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Synthesis, characterization, and cytotoxic activities of heterocyclic chalcones containing furan, and crystal structure of 1-(4-iodophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one

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ABSTRACT

A series of heterocyclic chalcone (**3a–e**, **5**, **7**) were synthesized and characterized by Infrared, ^{1}H and ^{13}C nuclear magnetic resonance, and mass spectra. Crystal structure of 1-(4-iodophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one was determined using single crystal X-ray diffraction. All the synthesized compounds were evaluated for their cytotoxic activities on MDA MB 231 and CHO cell by using MTT assay. Compound **3c** showed good cytotoxic effect on MDA MB 231 with IC_{50} values of 9.8 μg/mL.

KEYWORDS

Synthesis; breast cancer; crystal structure; cytotoxicity; heterocyclic chalcone

Introduction

Cancer is the second cause of human death in the world after cardiovascular diseases [1]. It reoccurs due to the presence of malignant tumor in the body with an ability to invade and spread to other tissues [2]. Breast cancer, for example, is one of the most common cancers among women [3, 4] and the first of the most frequent in Malaysia followed by colorectal and lung cancers [5]. There are various types of treatments, such as chemotherapy, radiotherapy, hormone therapy, surgery [6], and hyperthermia [7], offered to patients diagnosed with cancer. However, some of the treatments resulted with undesirable side effects to patients such as nausea, vomiting, poor oral intake, cachexia, and lethargy [8].

Bacterial infections are other types of health problems infect humans around the world [9]. The emergence of antibiotics to fight the microbes and other bacteria has resulted in the presence of pathogens that are resistant to antibiotics. The presence of drugs, such as amoxicillin, norfloxacin, and ciprofloxacin, can help to fight against the bacterial infection. Nevertheless, these drugs have side effects such as dizziness, nausea, hypertension, et al. [9, 20].

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Crystallographic data for compound **3e** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (CCDC) with CCDC number 1409270.

Therefore, chalcone can be one of the compounds that can act in tumor cell and fight against bacteria as it exhibit a broad range of pharmacological activities such as antibacterial, anticancer, antifungal, anti-inflammatory, antioxidant, et al. [10]. Secondary metabolites [11] are found abundantly in nature from fern to higher plants. The presence of α,β-unsaturated carbonyl group in its compound is the indication of the formation of chalcone from two aromatic rings, and the removal of this group could obstruct its various pharmacological activities [8].

Based on its diversity of biological activities, chalcone has attracted our attention to synthesize instead of isolating it from natural sources, as the extraction method is time-consuming and only small amount of product is formed. Previous study carried out by Solomon and Lee [4] reported that heteroarylchalcone showed an improved anti-proliferative activity on cancer cells than the commonly prescribed cisplatin. Encouraged by this, a series of heterocyclic chalconeanalogues were synthesized by focusing on the heterocyclic electron-rich oxygen with different substituents of phenyl ring. All the compounds were tested for their ability to act as antibacterial and anticancer agents to develop a novel therapeutic agent for better treatment of cancer and fight bacterial infections.

Materials and methods

5-Methylfurfural (99%), 4-chloroacetophenone (97%), 4-fluoroacetophenone (99%), 2-acetylpyrazine (≥99%), 4-acetylpyridine (97%), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reagent, and pyridine (>99%, Fluka) were purchased from Sigma, Subang Jaya, Selangor, Malaysia. Chemical reagents, 4-bromoacetophenone (98%, Acros Organic) and 4-iodoacetophenone (98%, Acros Organic) were purchased from Fischer Scientific, Shah Alam, Selangor, Malaysia. Benzaldehyde (>99%) and dimethlyamine were bought from Merck, Malaysia. Sodium hydroxide pellets, potassium hydroxide pellets, and hydrochloric acid (37%) were of analytical grade and obtained from Qrec, Rawang, Selangor, Malaysia. Melting points (uncorrected) were determined using a Barnstead Electrothermal 9100 melting point apparatus. Infrared (IR) spectra were recorded on a Perkin-Elmer Universal ATR spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were performed on BrukerAvance spectrometer at 400 MHz. The chemical shifts were recorded in parts per million (δ) in deuterochloroform solution. Results of mass spectra (MS) were obtained from NUS Mass Spectrometry Service, Singapore. Reactions were monitored using precoated silica gel 60 F_{254} TLC plates and observed under ultraviolet $(UV)_{254}$ light.

Synthesis of chalcones (3a–e)

A mixture of 5-methyl-2-furaldehyde (21.72 mmol) with substituted acetophenone (21.72 mmol) was dissolved in absolute ethanol (20 mL). Slowly, aqueous NaOH solution (4.3 mL, 2%) was added drop-wise [12] at 0°C and stirred for 24 hr at 25°C. After the completion of reaction was tested on pre-coated TLC plates, ice water (100 mL) was added and neutralized with 10% HCl. The precipitate formed was collected by vacuum filtration, washed with cold water, dried, and recrystallized using ethanol.

3-(5-Methylfuran-2-yl)-1-phenylprop-2-en-1-one (3a) [12]

Bright yellow powder (70.1% yield), m.p. 64.1–66.0°C, IR (cm−1): 3125 (C-H aromatic stretching), 2920 (C-H aliphatic), 1653 (C=O), 1586 (C=C olefinic), 1551 and 1444 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.42 (3H, s, CH3-6′), 6.16 (^{1}H, *dd*, $J = 0.8$ and 3.2 Hz, H-4′), 6.65 (^{1}H, *d*, $J = 3.2$ Hz, H-3′), 7.39 (^{1}H, *d*, $J = 15.2$ Hz, H-2), 7.50 (3H, *m*, phenyl-H), 7.55 (^{1}H, *d*, $J = 15.2$ Hz, H-3), 8.05 (2H, *m*, phenyl-H).

