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# Synthesis and *In vitro* Cytotoxic Evaluation of New Quinazolinone-Based Chalcones

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**Abstract**

*Chalcones are a class of compounds with a wide range of biological activities. In addition, derivatives based on the quinazolinone skeleton are currently of interest to research in the screening of compounds with cytotoxic effects. Compounds containing chalcone structures on the basis of quinazolinone can yield new structures with cytotoxic effects. This article presents the synthesis of new quinazolinone-based chalcones **8a-j** via a three step-procedure. The first step is the condensation of 5-hydroxyanthranilic acid (**5**) with acetic anhydride at reflux for 2 h to afford intermediate **6** in 87%. This intermediate was then reacted with 4-aminoacetophenone in acetic acid at reflux for 14 h to give **7** in 77 %. Finally, the reaction of **7** with different aldehydes in ethanol in the presence of NaOH at room temperature for 14 h furnished target compounds **8a-j** in 57 - 75%. The structure of synthesized compounds was confirmed using $^{1}H$, $^{13}C$ NMR and MS spectra. The bioassay results showed that several compounds displayed cytotoxic activity against two cell lines including HepG-2 and SKLu-1. Among synthesized compounds, **8c** exhibited the strongest cytotoxic activity against SKLu-1 with $IC_{50}$ value of 20.10 μM.*

Keywords: Cancer, chalcone, cytotoxicity, quinazolinone.

## 1. Introduction

Chalcone is one of the most important groups of flavonoids in the entire plant kingdom [1]. Studies showed that some chalcones possess a wide variety of cytoprotective and modulatory functions, which may have therapeutic potential for multiple diseases. In terms of structure, chalcones are open-chain precursors for the biosynthesis of flavonoids and iso-flavonoids. Chalcone occurs mainly as colored polyphenolic compounds [1]. Chalcone exists as a *trans* (*E*) or *cis* (*Z*) isomer where the two aromatic rings are linked together by a conjugated ketone system. In most cases, the *E* isomer (**1**) is more stable from a thermodynamic point of view, which makes it the predominant configuration among chalcones. The configuration of the *Z* isomer (**2**) is unstable due to the steric effect between the carbonyl group and the A ring [1] (Fig. 1).

The chemistry of chalcone is attracting the research interest of chemists because a large number of new chalcone derivatives can be generated by substituting the atomic hydrogens in the chalcone's structure. Many chalcone derivatives have promising biological activities, including anti-inflammatory, anti-gout, antihistamine, antioxidant, anti-obesity [1]. In particular, metochalcone (**3**) has been approved as a choleretic [2], and sofalcone (**4**) as an anti-ulcer agent increases mucosal prostaglandins, conferring gastric protective effect against *Helicobacter pylori* [2].

Quinazolinone is a class of nitrogen-containing heterocyclic substances that forms an important class of pharmacophores in medicinal chemistry due to their potential in H bonding and π–π stacking interactions with aromatic amino acid residues of receptors [3-5]. Therefore, quinazolinone is often used as a scaffold in the design and synthesis of compounds with cytotoxic effects, and a lot of drugs containing quinazolinone skeleton have been invented and used effectively in therapy such as anti-cancer (raltritrexed), anti-fungal (albaconazole), sedation (methaqualone) and non-steroidal anti-inflammatory (proquazone) compounds [6-8] (Fig. 2).

Chalcones and their analogues have attracted increasing interest due to their broad biological activities with clinical potential against various diseases, particularly for antitumor activity. A lot of chalcone derivatives have demonstrated potential *in vitro* and *in vivo* activity against cancers via multiple mechanisms, including cell cycle disruption, autophagy regulation, apoptosis induction, and immunomodulatory and inflammatory mediator [1].

Recently, several quinazolinone-based chalcones have been synthesized and displayed potent activity against some cancer cell lines [9,10]. Being intrigued by this observation, in this report, we present a synthesis of new quinazolinone-based chalcones and evaluate cytotoxic activity against several cancer cell lines.

