
5- Fluorouracil	-	-	-	25.57	-		-	-	-	-

The index obtained by dividing the IC_{50} values against Vero cell line by the values of each cancer cell lines is considered to be compounds selectivity. It was said that compound with ratio values higher than 6 indicate high selectivity, ratio values between 3-6 indicate moderate selectivity, while ratios values lower than 3 are nonselective[35]. In this context, chalcone **4** is considered to be nonselective to normal cell line despite having a broad spectrum of anticancer properties against all tested cancer cell lines with high activity. While chalcones **1**,**2**,**3** and **5** are considered to have high selectivity toward normal cell line. Based on this result, chalcone **3** is the most potent anticancer agent compare to the other synthesized chalcones. Moreover, compared to other studies that are reporting anticancer activity of non-chloro chalcone derivatives[36,37], the synthesized chloro chalcone **3** on this study also has better IC_{50} values. This finding gave an understanding that the modification on chloro gives positive effect to the anticancer activities.

Docking Study

Molecular docking study was performed in order to have an understanding of how chalcones **1-5** interact with the receptor. EGFR receptor was used since this protein is overexpressed in many types of cancer[14]. The redocking analysis of EGFR with the original ligand (Erlotinib) showed its binding site on MET769 residue. This binding site was then used to perform docking simulation of chalcone compounds. The binding models of chalcones with EGFR is shown in Fig.-3.

Chalcone **3** is nicely bound to EGFR receptor via two hydrogen bonds. The value of its binding energy is -7.5 kcal/mol. The two hydrogen bondings are formed between the oxygen atom of the carbonyl with MET769 residue (distance: 1.755 Å) and oxygen atom on *p*-OCH_3 with LYS721 (distance: 2.035 Å). It can be said that these interactions play an important factor in their anticancer activity. For further understanding, we also performed a docking simulation for the rest of the chalcones. Chalcone **1** is poorly bound to EGFR with a binding energy of -6.74 kcal/mol via one hydrogen bonding formed between the oxygen atom of the carbonyl with MET769 residue (distance: 1.919 Å). Chalcone **2** is bound to EGFR with a binding energy of -7.23 kcal/mol via one hydrogen bonding between the oxygen atom of the carbonyl with LYS828 (distance: 1.855 Å) and two π-cation interactions with LYS721. Chalcone **4** is bound to EGFR with a binding energy of -7.49 kcal/mol via two hydrogen bonds formed between the oxygen atom of the carbonyl with MET769 (distance: 1.977 Å) and the oxygen atom of *m*-OCH_3 with LYS721 (distance: 2.081 Å). Chalcone **5** is bound to EGFR with a binding energy of -7.17 kcal/mol via one hydrogen bond formed between the oxygen atom of the carbonyl with MET769 (distance: 1.849 Å). This molecular docking results of chalcones are consistent with their cytotoxicity evaluation data. It is found that the carbonyl group on chalcones is the key factor responsible for their activity. Moreover, the addition of methoxy substituent on chalcones can greatly increase their anticancer activity. Meanwhile, chloro substituent on ring A of all chalcones has no effect on their activity as there is no interaction between chloro and EGFR receptor.

CONCLUSION

The series of chloro chalcones **1-5** were successfully synthesized via base-catalyzed aldol condensation reaction at room temperature. The sonochemical method of chalcones synthesis provides significant benefits over the conventional method with respect to reaction time and yield. Cytotoxicity evaluation

revealed that chalcone **3** has the best anticancer activity against all tested cancer cell lines and the best selectivity toward normal cell line. Docking study showed that carbonyl group influences the anticancer potential and the presence of the methoxy group can enhance the activity.