1-(4-Fluorophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (3b) [12]

Yellow needles (88.6% yield), m.p. 65.6–68.9°C, IR (cm−1): 3097 (C-H aromatic), 2924 (C-H aliphatic), 1657 (C=O), 1601 (C=C olefinic), 1563 and 1504 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.42 (3H, s, CH3-6′), 6.16 (^{1}H, *dd, J* = 0.8, and 3.2 Hz, H-4′), 6.66 (^{1}H, *d, J* = 3.2 Hz, H-3′), 7.16 (2H, *m*, phenyl-H), 7.35 (^{1}H, *d, J* = 15.2 Hz, H-2), 7.55 (^{1}H, *d, J* = 15.2 Hz, H-3), 8.07 (2H, *m*, phenyl-H).

1-(4-Chlorophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (3c) [12]

Yellow crystals (85.3% yield), m.p. 104.7–107.8°C, IR (cm−1): 3113 (C-H aromatic), 2921 (C-H aliphatic), 1655 (C=O), 1597 (C=C olefinic), 1562 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.42 (3H, s, CH3-6′), 6.16 (^{1}H, *dd, J* = 0.8 and 3.2 Hz, H-4′), 6.67 (^{1}H, *d, J* = 3.2 Hz, H-3′), 7.33 (^{1}H, *d, J* = 15.2 Hz, H-2), 7.48 (2H, br *d, J* = 8.4 Hz, H-3″, H-5″), 7.55 (^{1}H, *d, J* = 15.2 Hz, H-3), 7.99 (2H, br *d, J* = 8.4 Hz, H-2″, H-6″).

1-(4-Bromophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (3d) [12]

Yellow crystal (90% yield), m.p. 109.6–111.9°C, IR (cm−1): 3110 (C-H aromatic), 2921 (C-H aliphatic), 1654 (C=O), 1598 (C=C olefinic), 1560 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.42 (3H, s, CH3-6′), 6.16 (^{1}H, *dd, J* = 0.8 and 3.2 Hz, H-4′), 6.67 (^{1}H, *d, J* = 3.2 Hz, H-3′), 7.32 (^{1}H, *d, J* = 15.2 Hz, H-2), 7.55 (^{1}H, *d, J* = 15.2 Hz, H-3), 7.64 (2H, br *d, J* = 8.4 Hz, H-3″, H-5″), 7.91 (2H, br *d, J* = 8.4 Hz, H-2″, H-6″).

1-(4-iodophenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one (3e)

Orange crystal (71.7% yield), m.p. 101.8–103.0°C, IR (cm−1): 3108 (C-H aromatic), 2917 (C-H aliphatic), 1651 (C=O), 1599 (C=C olefinic), 1559 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.42 (3H, s, CH3-6′), 6.16 (^{1}H, *dd, J* = 0.8 and 3.2 Hz, H-4′), 6.67 (^{1}H, *d, J* = 3.2 Hz, H-3′), 7.32 (^{1}H, *d, J* = 15.2 Hz, H-2), 7.55 (^{1}H, *d, J* = 15.2 Hz, H-3), 7.75 (2H, br *d, J* = 8.4 Hz, H-3″, H-5″), and 7.86 (2H, br *d, J* = 8.4 Hz, H-2″, H-6″). 13C NMR (100 MHz, CDCl3): δ 14.04 (C-6′), 100.32 (C-1″), 109.52 (C-4′), 116.82 (C-3), 118.76 (C-3′), 129.83 (C-3″, C-5″), 131.25 (C-2), 137.84 (C-2″, C-6″), 150.26 (C-2′), 156.22 (C-5′), 188.98 (C=O). Found (ESI): 338.9876 [M+ H+], (C14H12IO2 requires 338.9882).

Synthesis of 3-(5-methylfuran-2-yl)-1-(pyrazin-2-yl)prop-2-en-1-one (5) [13]

To a solution of 5-methyl-2-furaldehyde (24.59 mmol) and 2-acetylpyrazine (24.59 mmol) was added 20 mL of ethanol. The mixture was dissolved first before addition of 2% ethanolic KOH (9.63 mL) at 0°C and stirred for 24 hr at 25°C. After the completion of reaction was monitored by TLC, crushed ice was added to the reaction mixture. The precipitate formed was filtered, washed with cold water, dried, and recrystallized by absolute ethanol.

3-(5-methylfuran-2-yl)-1-(pyrazin-2-yl)prop-2-en-1-one (5)

Yellow crystal (77.4% yield) m.p. 113.4–115.7°C, IR (cm−1): 3104 (C-H aromatic), 2923 (C-H aliphatic), 1662 (C=O), 1598 (C=C olefinic), 1558 and 1518 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): Table 1); 13C NMR (100 MHz, CDCl3): Table 1; Found (ESI): 215.0815 [M+ H+], (C12H11N2O2 requires 215.0821).