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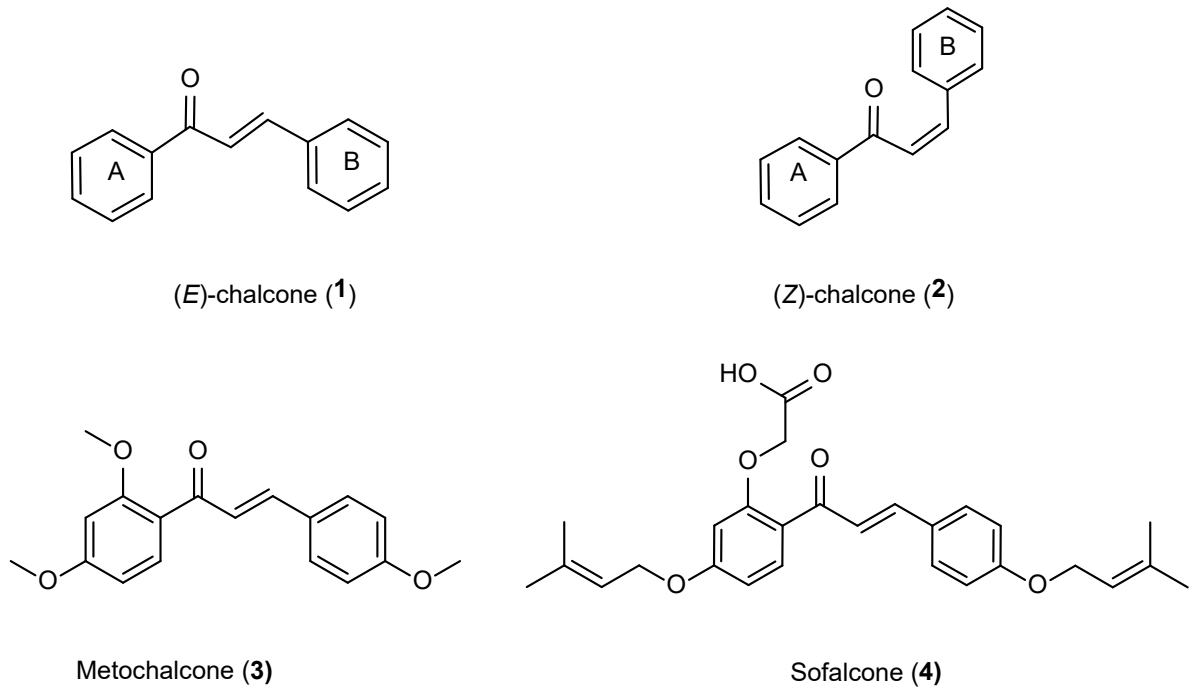



Fig. 1. *E* and *Z* isomers of chalcone and drugs with chalcone structures **3** and **4**

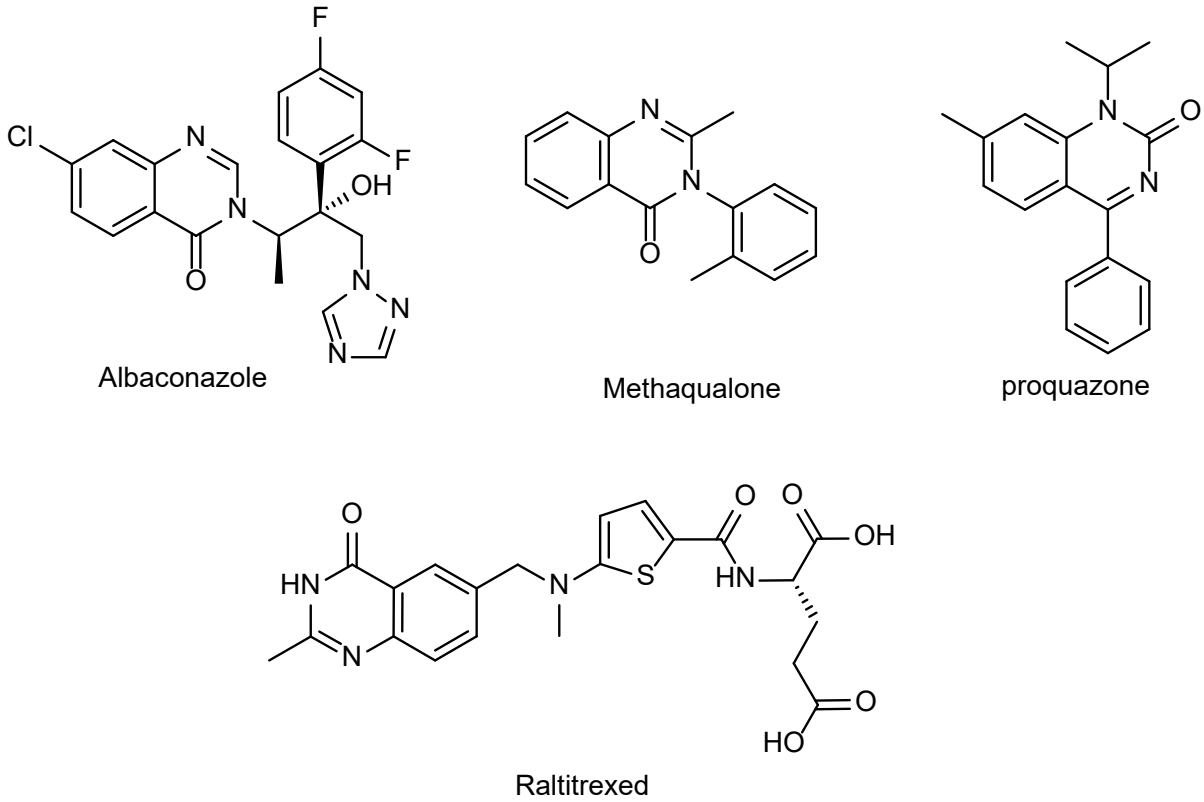



Fig. 2. Several quinazolinone-based drugs.