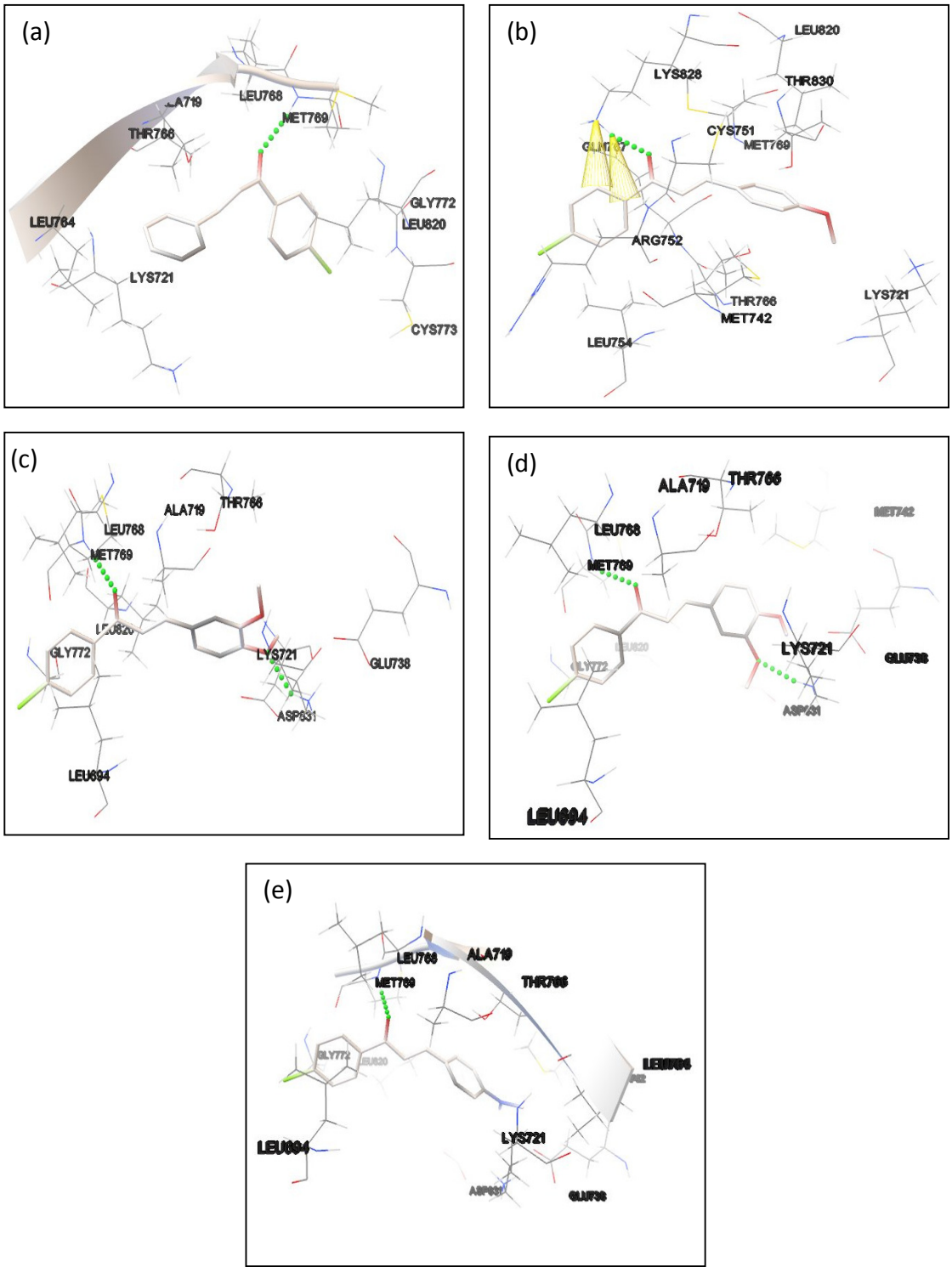

Fig.-3: Molecular Docking Result of EGFR Receptor with (a) Chalcone **1**, (b) Chalcone **2**, (c) Chalcone **3**, (d) Chalcone **4**, and (e) Chalcone **5**. The H-Bonds are Displayed as Green Dotted Lines. The π-Cation Interactions are Displayed as Yellow Lines.

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