Synthesis of 3-(5-methylfuran-2-yl)-1-(pyridine-4-yl)prop-2-en-1-one (7) [14]

5-Methyl-2-furaldehyde (8.26 mmol) was dissolved in 2 mL of pyridine before addition of 4-acetylpyridine (4.13 mmol) and dimethylamine (8.26 mmol). The reaction mixture was stirred at room temperature for 24 hr. After the completion of reaction was tested by TLC,

it was poured into 100 mL of water and kept at 4°C overnight. The solid formed was filtered, washed with cold water, dried, and recrystallized using ethanol.

3-(5-Methylfuran-2-yl)-1-(pyridine-4-yl)prop-2-en-1-one (7)

Black solid (73.9% yield), m.p. 103.5–107.3°C, IR (cm−1): 3116 (C-H aromatic), 2914 (C-H aliphatic), 1661 (C=O), 1597 (C=C olefinic), 1551 (C=C aromatic). ^{1}H NMR (400 MHz, CDCl3): δ 2.44 (3H, s, CH3-6′), 6.20 (^{1}H, *dd, J* = 0.4 and 3.2 Hz, H-4′), 6.75 (^{1}H, *d, J* = 3.2 Hz, H-3′), 7.28 (^{1}H, *d, J* = 15.2 Hz, H-2), 7.58 (^{1}H, *d, J* = 15.2 Hz, H-3), 7.88 (2H, *d, J* = 6.4 Hz, H-2″, H-6″), 8.85 (2H, *d, J* = 6.4 Hz, H-3″, H-5″). 13C NMR (100 MHz, CDCl3): δ 14.05 (C-6′), 109.79 (C-4′), 116.48 (C-3), 119.71 (C-3′), 121.43 (C-2″, C-6″), 132.25 (C-2), 144.65 (C-2″), 150.01 (C-3″, C-5″), 150.72 (C-2′), 156.85 (C-5′), 189.01 (C=O). Found (ESI): 214.0863 [M+H+], (C13H12NO2 requires 214.0868).

Cell lines

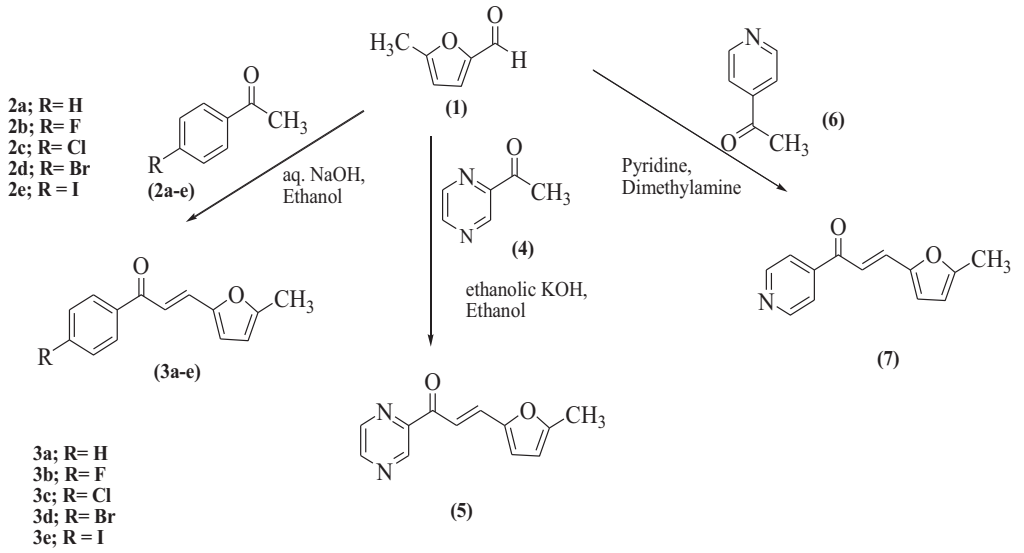
The human breast cancer cell (MDA MB 231) and Chinese Hamster Ovary (CHO) cell were obtained from Tissue Engineering Laboratory, Faculty of Bioscience and Medical Engineering, UTM as a gift from Dr. Salehhuddin Hamdan. MDA MB 231 cell was cultured in Dulbecco's Modified Eagle Medium (DMEM), and CHO cell was cultured in Roswell Park Media Institute (RPMI 1640). Both media were supplemented with 10% fetal bovine serum (FBS), 100 IU/mL of penicillin, and 100 µg/mL of streptomycin (GIBCO, Bio-Diagnostics, Petaling Jaya, Selangor, Malaysia). The cells were maintained at 37°C in 5% CO_2 (Panasonic, model MCO-175 series).

MTT assay

The viability of cell was carried out using MTT assay. MDA MB 231 and CHO cells were cultured in 96-well plates with a seeding density of 5×10^4 cells/mL, and were allowed to stay for 24 hr in CO_2 incubator. All chalcones were dissolved in dimethyl sulfoxide (DMSO) and diluted with different concentrations ranging from 0.39 to 50 µg/mL. The final percentage of DMSO used did not exceed 0.1%. Each sample, 100 µL, was added into 96-well plate, and incubated for 72 hr. The cells were then treated with 20 µL of MTT reagent (5 mg/1 mL in phosphate buffer solution (PBS)) into each well and incubated for an additional 4 hr. Buffer solution, 225 µL (10% 0.1 M HCl and 90% isopropanol), was added in each well to solubilize the formation of purple formazan produce by viable cells. The amount of formazan produced was measured at 575 nm and 570 nm (as reference) recorded by microplate reader (BioTek Instruments Inc., model Epoch, USA) [5, 16].