## 2. Material and Methods

### *2.1. Chemistry*

All products were examined by thin-layer chromatography (TLC), performed on Whatman® 250 μm Silica Gel GF Uniplates and visualized under UV light at 254 nm. Melting points were determined in open capillaries on Electrothermal IA 9200 Shimazu apparatus and uncorrected. Purification was done by crystallization and the open flash silica gel column chromatography using Merck silica gel 60 (240 to 400 mesh). $^{1}$H, $^{13}$C NMR and ESI-MS were performed at the Institute of Chemistry, Vietnam Academy of Science and Technology. Nuclear magnetic resonance spectra ($^{1}$H and $^{13}$C NMR) were recorded using tetramethylsilane (TMS) as an internal standard on a Bruker 500 MHz spectrometer with DMSO-*d6* as solvents. Chemical shifts are reported in parts per million (ppm) downfield from TMS as internal standard and coupling constants (*J*) are expressed in hertz (Hz). Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on FTICR MS Varian. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp or Merck unless noted otherwise. Solvents were distilled and dried before use.

### *2.2. Bioassay*

All media, sera and other reagents used for cell cultures were obtained from GIBCO Co. Ltd. (Grand Island, New York, USA) and two human cancer cell lines for testing including HepG-2 (liver cancer), and SKLU-1 (lung cancer) were provided by Institute of Biotechnology, Vietnam Academy of Science and Technology. The cytotoxicity of synthesized compounds was determined by a method of the American National Cancer Institute (NCI) as described in literature [12]. Briefly, these cancer cell lines were grown as monolayers in 2 mM of L-glutamine, 10 mM of HEPES, 1.0 mM of sodium pyruvate, and supplemented with 10% fetal bovine serum-FBS (GIBCO). Cells were cultured for 3-5 days after transfer and maintained at 37 $^{o}$C in a humidified atmosphere containing 5% $CO_2$. Assay samples were initially dissolved in DMSO and serially diluted to appropriate concentrations with a culture medium right before the assay. Then the cells in each well, incubated for 24 hours as described above, were treated with 20 μL of samples at 20 μg/mL; 0.8 μg/mL; 0.16 μg/mL. The plates were further incubated for 48 hours. The medium was removed and the cells were fixed with 10% solution of trifluoroacetic acid. The fixed cells were stained for 30 minutes by a staining solution (RSB method). Protein-bound dye was dissolved in a 10 mM tri-base solution and the ODs were measured at 510 nm using an Elisa reader. The $IC_{50}$ values were then calculated using Probits method. Ellipticine (Sigma) was used as a positive control and the values reported for the compounds are presented as an average of three determinations.

*2.2.1. Synthesis of 6-Hydroxy-2-methyl-4H-benzo[d][1,3] oxazin-4-one (**6**)*

A mixture of 5-hydroxy anthranilic acid (**5**) (5.0 g, 32.67 mmol) in acetic anhydride (15 mL) was refluxed for 2 h. The mixture was then poured in ice water. The resulting precipitate was filtered, washed with distilled water and dried in a vacuum to afford **6** (5.03 g, 87 %) which was used for the next step.

*2.2.2. Synthesis of 3-(4-Acetylphenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (7)*

A mixture of **6** (862 mg, 5.64 mmol) and 4-aminoacetophenone (2284 mg, 16.92 mmol, 3eq) in acetic acid (10 mL) was refluxed for 14 h. The reaction was monitored by TLC ($CH_2Cl_2$: MeOH = 100 : 1). The reaction mixture was then neutralized with 50 % $NaHCO_3$ to pH = 7 and extracted with $CH_2Cl_2$ (3 × 20 mL). The organic phase was separated, dried on anhydrous $Na_2SO_4$ and evaporated in reduced vacuum to afford the corresponding residue which was subjected to column chromatography on silica gel using n-hexane/ethyl acetate as eluting systems to give desired **7** as a white solid (1276 mg, 77%); $^{1}$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.03 (s, 1H, OH), 8.13 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* = 8.50 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 1H, H-8), 7.40 (d, *J* = 3.0 Hz, 1H, H-5), 7.30 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H, H-7), 2.65 (s, 3H, $CH_3$), 2.08 (s, 3H, $CH_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 197.3 (C=O), 170.3 (C-4), 160.9 (C-6), 155.9 (C-2), 150.2, 142.1, 140.5, 136.9, 129.4, 128.3, 124.0, 121.2, 109.1, 26.8($\underline{C}H_3CO$), 23.6 ($\underline{C}H_3$).