X-ray crystallography

X-ray crystallography analysis was performed to provide important information for future structure–activity relationship studies as a compliment to NMR results. However, from the seven studied chalcone derivatives, we were able to obtain a good crystal quality suitable for X-ray crystallography analysis for compound **3e** only. X-ray diffraction data for compound **3e** were collected from single crystal by using a Bruker APEX DUO CCD diffractometer with a graphite monochromatic Mo-K_α radiation at a detector distance of 5 cm with APEX2 software [17]. The collected data were reduced by using SAINT program and the empirical absorption corrections were performed with the SADABS program [17]. The structure of compound **3e**



Scheme 1. Route of synthesis for the preparation of chalcone derivatives.

was solved by direct methods and was refined using a full-matrix least-squares method on F^2 using the SHELXTL program [18]. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in calculated positions with C–H = 0.95–0.98 Å and refined using a riding model with $U_{iso}(H) = 1.2\ U_{eq}(C)$ and $U_{iso}(H) = 1.5\ U_{eq}$(C-methyl). A rotating group model was used for methyl groups. The final refinement converged well. Materials for publication were prepared by using SHELXTL [18] and PLATON [19].

Results and discussion

Chemistry

In the current study, seven chalcone derivatives (**3a–3e**, **5**, **and 7**) were successfully synthesized in high yield under the Claisen–Schmidt's condensation by reaction between 5-methyl-2-furaldehyde and different types of substituents of aromatic ring using basic conditions as depicted in Scheme 1. The confirmations of the structure of compounds were based on their spectral and physical data. Compound **5** is a new compound, synthesized from

Table 1. ^{1}H and ^{13}C NMR data for compound **5**.

Position	δ (H)	δ (C)	COSY	HMBC
1		188.27		
2	7.93 (d, J = 15.6 Hz)	116.04	H-3	C-2′, C-3, C-1
3	7.68 (d, J = 15.6 Hz)	131.54	H-2	C-2′, C-1
2′	-	150.53		
3′	6.75 (d, J = 3.2 Hz)	119.40	H-4′	C-4′, C-2′, C-5′
4′	6.18 (dd, J = 0.8 and 3.2 Hz)	109.68	H-3′	C-3′, C-2′, C-5′
5′	-	156.78		
6′	2.43	14.06		C-4′, C-5′
2″		148.83		
3″	9.38 (d, J = 1.6 Hz)	144.73		C-2′
5″	8.72 (dd, J = 1.6 and 2.4 Hz)	143.34		C-6′
6″	8.78 (d, J = 2.4 Hz)	147.15		C-5′

δ: ppm

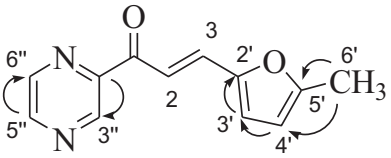


Figure 1. HMBC experiment for compound **5**.

2-acetylpyrazine and 5-methyl-2-furaldehyde under basic conditions. This compound was characterized by IR, ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS.

The IR spectrum for compound **5** showed two stretching bands at 3104 and 2923 cm^{-1}, which were attributed for C-H sp^2 aromatic and sp^3 of methyl group respectively. The absorption band at 1662 cm^{-1} was assigned for carbonyl group, which indicated the formation of chalcone. This band was slightly shifted to a lower frequency due to the conjugation effect with the presence of aryl group and also α,β double bond on enone linkage at 1598 cm^{-1} [9]. The stretching frequency for C=C aromatic was detected at 1558 and 1518 cm^{-1}, while for C-H vinyl, it was observed at 1015 cm^{-1}. These absorption bands were present in compounds **3a–e** and **7** as they all have the same skeleton.

The condensation reaction for the formation of chalcone was proved successful by ^{1}H NMR spectrum. Compound **5** exhibited two doublet signals at δ 7.68 (d, $J = 15.6$ Hz) and δ 7.93 (d, $J = 15.6$ Hz), which were assignable for H-2 and H-3 respectively. The presence of these two signals gave evidence of the presence of ethylene moiety in the enone linkage in trans-conformation. The signal for the carbonyl group could be found farther downfield at δ 188.27. These signals can also be found in other synthesized compounds (**3a–e** and **7**).

The data from ^{1}H NMR were supported with ^{13}C NMR as it showed the presence of 12 carbon atoms (Table 1), which were represented by one methyl, three quaternary carbon atoms, four methane carbons, three unequivalent carbon atoms of aromatic rings, and one carbonyl carbon. The heteronuclear multiple quantum correlation (HMQC) data showed correlation between proton and its carbon atoms. In this experiment, ethylene group, as a significant indication of chalcone formation, showed correlation between 7.68 and 131.54, and 7.93 and 116.04, while the connection observed between 6.18 and 109.68, and 6.75 and 119.40 were assigned for two methane carbons in the furan ring. The confirmation for the position such as quaternary carbon atoms could be observed through the heteronuclear multiple bond correlation (HMBC) experiment (Figure 1), which showed correlation of connected bonds between proton and carbon separated by two or three bonds. The results from MS (ESI) gave evidence of the formation of targeted heterocyclic compound with $m/z = 215.0815$, matched with its molecular formula $C_{12}H_{10}O_2N_2$.

Table 2. Cytotoxic activity of heterocyclic chalcone derivatives on cancer and non-cancer cells.

Compounds[a]	*R*	IC_{50} (μg/mL)[b]	
		CHO	MDA MB 231
3a	Phenyl	15.0	14.8
3b	4-FPh	17.2	16.2
3c	4-ClPh	14.5	9.8
3d	4-BrPh	13.5	16.2
3e	4-IPh	15.0	20.0
5	4-pyrazyl	23.0	19.0
7	4-pyridyl	22.5	16.2

[a] The structure of these compounds can be referred in Scheme 1.
[b] IC_{50}: growth inhibition.

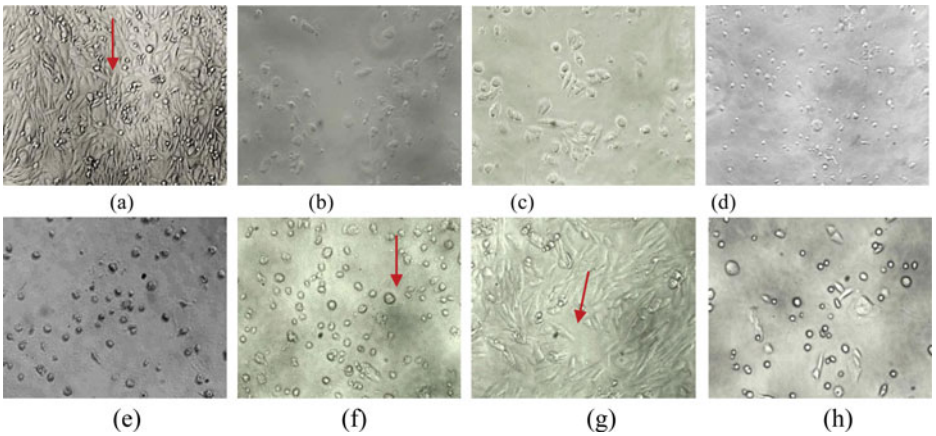


Figure 2. Morphologic changes in CHO cell on treatment with heterocyclic chalcone: (a) cell without treatment; (b) **3a**; (c) **3b**; (d) **3c**; (e) **3d**; (f) **3e**; (g) **5**; (h) **7**, based on their IC_{50} values at 72 hr. The red arrow in the picture of cells showed the morphology of cell.

Heterocyclic chalcone **7** was synthesized first by using the same basic condition as used for compounds **3a–e**. However, this condition has resulted in the failure of chalcone formation. It was in agreement with Szües et. al. [14], who synthesized this chalcone using other acid and basic condition but still had no improvement, and therefore they replaced the reagent with diethylamine, which acted as a catalyst. The basicity of diethylamine is sufficient to form the desired chalcone and avoid the formation of a complex product. However, in this study, we use dimethylamine instead of diethylamine. Although its basicity is slightly lower than diethylamine, changing the catalyst could still give the desired chalcone.

Cytotoxicity study of heterocyclic chalcones

Each synthesized compound was tested for its toxicity effects against breast cancer cell line (MDA MB 231) and Chinese hamster ovary (CHO) cell as a positive control cell group. The toxicity of the investigated compounds was expressed in IC_{50} values as summarized in Table 2.

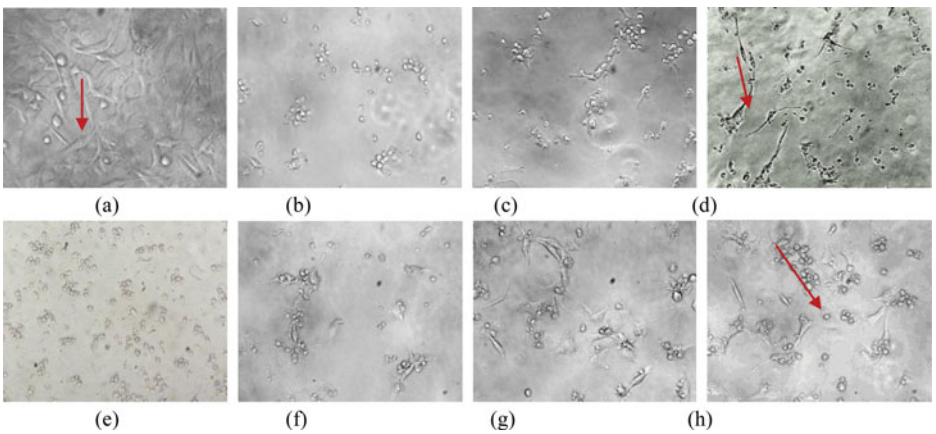


Figure 3. Morphologic changes in MDA MB 231 cell on treatment with heterocyclic chalcone: (a) cell without treatment; (b) **3a**; (c) **3b**; (d) **3c**; (e) **3d**; (f) **3e**; (g) **5**; (h) **7**, based on their IC_{50} values. The red arrow in the picture of cells showed the morphology of cell.