*2.2.3. General procedure for the synthesis of quinazolinone-based chalcone*

A mixture of **7** (400 mg, 1.36 mmol), NaOH (54 mg, 1 eq) and corresponding aldehydes (1.5 eq) in ethanol (10 mL) was stirred at room temperature for 10 h. The reaction was monitored by TLC ($CH_2Cl_2$: MeOH = 100 : 2). The reaction mixture was then neutralized to pH = 7 using HCl 5% and extracted with $CH_2Cl_2$ (3 × 20 mL). The organic phase was separated, dried on anhydrous $Na_2SO_4$ and evaporated in reduced vacuum to afford the corresponding residues which was subjected to column chromatography on silica gel using $CH_2Cl_2$ : MeOH=100 : 1 as eluting systems to give desired chalcones **8a-j**.

**(*E*)-3-(4-Cinnamoylphenyl)-6-hydroxy-2-methylquinazolin-4(3H)-one (8a)**

Bright yellow solid, yield 75%. Mp 132-134 $^{o}$C; $^{1}$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.05 (s, 1H, OH), 8.34 (d, *J* = 8.5 Hz, 2H), 8.03 (d, *J* = 15.5 Hz, 1H, H-β), 7.93-7.91(m, 2H), 7.83 (d, *J* = 15.5 Hz, 1H, H-α), 7.67 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 9.0 Hz, 1H, H-8), 7.48 (m, 2H), 7.42 (d, *J* = 2.5 Hz, 1H, H-5), 7.31 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H,

H-7), 2.11 (s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.6 (C=O), 161.0 (C=O), 155.9 (C-6), 150.3 (C-2), 144.4, 142.1, 140.5, 137.7, 134.6, 130.7, 129.7, 129.2, 128.96, 128.90, 128.3, 124.0, 121.9, 121.2, 109.1, 23.6 (C$\underline{H}_3$). ESI-MS *m/z*: 383.2 $[M+H]^+$.

**(*E*)-3-(4-(3-(2-Fluorophenyl)acryloyl)phenyl)-6-hydroxy-2-methylquinazoline-4(3H)-one (8b)**:

Bright yellow solid, yield: 62%; Mp 139-141 °C $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.05 (s, 1H, OH), 8.32 (d, *J* = 8.5 Hz, 2H), 8.16 (t, *J* = 8.0 Hz, 1H), 8.07 (d, *J* = 15.5 Hz, 1H, H-β), 7.91 (d, *J* = 15.5 Hz, 1H, H-α), 7.67 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 2H), 7.41 (d, *J* = 3.0 Hz, 1H), 7.33-7.29 (m, 3H), 2.08 (s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.4 (C=O), 161.9 (C-F), 161.0 (C-4), 159.9 (C-6), 155.9 (C-2), 150.2, 142.3, 140.5, 137.5, 135.5, 132.8, 129,8, 129.2, 128.3, 124.9, 124.0, 122.3, 122.2, 121.2, 116.2, 109.1, 23.6(C$\underline{H}_3$). ESI -MS *m/z*: 401.1 $[M+H]^+$.

**(*E*)-3-(4-(3-(4-fluorophenyl)acryloyl)phenyl)-6-hydroxy-2-methylquinazoline-4(3*H*)-one (8c)**

Bright yellow solid, yield 65%. Mp Mp 152-154 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.05 (s, 1H, OH), 8.34 (d, *J* = 8.5 Hz, 2H), 8.03-8.0 (m, 2H), 7.97 (d, *J* = 16.0 Hz, 1H, H-β), 7.83 (d, *J* = 16.0 Hz, 1H, H-α), 7.67 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 1H, H-8), 7.41 (d, *J* = 2.5 Hz, 1H, H-5), 7.34 (d, *J* = 8.5 Hz, 2H), 7.30 (dd, *J* = 3.0 Hz, 8.5 Hz, 1H), 2.1 (s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.4 (C=O), 164.5 (C-F), 162.5 (C=O), 161.0 (C-6), 155.9 (C-2), 150.3, 143.2, 142.1, 140.5, 137.7, 131.4, 129.7, 129.2, 128.3, 124.0, 121.8, 121.2, 116.0, 109.1, 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 401.3 $[M+H]^+$.