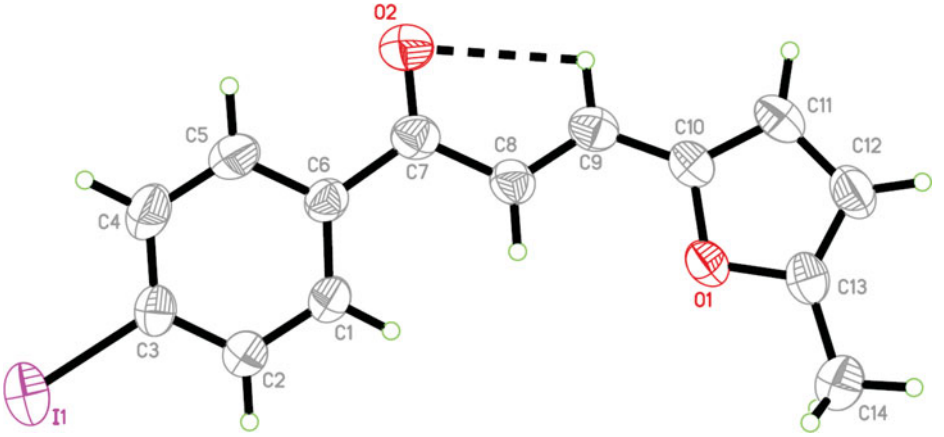


Figure 4. The molecular structure of compound **3e**, drawn with 30% probability displacement ellipsoids and the atom-numbering scheme. Dash lines show the C–H···O intramolecular hydrogen bonds.

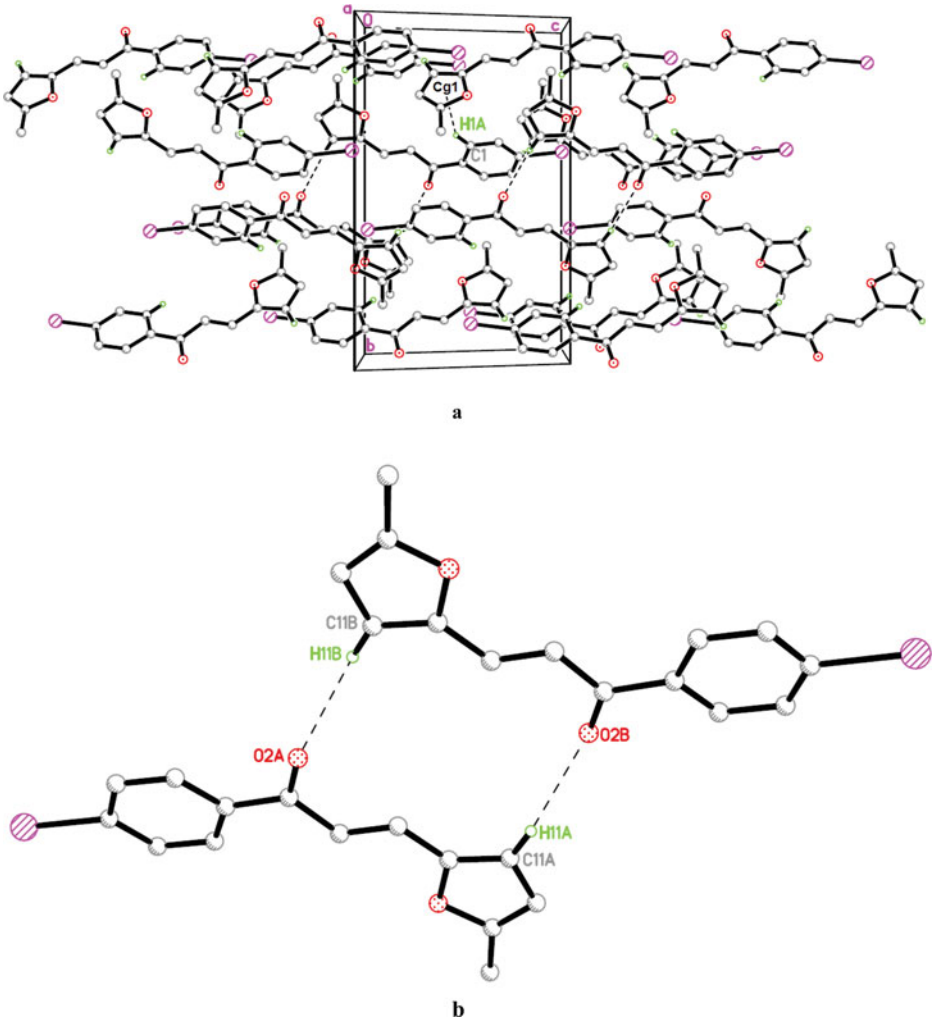


Figure 5. (a) Crystal packing of compound **3e**, hydrogen bond is shown as s dash lines. **(b)** Dimer in compound **3e**.

Table 3. Crystal data and structure refinement of compound **3e**.

CCDC	1409270
Formula	$C_{14}H_{11}IO_2$
Formula weight (gm·mole^{-1})	338.13
Temperature (K)	297
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	$P2_1/n$
Unit cell (Å, °)	$a = 8.5647(14)$, $b = 15.793(3)$, $c = 10.2310(17)\beta = 109.124(2)$
Volume (Å^3)	1307.5(4)
Z	4
Calculated density (g·cm^{-3})	1.718
Absorption coefficient (mm^{-1})	2.436
$F(000)$	656
Crystal size (mm)	0.09×0.24×0.25
θ (min, max)	2.5°, 27.5°
Dataset	$-11 \le h \le 11$; $-20 \le k \le 20$; $-13 \le l \le 13$
Total/unique data/R (int)	38318, 3008, 0.060
Observed data [$I > 2.0\sigma(I)$]	1925
Data/parameters	3008, 155
R/wR^2/Goodness-of-fit	0.0531, 0.0882, 1.20
Largest diff. peak and hole (e·Å^{-3})	−0.50, 0.51

These values represent the concentration of the samples that caused a 50% decrease in cell growth [1].

The morphologic changes against MDA MB 231 and CHO cells were investigated to confirm the potent cytotoxicity of heterocyclic chalcones (Figures 2 and 3). It obviously showed that the treatment of chalcones at their IC_{50} values caused both of the cells to be rounded in shape, and retracted from their neighboring cells different from the non-treated cells as shown in Figure 2(f). However, Figure 2(g) with $IC_{50} = 23.2$ µg/mL shows that the morphology of CHO cell is the same as that of control (Figure 2a), except that the number of viable cells was reduced (referred with red arrows).

Apart from this, it can be observed from the red arrows in Figures 2(d) and (h) of MDA MB 231 that few of the cells still maintained their morphology when compared with their control (Figure 3a).

Results from the data of IC_{50} values revealed that all chalcones possess cytotoxic activity in a range of 9.8 to 20 µg/mL against MDA MB 231. A previous study has reported that the compound exhibited the concentration that inhibited 50% of cell growth of CHO or MDA 231, with $IC_{50} < 10\ \mu$g/mL considered to be as active, while $IC_{50} > 50\ \mu$g/mL was considered as non-cytotoxic to the cell [21]. Among all heteroarylchalcone bearing electron withdrawing groups, Cl**(3c)** exhibited the highest cytotoxic activity against MDA MB 231 cell ($IC_{50} = 9.8\ \mu$g/mL). It was supported by Solomon and Lee [4], who found that compound with 4-chloro group attached to the phenyl ring in furan chalcone emerged as the most potent compound.

However, the introduction of halogen groups at the phenyl ring of heterocyclic chalcone, especially compounds **(3b**, **3d**, **and 3e)** caused decrease in cytotoxic activity on MDA MB 231 cancer cell than the unsubstituted chalcone **(3a)**. Previous study showed that the introduction of hydrophobic substituent of halogen groups (fluoro, bromo, and chloro) at the para position of phenyl ring, especially in furan chalcone, resulted in an increase in anti-proliferative activity than the parental compound [4].

These contradictory results have been influenced by the effect of electron affinity of the compounds [22, 23]. According to Olive [23], a compound with higher electron affinity (ability to accept electron) required low concentration of drug. The increasing toxicity effects of chalcone against MDA MB 231 cell by the addition of halogen groups at para position were in

Table 4. Bond lengths (Å), angles (◦), and torsion angles (◦) for compound **3e**.

Bond lengths			
I1—C3	2.085 (5)	C7—C8	1.459 (7)
O1—C10	1.368 (6)	C8—C9	1.331 (6)
O1—C13	1.374 (6)	C8—H8A	0.9500
O2—C7	1.226 (6)	C9—C10	1.419 (7)
C1—C6	1.360 (7)	C9—H9A	0.9500
C1—C2	1.380 (7)	C10—C11	1.356 (7)
C1—H1A	0.9500	C11—C12	1.407 (8)
C2—C3	1.355 (7)	C11—H11A	0.9500
C2—H2A	0.9500	C12—C13	1.330 (7)
C3—C4	1.355 (7)	C12—H12A	0.9500
C4—C5	1.380 (8)	C13—C14	1.479 (7)
C4—H4A	0.9500	C14—H14A	0.9800
C5—C6	1.379 (7)	C14—H14B	0.9800
C5—H5A	0.9500	C14—H14C	0.9800
C6—C7	1.490 (7)		
Bond angles			
C10—O1—C13	107.1 (4)	C9—C8—H8A	119.0
C6—C1—C2	121.3 (5)	C7—C8—H8A	119.0
C6—C1—H1A	119.4	C8—C9—C10	126.7 (5)
C2—C1—H1A	119.4	C8—C9—H9A	116.7
C3—C2—C1	120.3 (5)	C10—C9—H9A	116.7
C3—C2—H2A	119.8	C11—C10—O1	108.7 (5)
C1—C2—H2A	119.8	C11—C10—C9	132.7 (6)
C2—C3—C4	119.7 (5)	O1—C10—C9	118.6 (4)
C2—C3—I1	120.3 (4)	C10—C11—C12	107.1 (5)
C4—C3—I1	120.0 (4)	C10—C11—H11A	126.4
C3—C4—C5	119.9 (5)	C12—C11—H11A	126.4
C3—C4—H4A	120.1	C13—C12—C11	107.5 (5)
C5—C4—H4A	120.1	C13—C12—H12A	126.3
C6—C5—C4	121.3 (6)	C11—C12—H12A	126.3
C6—C5—H5A	119.4	C12—C13—O1	109.6 (5)
C4—C5—H5A	119.4	C12—C13—C14	135.0 (6)
C1—C6—C5	117.5 (5)	O1—C13—C14	115.4 (5)
C1—C6—C7	123.7 (5)	C13—C14—H14A	109.5
C5—C6—C7	118.8 (5)	C13—C14—H14B	109.5
O2—C7—C8	120.6 (5)	H14A—C14—H14B	109.5
O2—C7—C6	119.7 (5)	C13—C14—H14C	109.5
C8—C7—C6	119.7 (5)	H14A—C14—H14C	109.5
C9—C8—C7	122.0 (5)	H14B—C14—H14C	109.5
Torsion angles			
C6—C1—C2—C3	−1.0(10)	O2—C7—C8—C9	−1.8(8)
C1—C2—C3—C4	0.2 (9)	C6—C7—C8—C9	178.7 (5)
C1—C2—C3—I1	−179.8(5)	C7—C8—C9—C10	177.6 (5)
C2—C3—C4—C5	−0.3(10)	C13—O1—C10—C11	−0.3 (6)
I1—C3—C4—C5	179.7 (5)	C13—O1—C10—C9	−179.3 (4)
C3—C4—C5—C6	1.2 (11)	C8—C9—C10—C11	−178.6 (6)
C2—C1—C6—C5	1.9 (9)	C8—C9—C10—O1	0.1 (8)
C2—C1—C6—C7	−178.3 (5)	O1—C10—C11—C12	−0.4 (7)
C4—C5—C6—C1	−2.0 (9)	C9—C10—C11—C12	178.4 (6)
C4—C5—C6—C7	178.2 (6)	C10—C11—C12—C13	1.0 (7)
C1—C6—C7—O2	171.7 (5)	C11—C12—C13—O1	−1.1 (7)
C5—C6—C7—O2	−8.5 (8)	C11—C12—C13—C14	177.6 (6)
C1—C6—C7—C8	−8.7 (8)	C10—O1—C13—C12	0.9 (6)
C5—C6—C7—C8	171.1 (5)	C10—O1—C13—C14	−178.1 (5)