**(*E*)-6-Hydroxy-3-(4-(3-(4-hydroxyphenyl) acryloyl)phenyl)-2-methylquinazoline-4(3*H*)-one (8d)**

Bright yellow solid, yield: 64%; Mp 166-168 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.08 (s, 2H, OH), 8.29 (d, *J* = 8.0 Hz, 2H), 7.81-7.72 (m, 4H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.56 (*J* = 8.5 Hz, 1H), 7.41 (d, *J* = 1.5 Hz, 1H), 7.31 (dd, *J* = 1.5 Hz, 8.5 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 2H), 2.11 (s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)) 188.3 (C=O), 161.0 (C-4), 160.3 (C-OH), 155.9 (C-OH), 150.3 (C-2), 145.0, 141.8, 140.5, 138.2, 131.2, 129.5, 129.1, 128.3, 125.7, 124.0, 121.3, 118.4, 115.8, 109.1, 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 399.5 $[M+H]^+$.

**(*E*)- 6-Hydroxy-3-(4-(3-(2-hydroxyphenyl) acryloyl)phenyl)-2-methylquinazoline-4(3*H*)-one (8e)**

Bright yellow solid, yield 68%; Mp 147-149 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.04 (s, 1H, OH), 8.30 (d, *J* = 8.5 Hz, 2H), 8.13 (d, *J* = 15.5 Hz, 1H, H-β), 8.02 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 15.5 Hz, 1H, H-α), 7.65 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.47 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 3.0 Hz, 1H), 7.31 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 3.92 (s, 3H, OC$\underline{H}_3$), 2.11 (s, 3H, $CH_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.7 (C=O), 161.0 (C-4), 158.3, 155.9, 150.3 (C-2), 142.0, 140.5, 138.9, 137.9, 132.5, 129.6, 129.1, 128.6, 128.3, 124.0, 122.8, 121.7, 121.2, 120.7, 111.8, 109.1, 55.7 (O$\underline{C}H_3$), 23.6 (C$\underline{H}_3$). ESI-MS *m/z*: 413.6 $[M+H]^+$.

**(*E*)- 6-Hydroxy-3-(4-(3-(4-methoxyphenyl) acryloyl)phenyl)-2-methylquinazoline-4(3*H*)-one (8f)**

Bright yellow solid, yield 66%; Mp 122-124 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.04 (s, 1H, OH), 8.31 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 9.0 Hz, 2H), 7.86 (d, J = 15.5 Hz, 1H, H-β), 7.80 (d, *J* = 15.5 H, 1H, H-α), 7.64 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 1H, H-8), 7.41 (d, *J* = 3.0 Hz, 1H, H-5), 7.31 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H, H-7), 7.05 (d, *J* = 9.0 Hz, 2H), 3.84 (s, 3H, OC$\underline{H}_3$), 2.11 (s, 3H, $CH_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.4 (C=O), 161.5 (C=O), 161.0, 155.9, 150.3 (C-2), 144.5, 141.9, 140.5, 138.0, 130.92, 129.6, 129.1, 128.3, 127.2, 124.0, 121.2, 119.4, 114.4, 109.1, 55.4 (CO$\underline{C}H_3$), 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 413.2 $[M+H]^+$.

**(*E*)-6-Hydroxy-3-(4-(3-(2,4-dimethoxyphenyl) acryloyl)phenyl)-2-methylquinazoline-4(3H)-one (8g)**

Bright yellow solid, yield 65%. Mp 162-164 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.04 (s, 1H, OH), 8.26 (d, *J* = 8.5 Hz, 2H), 8.07 (d, *J* =15.5Hz, 1H, H-β), 7.97 (d, *J* = 8.5Hz, 1H), 7.84 (d, *J* =15.5Hz, 1H, H-α), 7.63 (d, *J* = 8,5 Hz, 2H), 7.55 (d, *J* = 9.0 Hz, 1H, H-8), 7.41 (d, *J* = 3.0 Hz, 1H, H-5), 7.31 (dd, *J* =3.0 Hz, 9.0 Hz, 1H, H-7), 6.67-6.63 (m, 2H), 3.92 (s, 3H, OC$\underline{H}_3$), 3.85 (s, 3H, OC$\underline{H}_3$), 2.10 (s, 3H, $CH_3$).$^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.47 (C=O), 163.3 (C-4), 161.0, 160.1, 156.1, 150.2, 141.8, 140.5, 139.2, 138.3, 130.2, 129.4, 129.1, 128.3, 124.1, 121.2, 118.9, 115.8, 109.1, 106.4, 98.3, 55.8 ($OCH_3$), 55.5 (O$\underline{C}H_3$), 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 443.3 $[M+H]^+$.