the following order: **3e** < **3d** < **3b** < **3c**. These halogen groups, which act as electron attracting substituents, help to increase electron affinity. Conversely, literature revealed that the order of halogen groups, as substituents were slightly different with the following order: $-NO_2 > -Br = -Cl > -F$ [24]. This was due to several factors, such as hydrophobic parameter and molecular

Table 5. Hydrogen bond lengths (Å) and angles (◦) for compound **3e**.

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (◦)
C9—H9A···O2	0.95	2.4600	2.790(6)	100.00
C11—H11A···O2[a]	0.95	2.5300	3.434(8)	159.00
C1—H1A···Cg1[b]	0.95	2.8200	3.642	145.00

Symmetry operation: (a) $x, y, -1+z$, (b) $\frac{1}{2}+x, \frac{1}{2}-y, \frac{1}{2}+z$.
Cg1: O1/C10–C13.

hardness, that influenced the cytotoxicity of MDA MB 231 cell [22]. Meanwhile, the substitution of phenyl ring with pyrazine **(5)** and pyridine **(7)** showed weak cytotoxic effect on MDA MB 231 cell ($IC_{50} > 10\ \mu g/mL$). According to Ellithey et al. [25], the compound that exhibited $IC_{50} > 10\ \mu g/mL$ was considered as a weak cytotoxic to the cell.

In addition, our results clearly showed that chalcone with substituent bromine **(3d)** and iodine **(3e)** showed greater toxicity on CHO, non-cancer cell line (13.5 and 15.0 $\mu g/mL$) compared with MDA MB 231 (16.2 and 20.0 $\mu g/mL$). This SAR study implies that the toxicity of the tested compounds on cells is possibly due to some factors such as the conjugation effect of the presence of double bond in the structure of heterocyclic chalcone [26], the lipophilic nature (dissolved in lipid) [4], and the presence of substituent at both aromatic and heteroaryl rings. Overall, the synthesized compounds exert cytotoxic effect on CHO cell line, which is in agreement with Pittella et al. [27], who reported that the chemotherapeutic agents available in the market worked on both cancer and non-cancer cells. Apart from this, a commonly prescribed anticancer agent, cisplatin, has been shown to exert cytotoxic activity on MDA MB 231 (7.10 $\mu g/mL$) and non-cancer breast cell, 184B5 (7.66 $\mu g/mL$) [4]. Survey of literature reported that 42% of patients who were treated with high dose of cisplatin for solid tumor suffered from nephrotoxic injury [28]. Meanwhile, tamoxifen also showed cytotoxic activity against MDA MB 231 (12 $\mu g/mL$) and CHO cells (8 $\mu g/mL$) [5]. Based on the IC_{50} values between MDA MB 231 and CHO cells, it revealed that both of these drugs are toxic toward normal cell lines.

X-ray crystallography

All bond lengths and angles in compound **3e** were within normal ranges, comparable with the related structure [29], and listed in Table 3. The molecule exists in *E* configuration with respect to C8=C9 double bond (1.331(6) Å). Dihedral angle between benzene and furan rings is 12.3(3)°.

An *S*(5) ring motif was observed in the molecules, formed by intramolecular interaction of C9–H9A···O2 (Table 4, and Figure 4). The crystal structure of compound **3e** (Figure 5a) was also stabilized by the intermolecular interaction of C11–H11A···O2, and formed a dimer as shown in Figure 5b. C–H···π interaction [30] (Figure 5a) involving furan ring was also found in the crystal structure (Table 5).

Conclusions

A series of heterocyclic chalcone containing halogen groups, pyrazine, and pyridine, replacing the phenyl ring, have been successfully synthesized in high yields. The potential bioactive compounds were evaluated for their cytotoxic activities using MTT assay. Based on the IC_{50} values of cytotoxic activities of heterocyclic chalcone, the overall compounds were found to be toxic on MDA MB 231 and CHO cell as well. Since this is a preliminary study and the

normal cell used is from a mouse, it cannot be compared with human breast normal cell line. In future, this series of chalcone needs to be further tested on normal breast cell lines such as 184B5 (non-cancer breast 270 epithelial cells), as compound **3c** (IC_{50} = 9.8 μg/mL) was found to be an active compound against MDA MB 231.

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