**(*E*)-6-Hydroxy-3-(4-(3-(3-hydroxy-4-methoxyphenyl)acryloyl)phenyl)-2-methylquinazoline-4(3H)-one (8h)**

Bright yellow solid, yield 67%; Mp 157-159 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.04 (s, 1H, OH), 9.16 (s, 1H, OH), 8.30 (d, *J* = 8.5 Hz, 2H), 7.78 (d, *J* = 15.5 Hz, 1H, H-β), 7.70 (d, *J* = 15.5 Hz, 1H, H-α), 7.64 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.41 (d, *J* = 2.5 Hz, 1H), 7.41-7.29 (m, 3H), 7.02 (d, *J* = 9.0 Hz, 1H), 3.85 (s, 3H, OC$\underline{H}_3$), 2.11 (s, 3H, $CH_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.3 (C=O), 161.0 (C-4), 160.3 (C-6), 155.9 (C-2), 150.3, 145.0, 141.9, 140.5, 138.2, 131.2, 129.6, 129.1, 128.3, 125.7, 124.0, 121.3, 118.4, 115.8, 109.1, 55.7 (O$\underline{C}H_3$), 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 429.2 $[M+H]^+$.

**(*E*)-6-Hydroxy-3-(4-(3-(4-chlorophenyl) acryloyl)phenyl)-2-methylquinazoline-4(3*H*)-one (8i)**

Light yellow solid, yield 57%. Mp 166-168 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.03 (s, 1H, OH), 8.34 (d, *J* =8.5 Hz, 2H), 8.06 (d, *J* = 16Hz, 1H, H-β), 7.98 (d, *J* = 8.5 Hz, 2H), 7.81 (d, *J* =15.5Hz, 1H, H-α), 7.66 (d, *J* =8.5Hz, 2H), 7.56-7.53 (m, 3H), 7.41 (d, *J* = 2.5Hz, 1H), 7.31 (dd, *J* = 3Hz, 9.0 Hz, 1H), 2.11 (s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)): 188.4 (C=O), 161.0 (C-4), 155.9 (C-6), 150.2 (C-2), 142.9, 142.2, 140.5, 137.6, 135.2, 133.6, 130.7, 129.8, 129.2, 128.9, 128.3, 124.0, 122.7, 121.2, 109.1, 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 417.0 $[M+H]^+$.

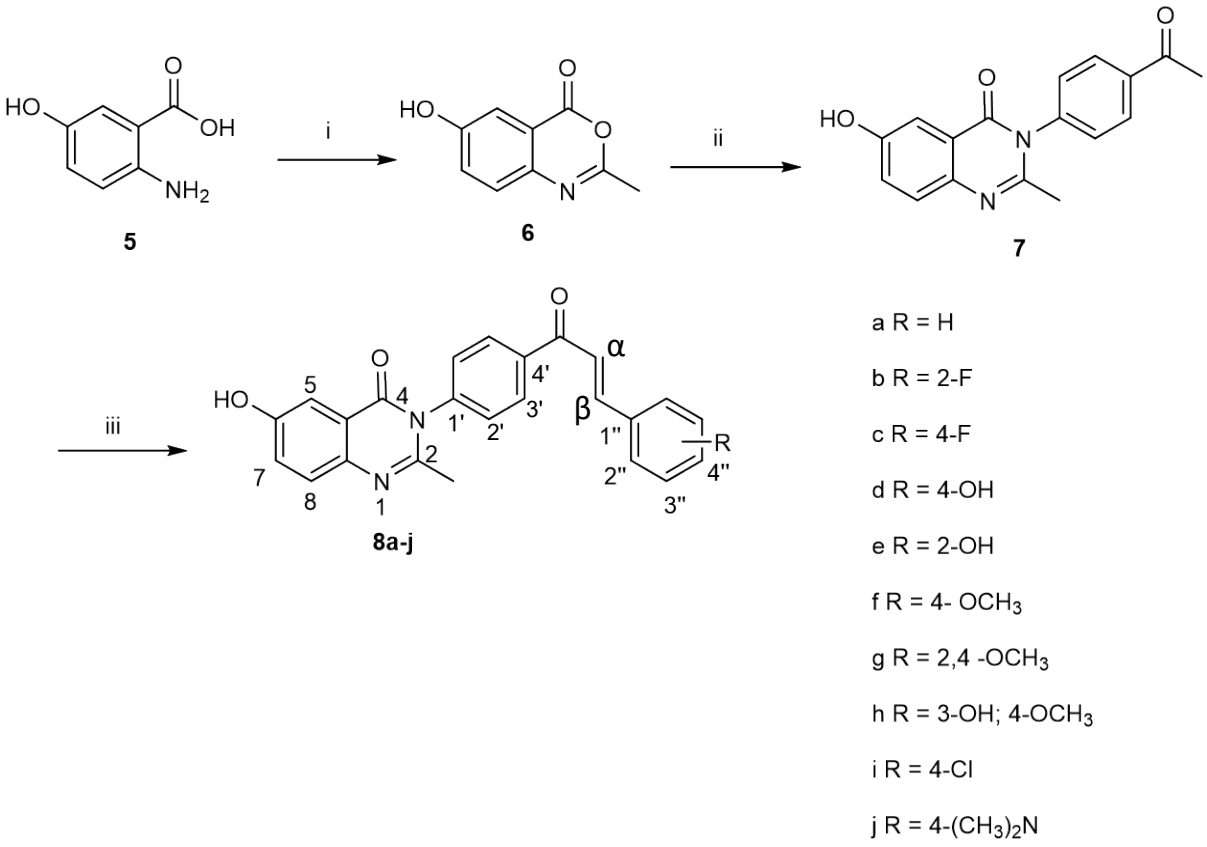
**(*E*)-3-(4-(3-(4-(Dimethylamino)phenyl) acryloyl)phenyl)-6-hydroxy-2-methylquinazolin-4(3*H*)-one (8j)**

Light yellow solid, yield 71%; Mp 163-165 °C; $^1$H NMR (500 MHz, DMSO-$d_6$, δ (ppm)): 10.04 (s, 1H, OH), 8.27 (d, *J* = 8.5 Hz, 2H), 7.76-7.71 (m, 4H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 9.0 Hz, 1H, H-8), 7.42 (d, *J* = 3.0 Hz, 1H, H-5), 7.31 (dd, *J* = 3.0 Hz, 9.0 Hz, 1H, H-7), 6.77 (d, *J* = 9.0 Hz, 2H), 3.02 (s, 6H, 2C$\underline{H}_3$), 2.11(s, 3H, C$\underline{H}_3$). $^{13}$C NMR (125 MHz, DMSO-$d_6$, δ (ppm)) 197.4 (C=O), 170.5, 165.4, 161.6, 159.8, 155.2, 151.0, 150.0, 148.1, 140.4, 138.8, 138.4, 137.8, 133.5, 131.4, 130.8, 125.4, 121.2, 118.6, 33.1 $(NCH_3)_2$, 23.6 (C$\underline{H}_3$). ESI -MS *m/z*: 426.2 $[M+H]^+$.

## 3. Results and Discussion

### *3.1. Chemistry*

A series of new quinazolinone-based chalcones **8a-j** was synthesized in good yields via a three-step procedure (Scheme 1). 6-Hydroxyanthranilic acid (**5**) was first condensed with the excess of acetic anhydride at reflux for 2 h to afford benzoxazinone **6** in 87 % yield [11]. The purification of compound **6** was obtained by pouring the reaction mixture into the ice-water. The resulting precipitate was filtered, washed with distilled water, and dried in a vacuum. Compound **6** was next reacted with 4-aminoacetophenone in acetic acid at reflux for 14 h to give the intermediate **7** in 92 % yield.



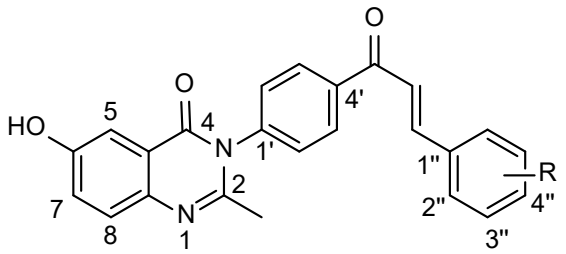

Scheme 1. Condition and reagents: i) $(CH_3CO)_2O$, reflux, 2h; 87%; ii) 4-aminoacetophenone, $CH_3COOH$, reflux, 14 h, 77%; iii) aldehydes, EtOH, NaOH, 14 h, 57-75%.

Finally, the reaction of **7** with different aldehydes in ethanol in the presence of NaOH at room temperature for 14 h to furnish new quinazolinone-based chalcones **8a-j** in 57 - 75 % yields. The structure of target compounds was characterized by $^{1}$H NMR, $^{13}$C NMR, and MS spectra. Due to the structural similarity of target compounds, compound **8c** was used as an example to elucidate the structure. The $^{1}$H NMR spectrum of compound **8c** indicated the presence of 17 protons in the molecule in which a singlet signal at $\delta_H$ 10.05 ppm is attributed to OH group.

It is easy to observe the resonance signals of two protons β and α of the conjugated system. The signal at $\delta_H$ 8.0 ppm is attributed to proton β and the other at $\delta_H$ 7.83 ppm with a coupling constant ($J$ = 15.5 Hz) confirming its trans (*E*) configuration. In addition, the characteristic splitting pattern of 3 protons H-5, H-7 and H-8 as a ABX system of quinazolinone skeleton was easily observed, in which the proton H-5 resonates as a doublet at $\delta_H$ 7.41 ppm ($J$ = 3.0 Hz) resulting from a long coupling with H-7. The proton H-8 resonates as a doublet at $\delta_H$ 7.56 ($J$ = 8.5 Hz) due to a near coupling with H-7 and H-7 was observed as a doublet of doublet at $\delta_H$ 7.30 (d, $J$ = 3.0 Hz, 8.5 Hz) due to coupling with both H-8 and H-7. In addition, four protons of the quinazolinone phenyl ring were also observed as doublets at $\delta_H$ 8.36 and 7.67 ppm ($J$ = 8.5 Hz), and four protons of the chalcone phenyl ring at $\delta_H$ 8.03 and 7.34 ppm ($J$ = 9.0 Hz). In the highest field, a singlet resonance signal of the quinazolinone methyl is observed at $\delta_H$ 2.11 ppm.

The $^{13}$C NMR spectrum of **8c** showed the presence of 19 aromatic carbons in the molecule, in which resonance signal in the lowest field at $\delta_C$ 188.4 ppm belongs to the conjugated system carbon. The signal at $\delta_C$ 164.5 ppm is attributed to the carbon attached to F. C-4 and C-6 resonate at $\delta_C$ 162.5.4 and 161.0 ppm, respectively, and C-2 at $\delta_C$ 150.1 ppm.

Table 1. *In vitro* cytotoxic activity of chalcones **8a-j**




**8a-j**

| N₀ | Compounds | R | IC₅₀ (µM)[a] | |
|---|---|---|---|---|
| | | | HepG-2[b] | SKLu-1[b] |
| 1 | **8a** | H | > 100 | > 100 |
| 2 | **8b** | 2-F | 37.45 ± 2.09 | 34.97± 1.08 |
| 3 | **8c** | 4-F | 24.75 ± 1.07 | 20.10± 0.39 |
| 4 | **8d** | 4-OH | >100 | >100 |
| 5 | **8e** | 2-OH | >100 | >100 |
| 6 | **8f** | 4-$OCH_3$ | > 100 | > 100 |
| 7 | **8g** | 2,4-$OCH_3$ | >100 | >100 |
| 8 | **8h** | 3-OH, 4-$OCH_3$ | >100 | >100 |
| 9 | **8i** | 4-Cl | 36.83 ± 2.37 | 32.31 ± 1.97 |
| 10 | **8j** | 4-$(CH_3)_2N$ | 47.51 ± 2.01 | 40.21 ± 1.11 |
| | **Ellipticine** | | **1.63** | **1.75** |

[a]Concentration (µM) that produces a 50 % reduction in cell growth or enzyme activity, the numbers represent the averaged results from triplicate experiments with deviation of less than 10 %. [b]Cell lines: HepG2, liver cancer; SKLU-1, lung cancer.

### *3.2. Bioassay*

All target compounds **8a-j** were evaluated for their *in vitro* cytotoxicity against HepG-2 (liver cancer), SKLU-1 (lung cancer) using SRB method [12]. All compounds were initially screened at a fixed concentration of 100 µg/mL. If the compounds are active, they will be further screened at smaller concentrations (e.g., 20 µg/mL, 4 µg/mL, 0.8 µg/mL and 0.16 µg/mL), and $IC_{50}$ values were calculated and shown in Table 1.

As can be seen in the Table 1 that 5 compounds including **8b**, **8c**, **8f**, **8i** and **8j** displayed cytotoxic activity on the two human cancer cell lines tested with $IC_{50}$ values ranging from 47.51 to 20.10 µM, and no compounds was comparable to ellipticine in terms of cytotoxicity. It was observed that compounds **8d**, **8e**, **8f**, **8g** and **8h** containing electron-donating groups (-OH, -$OCH_3$) resulted in no activity against both cancer cell lines tested except the compound **8j** that showed moderate cytotoxicity against the HepG2 and SKLu-1 cell lines with $IC_{50}$ values of 36.83 and 32.31 µM, respectively. It seems that these compounds were more cytotoxic towards the SKLu-1 than the HepG2 cell line. Compound **8b**, **8c** and **8i** containing electron -withdrawing groups (-F, -Cl) exhibited better cytotoxic activity against the SKLu-1 than the HepG-2 cell line. Among synthesized compounds, compound **8c** exhibited the strongest cytotoxic effect against SKLu-1 and HepG2 with $IC_{50}$ values of 20.10 and 24.75 µM, respectively.

## 4. Conclusion

It is the first time a series of new quinazolinone-based chalcones **8a-j** have been synthesized and elucidated structure using different spectroscopic methods such as $^{1}H$, $^{13}C$ NMR and MS. All target compounds have been evaluated for their *in vitro* cytotoxicity against two human cancer cell lines, including HepG-2 and SKLu-1. The result showed that several compounds exerted cytotoxic activity in which **8c** exhibited the strongest cytotoxic activity against SKLu-1 with $IC_{50}$ value of 20.10 µM. This compound can be considered as a template for future structural modification studies of new chalcones based on the quinazolinone skeleton.

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